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Functional genomics of the bacterial degradation of the emerging water contaminants: 1,4-dioxane and *N*-nitrosodimethylamine (NDMA)

by

Christopher Michael Sales

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Committee in charge:

Professor Lisa Alvarez-Cohen, Chair

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Professor Mary K. Firestone

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Abstract

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The emerging water contaminants 1,4-dioxane and *N*-nitrosodimethylamine (NDMA) are toxic and classified as probable human carcinogens. Both compounds are persistent in the environment and are highly mobile in groundwater plumes due to their hydrophilic nature. The major source of 1,4-dioxane is due to its use as a stabilizer in the chlorinated solvent 1,1,1-trichloroethane. The presence of NDMA as a water contaminant is related to the release of rocket fuels and its formation in the disinfection of water and wastewater. Prior studies have demonstrated that bacteria expressing monooxygenases are capable of degrading 1,4-dioxane and NDMA. While growth on 1,4-dioxane as a sole carbon and energy source has been reported in *Pseudonocardia dioxanivorans* CB1190 and *Pseudonocardia benzenivorans* B5, it is also co-metabolically degradable by a variety of monooxygenase-expressing strains. In contrast, NDMA has only been observed to biodegrade co-metabolically after growth on some monooxygenase-inducing substrates. The fastest rates of NDMA degradation occur in *Rhodococcus* sp. RR1 and *Mycobacterium vaccae* JOB5 after growth on propane. Pathways have been proposed for 1,4-dioxane biodegradation in *P. dioxanivorans* and for NDMA biodegradation in propane-induced *Rhodococcus* sp. RR1 based only on identified intermediates. The overall goal of this dissertation is to gain a better understanding of the genes and biological pathways responsible for degradation of 1,4-dioxane and NDMA.

Due to the lack of molecular sequence information for organisms capable of growth on 1,4-dioxane, the genome of *P. dioxanivorans* strain CB1190 was sequenced in this study. The genome has a total size of 7,440,794 bp and consists of four replicons: a circular chromosome, a circular plasmid pPSED01, an unclosed circular plasmid pPSED02, and a linear plasmid pPSED03. Analysis of this genome sequence revealed the presence of eight multicomponent monooxygenases: a propane monooxygenase, a phenol monooxygenase, a 4-hydroxyphenylacetate monooxygenase, four aromatic (toluene) monooxygenases, and a tetrahydrofuran (THF) monooxygenase. A total of 92 genes identified as putative dioxygenases were identified. Protein-encoding genes involved in transport systems, signal transduction systems, secretion systems, and heavy-metal and antibiotic resistance were identified. The presence of pathways for carbon and nitrogen metabolism was examined. A complete Calvin-Benson-Bassham carbon fixation pathway was found and a number of carboxylases that function in other carbon fixation pathways were identified. Although *P. dioxanivorans* has been reported to fix nitrogen, no nitrogenase genes

were found. The genome sequence of *P. dioxanivorans* was compared to other sequenced genomes of members in the family *Pseudonocardiaceae*, including *Amycolatopsis mediterranei* S699, *Amycolatopsis mediterranei* U32, *Pseudonocardia* sp. P1, *Pseudonocardia* sp. P2, *Saccharomonaspora azurea* NA-128, *Saccharomonospora paurometabolica* YIM 9007, *Sacharomonospora viridis* DSM43017, *Saccharopolyspora erythraea* NRRL2338, and *Saccharopolyspora spinosa* NRRL18395.

Genome-aided approaches were employed to identify the protein-encoding genes involved in the metabolic degradation of 1,4-dioxane in *P. dioxanivorans*. These approaches included whole genome expression microarray transcriptomics, quantitative reverse transcriptase PCR (qRT-PCR), enzyme assays, and heterologous expression clones. Genes differentially expressed during growth on 1,4-dioxane, glycolate (a previously identified degradation intermediate), and pyruvate (control) were analyzed to determine genes differentially expressed and involved in the metabolism of 1,4-dioxane. Based on the differentially expressed genes, the 1,4-dioxane degradation pathway was revised and annotated with the enzymes known to catalyze specific transformation steps. Up-regulation of genes were confirmed and quantified by qRT-PCR. Transcriptional analyses, isotopic tracer analyses with 1,4-[U-^{13}C]-dioxane, and glyoxylate carboligase enzymatic activity assays confirmed the role of glyoxylate as a central intermediate in the degradation of 1,4-dioxane. Specifically, transcriptional analyses indicated that the THF monooxygenase, encoded by *thmADBC*, is up-regulated during growth on 1,4-dioxane and THF. Furthermore, *Rhodococcus jostii* RHA1 clones heterologously expressing the *P. dioxanivorans* genes *thmADBC* demonstrated 1,4-dioxane and THF degradation activity. A surprising result with the THF monooxygenase expressing clones was the accumulation of the intermediate β-hydroxyethoxyacetic acid (HEAA). This result, combined with the non-inhibitory effect of acetylene on HEAA degradation by 1,4-dioxane grown *P. dioxanivorans* indicates that a monooxygenase is not involved in the transformation of HEAA as previously hypothesized. Transcriptomic microarray analysis of THF- and succinate-grown cells led to the first proposed THF metabolic degradation pathway for *P. dioxanivorans*. A novel finding of this transcriptomic microarray analysis was the identification of an alcohol dehydrogenase up-regulated during growth on THF that could catalyze the conversion of 2-hydroxytetrahydrofuran to γ-butyrolactone.

The genome sequence of *Rhodococcus jostii* RHA1 was utilized to determine the propane-induced monooxygenase responsible for NDMA degradation. The degradation of NDMA was characterized in *R. jostii* RHA1 grown on propane and on non-inducing substrates (LB medium, soy broth, and pyruvate). Propane enhanced the removal rate of NDMA by nearly two orders of magnitude compared to constitutive degradation during growth on non-inducing substrate. Transcriptomic microarray and qRT-PCR analyses demonstrated that propane elicits the up-regulation of a propane monooxygenase gene cluster. Genetic knockouts of the *prmA* gene encoding the large hydroxylase component of the propane monooxygenase were unable to grow on propane and degrade NDMA. These results indicate that the propane monooxygenase is responsible for NDMA degradation by *R. jostii* and explain the enhanced co-metabolic degradation of NDMA in the presence of propane. With the newly gained knowledge of the role of propane monooxygenase in NDMA degradation, oligonucleotide degenerate primers targeting *prmA* were designed and were identify and quantify the presence of propane monooxygenase genes

in *Rhodoccocus* sp. RR1 and *M. vaccae* JOB5. A homolog to *prmA* was found in *Rhodoccocus* sp. RR1 but not in *M. vaccae* JOB5. The *prmA* gene in *Rhodococcus* sp. RR1 was up-regulated during growth on propane relative to pyruvate. Characterization of the kinetics of propane-enhanced NDMA degradation showed that *Rhodococcus* sp. RR1 and *M. vaccae* possess similar maximum transformation rates (44 ± 5 and 28 ± 3 mg NDMA(mg protein)$^{-1}$h^{-1}, respectively). However, a comparison of half saturation constants ($K_{s,n}$) and NDMA degradation in the presence of propane revealed pronounced differences between the strains. The $K_{s,n}$ for *Rhodococcus* sp. RR1 was 36 ± 10 mg NDMA L^{-1} while the propane concentration needed to inhibit NDMA rates by 50% (K_{inh}) occurred at 7,700 mg propane L^{-1} (R^2 = 0.9669). In contrast, *M. vaccae* had a markedly lower affinity for NDMA verses propane with a calculated $K_{s,n}$ of 2,200 ± 1,000 mg NDMA L^{-1} and K_{inh} of 120 mg propane L^{-1} (R^2 = 0.9895). Differences between the propane monooxygenases in *Rhodococcus* sp. RR1 and the unidentified enzyme(s) in *M. vaccae* may explain the disparities in NDMA degradation and inhibition kinetics between these strains.

To Hilary

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Chapter 1

Introduction and Objectives

1.1. Introduction

Awareness of the presence of 1,4-dioxane and *N*-nitrosodimethylamine (NDMA) as groundwater contaminants has grown significantly over the last decade (Mitch *et al*., 2003a; Mohr *et al*., 2010). The concern with these compounds is their toxicological effects, primarily their ability to act as probable human carcinogens. The occurrence of 1,4-dioxane contamination is due to its use and unintentional formation as a by-product in industrial and commercial processes. The dominant source of 1,4-dioxane in the environment is due to its role as a solvent stabilizer to the widely used chlorinated solvent, 1,1,1-trichloroethane (1,1,1-TCA). The occurrence of NDMA contamination is attributable to its use as an additive to a few manufactured and commercial products, but the two main sources of NDMA in the environment are its formation in liquid rocket fuels containing unsymmetrical dimethylhydrazine (UDMH) and its formation during chlorine based disinfection of wastewater (Mitch *et al*., 2003b; Choi and Valentine, 2002; Mitch and Sedlak, 2002). Once 1,4-dioxane and NDMA are released into groundwater, they are highly mobile because of their hydrophilic characteristics. Conventional treatment strategies, such as air stripping, activated carbon adsorption, and chemical oxidation are not effective at removing either of these compounds. A number of advanced oxidation processes (AOPs) have been effective at removing 1,4-dioxane from water (Klecka and Gonsior, 1986; Stefan and Bolton, 1998ab; Maurino *et al*., 1997; Kim *et al*., 2006). For treatment of NDMA contamination, direct UV photolysis is preferred over AOPs (Lee *et al*., 2007; Lee *et al*., 2008). An alternative strategy for removing 1,4-dioxane and NDMA is biological treatment.

Biodegradation of 1,4-dioxane has been reported to occur in pure and mixed cultures of bacteria under mainly aerobic conditions. Aerobic biotransformation of 1,4-dioxane can occur metabolically or co-metabolically. A fair number of bacteria and fungi have been identified with the ability to co-metabolically degrade 1,4-dioxane after growth on an inducing substrate, such as methane, propane, tetrahydrofuran (THF), and toluene (Burback and Perry, 1993; Roy *et al*., 1994; Raj *et al*., 1997; Patt and Abebe, 1995; Kohlweyer *et al*., 2000; Zenker *et al*., 2000; Vainberg *et al*., 2006; Mahendra, 2006; Skinner *et al*., 2009). Only a handful of organisms can grow on 1,4-dioxane as a sole carbon and energy source, including a fungus *Cordyceps sinensis* (Nakaimaya *et al*., 2005) and four bacterial isolates, *Rhodococcus ruber* 219 (Bernhardt and Diekmann, 1991), *Mycobacterium* sp. PH-06 (Kim *et al*., 2009), *Pseudonocardia dioxanivorans* CB1190 (Parales *et al*., 1994; Mahendra and Alvarez-Cohen, 2005) and *Pseudonocardia benzenivorans* B5 (Mahendra and Alvarez-Cohen, 2006).

Aerobic and anaerobic biodegradation of NDMA has been observed to occur in multiple consortia of soil microorganisms (Mallik and Tesfai, 1981; Kaplan and Kaplan, 1985; Gunnison *et al*., 2000; Bradley *et al*., 2005; Sedlak *et al*., 2006; Yang *et al*., 2005; Gan *et al*., 2006; Arenzio *et al*., 2006; Zhou *et al*., 2009; Padhye *et al*., 2009). To date, no organisms have been identified that can use NDMA as a sole source of carbon and energy for growth. However, a number of organisms are capable of NDMA removal after growth on inducing substrates (Yoshinari and Shafer, 1985; Sharp *et al*., 2005). The fastest reported degradation of NDMA occurred in propane-grown *Mycobacterium vaccae* JOB5 (Sharp

et al., 2005). In the doctoral studies of Jonathan O. Sharp (2006), propane also enhanced the degradation of NDMA within a number of soil microcosms and in a bacterial isolate *Rhodococcus* sp. RR1.

Multiple lines of evidence have shown that monooxygenase enzymes are responsible for the aerobic biodegradation of 1,4-dioxane and NDMA (Sharp *et al*, 2005; Mahendra *et al*., 2006). Co-metabolic degradation of 1,4-dioxane has been observed in bacteria that can express a soluble methane monooxygenase, a propane monooxygenase, a toluene-2-monooxygenase (T2MO), a toluene-p-monooxygenase (TpMO), a toluene-4-monooxygenase (T4MO), and a THF monooxygenase (Mahendra and Alvarez-Cohen, 2006; Vainberg *et al*., 2006). Even metabolic growth on 1,4-dioxane by *P. dioxanivorans* and *P. benzenivorans* was proposed to be catalyzed by a monooxygenase enzyme based on positive results from a monooxygenase specific naphthol assay and acetylene inhibition of growth and degradation in these isolates (Mahendra *et al*., 2007). For NDMA, co-metabolic biodegradation was observed in strains expressing a soluble methane monooxygenase, a propane monooxygenase, and a toluene-2-monooxygenase and acetylene inhibition of degradation was observed (Sharp *et al*., 2005). Based on identified intermediates, degradation pathways for 1,4-dioxane (Mahendra *et al*., 2007) and NDMA (Sharp, 2006) were proposed. Although a monooxygenase is expected to catalyze the initial oxidation of 1,4-dioxane and NDMA, the identity of the enzymes catalyzing each transformation step in these proposed pathways has not been determined.

1.2. Research goal and specific objectives.

The goal of this research was to employ functional genomics to gain a better understanding of the genes and enzymes involved in the biodegradation of the emerging water contaminants 1,4-dioxane and NDMA. This new gained insight will improve the design, operation, and outcome of bioremediation processes in natural and engineered systems.

In order to achieve the goal of improving our knowledge of 1,4-dioxane biodegradation, the following objectives were pursued:

(A.1) Sequence, assemble, and annotate the genome of *P. dioxanivorans* CB1190, in order to understand its physiology and discover the genes that allow it the rare ability to grow on 1,4-dioxane as a sole carbon and energy source.

(A.2) Identify up-regulated genes involved in the 1,4-dioxane metabolic degradation pathway in *P. dioxanivorans*, by using a whole genome expression microarray and quantitative reverse transcriptase PCR.

(A.3) Verify and characterize the functions of enzymes catalyzing transformation steps in the metabolic degradation pathway of 1,4-dioxane using enzymatic assays and expression clones.

A similar approach was taken to reach the goal of improving our knowledge of NDMA

biodegradation:

(B.1) Identify the genes responsible for propane-enhanced NDMA degradation in the genome-sequenced *Rhodococcus jostii* RHA1 using transcriptomic microarray analysis and genetic knockouts.

(B.2) Characterize the kinetics of and the gene clusters involved in propane-enhanced degradation by *Rhodoccocus* sp. RR1 and *Mycobacterium vaccae* JOB5.

1.3. Dissertation Overview.

This dissertation is organized into eight chapters. **Chapter 1** introduces the background information leading to the primary research goal and outlines the specific objectives. **Chapter 2** presents relevant information from the literature that is pertinent to 1,4-dioxane and NDMA as contaminants and that demonstrates the need for this research. **Chapter 3** analyzes the genome sequence of the 1,4-dioxane degrading *Pseudonocardia dioxanivorans* strain CB1190. Most of Chapter 3 will be condensed into a manuscript for submission that focuses on the discovery of multicomponent monooxygenases in *P. dioxanivorans*. A short genome announcement for *P. dioxanivorans* has been published in the *Journal of Bacteriology* (Sales *et al*., 2011). **Chapter 4** includes a revised biodegradation pathway for 1,4-dioxane that highlights the role of glyoxylate as an intermediate and indentifies genes that are up-regulated on 1,4-dioxane and shown to be functionally involved in the transformation of glyoxylate. A manuscript consisting of the work described in Chapter 4 is being prepared for submission in collaboration with Ariel Grostern, a postdoctoral researcher in the Alvarez-Cohen research group. **Chapter 5** confirms the role of a THF monooxygenase in the initial oxidation of 1,4-dioxane and THF, proposes a THF biodegradation pathway for *P. dioxanivorans*, and discovers that a monooxygenase does not catalyze the transformation of β-hydroxyethoxyacetic acid, a 1,4-dioxane degradation intermediate, as previously proposed. The bulk of the research in Chapter 5 is in preparation for submission. **Chapter 6** describes the identification of a propane monooxygenase that is involved in NDMA degradation. Much of the research described in Chapter 6 has been published in Applied and Environmental Microbiology (Sharp *et al*., 2007) and permission was granted by the American Society for Microbiology to modify the material from the publication for this dissertation. **Chapter 7** characterizes propane-induced cometabolic degradation of NDMA by *Rhodococcus* sp. RR1 and *Mycobacterium vacace* JOB5 through kinetics, gene presence, and gene expression studies. Portions of Chapter 7 have been published in Biotechnology and Bioengineering (Sales *et al*., 2010) and permission was received from John Wiley & Sons, Inc. to include material from the publication in this dissertation. **Chapter 8** summarizes and draws conclusion from this research and suggests directions for future work.

Chapter 2

Background and Literature Review

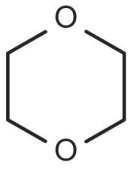
2.1. Introduction.

The compounds 1,4-dioxane and *N*-nitrosodimethylamine (NDMA) have become emerging water contaminants. Both chemicals lack drinking water regulation standards, but are classified as probable human carcinogens due to their observed carcinogenicity in animal studies. Contamination of water by 1,4-dioxane and NDMA are widespread because of their many uses and unintentional formation as by-products in industrial, commercial, and municipal processes. Both are highly mobile in aqueous systems and have shown resistance to removal by traditional treatment strategies. Treatment options, such as advanced oxidation processes and direct UV hydrolysis, have demonstrated effectiveness at removing these environmentally persistent compounds but are expensive options. Biological treatment of 1,4-dioxane and NDMA has also become a promising remediation strategy that has gained growing interest over the last decade. Although recent research has identified microbial organisms that degrade these contaminants as well as potential degradation intermediates, more research is necessary to understand the microbial processes that are involved in the biodegradation of 1,4-dioxane and NDMA.

2.2. 1,4-dioxane and NDMA as emerging contaminants.

1,4-Dioxane and NDMA have been classified by the United States Environmental Protection Agency (USEPA) as emerging contaminants (USEPA , 2008 and USEPA, 2010). 1,4-Dioxane is a cyclic organic compound containing two ether moieties (See Figure 2.1), with synonyms including dioxane, dioxan, *p*-dioxane, diethylene dioxide, diethylene oxide, diethylene ether, and glycol ethylene ether. The two molecular oxygen atoms in its ring structure make 1,4-dioxane hydrophilic and consequently highly soluble in water. The ring structure also makes 1,4-dioxane highly stable and resistant to rupture, except in the presence of concentrated acids and strong oxidizing agents (Mohr *et al*., 2010). These characteristics and others listed in Table 2.1 have made 1,4-dioxane a persistent compound in aqueous environments.

NDMA belongs to a class of compounds known as nitrosamines, which have a chemical structure R^1-N-(-R^2)-N=O. In NDMA, R^1 and R^2 are methyl groups. The nitroso group

A) 

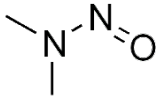
B) 

Figure 2.1. Chemical structure of 1,4-dioxane (A) and NDMA (B)

of NDMA makes it hydrophilic and highly mobile in aqueous systems. At room temperature, NDMA is a yellow liquid. Synonyms of NDMA include dimethylnitrosamine (DMNA), nitrosodimethylamine, *N*-methyl-*N*-nitrosomethenamine, and *N*,*N*-dimethylnitrosamine. A more detailed list of the physical and chemical properties of NDMA can be found in Table 2.1.

2.2.1. Drinking water regulations and health effects of 1,4-dioxane.

To date, the USEPA does not regulate 1,4-dioxane. Therefore, there is currently no federal maximum contaminant level (MCL) or MCL Goal (MCLG) for 1,4-dioxane in drinking water. Several USEPA regional offices use 6.1 µg/L as an advisory level for 1,4-dioxane concentrations in drinking water. In July 2006, the USEPA selected 1,4-dioxane as one of 32 from the 116 constituents on the Third Contaminant Candidate List (CCL) for further evaluation in its regulatory determination process (USEPA, 2011). This decision puts 1,4-dioxane one step closer to being federally regulated. In the absence of federal regulation, a number of states have adopted their own water quality standards or guidance values for 1,4-dioxane (Mohr *et al*., 2010). In March 2005, the Colorado Department of Public Health and Environment became the first state to enforce a water quality standard for 1,4-dioxane at 3.2 µg/L. California has a drinking water notification level (NL) of 3 µg/L for 1,4-dioxane but this is not a regulatory standard. Therefore, when 1,4-dioxane is found to exceed the NL, water utilities may continue to operate as long as they notify their customers of the presence of 1,4-dioxane in their water supply. Wells in California are recommended to be taken off-line if the concentration of 1,4-dioxane is 100 times the NL. Internationally, Canada established a guidance value for 1,4-dioxane in drinking water of 30 µg/L, while Japan has set a drinking water quality standard of 50 µg/L.

Table 2.1. Chemical and Physical Properties of 1,4-dioxane and NDMA

Property	1,4-dioxane	NDMA
Molecular weight	88.11	74.08
Density	1.028 g/cm^3	1.0059 g/cm^3
Water solubility	Miscible	Miscible
Boiling point	101.2°C	154°C
Vapor pressure	5.08 kPa at 25°C	0.36 kPa at 25°C
Octanol-water partition coefficient (log K_{ow})	-0.27	-0.57
Organic carbon partition coefficient (log K_{oc})	1.23	1.079
Henry's law constant (H_c)	4.80 x 10^{-6} atm-m^3/mol	2.63 x 10^{-7} atm-m^3/mol
Henry's law constant (dimensionless, H_{c*})	1.96 x 10^{-4}	1.1x10^{-5}

[a] Sources: USEPA, 2010; Mohr *et al*., 2010 and references therein

[b] Sources: ATSDR, 1989; USEPA, 2008

The possible health effects of 1,4-dioxane in humans are determined by considering toxicological data from studies in laboratory animals and a limited set of toxicity studies in humans. Toxicokinetics studies in laboratory animals and in human volunteers and workers demonstrated that the major metabolite of 1,4-dioxane is β-hydroxyethoxy acetic acid (HEAA) (Woo *et al*., 1977abc and 1978; Braun and Young, 1977; Young *et al*., 1976, 1977, 1978). Metabolism of 1,4-dioxane to HEAA is high following inhalation exposure, with 99% of 1,4-dioxane eliminated as HEAA in the urine in one study (Young *et al*., 1976). Oxidation of 1,4-dioxane to HEAA was shown to be mediated by the cytochrome P450 enzyme system in a variety of laboratory animals (Woo *et al*., 1977b and 1978; Pawar and Mungikar, 1976; Mungikar and Pawar, 1978 Nannelli *et al*., 2005).

The human toxicological data includes case reports of occupational poisoning, volunteer studies of acute inhalation exposure, and epidemiology studies of workers occupationally exposed to 1,4-dioxane (Mohr *et al*., 2010). From these toxicological studies, the primary exposure routes of 1,4-dioxane are inhalation and ingestion, with oral exposure due to consumption of contaminated drinking water. In an occupational setting, humans may be susceptible to 1,4-dioxane via major inhalation or dermal exposure. In fact, a handful of fatal cases of kidney and liver damage were attributed to inhalation and dermal exposure to 1,4-dioxane occupationally (Mohr *et al.*, 2010). Acute inhalation exposure of 1,4-dioxane to human volunteers caused irritation of the eyes, nose, and throat. Several acute and short-term studies of 1,4-dioxane in laboratory animals tested a variety of exposure routes, including dermal application, drinking-water exposure, gavage (force-fed to the stomach through a small tube), vapor inhalation, and intravenous (vein) or intraperitoneal (body cavity) injections. Severe liver and kidney degeneration and necrosis were frequently observed in these acute studies, with mortality seen in a number of acute-high dose experiments.

Laboratory animal studies also investigated the chronic effects associated with long-term, low-dose exposure to 1,4-dioxane. The majority of these chronic studies administered low-doses of 1,4-dioxane through drinking water. The highest doses in many of these chronic studies, using rats, mice, rabbits, and/or guinea pigs, led to mortality over time. In a study by Fairley *et al*. (1934), kidney enlargement, renal cortical degeneration, and necrosis in the cortex were observed in rats and mice exposed to 1,4-dioxane in their drinking water (qtd. in Mohr *et al*., 2010). A study by Hoch-Ligeti *et al*. (1970) exposed guinea pigs to low-doses of 1,4-dioxane in their drinking water. The guinea pigs developed 1,4-dioxane toxicity in a variety of locations, including the lungs, gall bladder, and kidneys (qtd. in Mohr *et al*., 2010). Argus *et al*. (1956) discovered that a portion of the rats that were exposed to 1,4-dioxane in their drinking water developed liver tumors (hepatocellular carcinomas) (qtd in. Mohr *et al*., 2010). In another study by the National Cancer Institute (NCI, 1978), visible nasal cavity tumors were discovered in rats exposed to 1,4-dioxane in their drinking water. These tumors obstructed the nasal passages, causing breathing difficulties and weight loss in the rats (Mohr *et al*., 2010). Giavini *et al*. (1985) examined the effects of 1,4-dioxane on rat reproduction. In this study, the fetuses of pregnant female rats given 1,4-dioxane by gavage in water showed evidence of developmental delays.

In summary, the non-cancer health effects associated with exposure to 1,4-dioxane in

humans and laboratory animals were liver and kidney toxicities. Additional non-cancer toxicological effects of 1,4-dioxane were discovered in the nasal cavity and respiratory tracts in laboratory animals. The cancer potential of 1,4-dioxane in humans is not well supported by human studies of occupational exposure to 1,4-dioxane because the size and number of reported cases are too small to make an accurate assessment. However, several carcinogenicity bioassays for 1,4-dioxane conducted in mice, rats, and guinea pigs observed various tumors in the liver and nasal cavity. Based on inadequate human evidence and sufficient evidence in experimental animals, the International Agency for Research on Cancer (IARC) classes 1,4-dioxane as possibly carcinogenic to humans (Group B2) (IARC, 1999). Similarly, the USEPA has classified 1,4-dioxane as a probable human carcinogen (Group B2).

2.2.2. Uses of 1,4-dioxane.

The occurrence of 1,4-dioxane in surface waters and groundwaters is predominantly due to its widespread use as a stabilizer for chlorinated solvents, such as 1,1,1-trichloroethane (1,1,1-TCA), also known as methyl chloroform (Mohr *et al*., 2010). Solvent stabilizers, as their name suggests, are compounds added to solvents to ensure they will not degrade during their intended application. Decomposition of chlorinated solvents can lead to acid formation by a number of routes: (1) hydrolysis; (2) oxidation, initiated by exposure to air, by thermal breakdown, or by ultraviolet light; and (3) condensation reactions with alkali metal salts (Mohr *et al*., 2010). As stated in Mohr *et al*. (2010), the stability of 1,1,1-TCA compared to other major chlorinated solvents (*i.e.*, carbon tetrachloride, dichloromethane, perchlorethene (PCE), and trichloroethene (TCE)) is rather poor because 1,1,1-TCA is especially unstable when exposed to alkali metals, such as aluminum. Chlorinated organics in contact with alkali metal results in the autocatalytic production of hydrochloric acid and the formation of a Lewis acid catalyst (*i.e.*, anhydrous aluminum trichloride [$AlCl_3$]), which catalyzes the condensation of chlorinated solvents onto themselves. Since 1,4-dioxane is a Lewis base (due to free electrons on its oxygen molecules), it competes with the solvent for electron-deficient sites on aluminum chloride that are responsible for breakdown of the chlorinated solvent. Therefore, in order to prevent the decomposition of 1,1,1-TCA via the aforementioned process, 1,4-dioxane was widely added to 1,1,1-TCA as a metal inhibitor (Mohr *et al.*, 2010). In fact, the largest demand for commercially produced 1,4-dioxane occurred in the late 1950s and early 1960s due to its use a stabilizer for 1,1,1-TCA (Mohr *et al*., 2010). The production of 1,4-dioxane for domestic consumption equaled approximately 3% of the production of 1,1,1-TCA. Only after the early 1990s, when the use of 1,1,1-TCA was reduced because of its ozone-depleting potential, did the production of 1,4-dioxane significantly decrease (Mohr *et al*., 2010).

In addition to being a chlorinated solvent stabilizer, 1,4-dioxane is used directly in a number of industrial and commercial processes. Solvents used to manufacture photosensitive resins and magnetic tapes for audio and data recording contain as much as 40% 1,4-dioxane (Mohr *et al*., 2010). 1,4-Dioxane is a key solvent in producing cellulose acetate

membrane filters for osmosis and kidney dialysis (Mohr *et al*., 2010). It is also a popular component of liquid scintillation cocktails, which are used for counting levels of radiation in various types of liquid media or tissue samples (Mohr *et al*., 2010). A number of liquid chromatography-mass spectroscopy methods use 1,4-dioxane as the organic mobile phase. 1,4-Dioxane has also been used as a dehydrating reagent for preparing tissues for histological slides (Mohr *et al*., 2010). Its presence in paints and inks is likely due to the addition of chlorinated solvents that contain 1,4-dioxane as a stabilizer (Mohr *et al*., 2010). The production of brominated fire retardants have used 1,4-dioxane as a solvent in the reaction of cyclodecatriene with bromide. Aircraft deicing fluids formulations are known to be comprised of ethylene glycol or propylene glycol which contain 1,4-dioxane as a residual solvent used in their production (Mohr *et al*., 2010). 1,4-Dioxane was also intentionally added to deicing fluids as a wetting and dispersing agent (Mohr *et al*., 2010). Many brands of automotive antifreeze also list 1,4-dioxane as a minor component, while some solvent-based automotive brake cleaning agents and rust inhibitors contain 1,4-dioxane. 1,4-Dioxane is also present in glues as a solvent and is found in 1,1,1-TCA-containing adhesives (Mohr *et al*., 2010). In order to create clear polyurethane for medical devices, 1,4-dioxane is commonly used as a solvent to dissolve the polymers (Mohr *et al*., 2010). Furthermore, cyclic ethers including 1,4-dioxane were often added as fuel supplement in racing vehicles to enhance the octane value (Mohr *et al*., 2010).

In addition to its direct uses, 1,4-dioxane is accidentally formed as a by-product in several chemical processes used to manufacture polyesters, soaps, and plastics (ATSDR, 2007; USEPA, 2010 ; Mohr *et al*., 2010). The sulfonation reaction with alcohol ethoxylates used to produce soaps and detergents also causes the formation of 1,4-dioxane. The alcohol ethoxylation process, where ethylene oxide is polymerized, allows the dimerization reaction of ethylene oxide to diethylene oxide, also known as 1,4-dioxane, to occur. Therefore, 1,4-dioxane has often been found as an impurity in cosmetics and shampoos that contain the surfactant sodium laureth sulfate and in contraceptive sponges and spermicidal lubricants containing nonoxynol-9 (Mohr *et al*., 2010). During the above manufacturing processes, the formation of 1,4-dioxane has been successfully limited by controlling various reaction parameters and, recently, stripping has been employed to remove any remaining 1,4-dioxane impurities from ethoxylated surfactants (ATSDR, 2007).

1,4-Dioxane has also been found as an impurity in polyethylene glycol (PEG) compounds used in a wide variety of products, including pharmaceuticals, plastics, resins, paper, lubricants, and cosmetics. PEG compounds are produced by the esterification of polyakyl alcohols with lauric acid and oleic acid and often contain 1,4-dioxane levels as high as 5 ppm (Mohr *et al.*, 2010). 1,4-Dioxane has also been found as an impurity of polyoxyethyleneamine, which is a major ingredient in herbicides such as Roundup®. Other ingredients in herbicides and pesticides, such as *p*-chlorophenyl isocyanate, rimsulfuron, and Dursban, are produced by reacting them with or in a solution of 1,4-dioxane. The esterification process for manufacturing polyethylene terephthalate (PET), commonly used in plastics for storing beverages and foods, forms 1,4-dioxane as a by-product.

Thus, the potential widespread impact of 1,4-dioxane on human health and the environment is evident in its use as a chlorinated solvents stabilizer, its direct uses in various industrial processes, and its accidental formation as an impurity in a number of consumer

products.

2.2.3. Occurrence and persistence of 1,4-dioxane in the environment.

Despite being widely used, efforts to detect 1,4-dioxane in the environment have been minimal. The few studies that have sought to detect 1,4-dioxane in the environment have shown its occurrence in ambient air, wastewater streams, surface water sources, groundwater aquifers, and drinking water supplies (Abe, 1999; Jackson & Dwarkanath, 1999; Johns *et al*., 1993; Zenker *et al*., 2003). In Washtenaw, Michigan, where Pall-Gelman Sciences Coporation manufactured medical filters, the sole pollutant in the groundwater aquifers was 1,4-dioxane, at concentrations up to 212,000 µg/L (Fotouhi, 2006). The City of Ann Arbor, Michigan conducted a study that found 1,4-dioxane to be present in raw wastewater and treated effluent, during all sampling events, at average concentrations of 3 ppb and 2 ppb, respectively (Skadsen *et al*., 2004). A few studies have also shown 1,4-dioxane to be present in the leachate beneath municipal and industrial landfills that impact groundwater aquifers (Mohr *et al*., 2010). A study by Abe (1999) investigated the distribution of 1,4-dioxane in surface waters and groundwater wells in a province adjacent to Tokyo, Japan. In this study, 1,4-dioxane was detected in all samples from three different rivers and in most of the groundwater samples, predominantly at low levels (<1 µg/L). One of the groundwater sampling locations had high concentrations of 1,4-dioxane, ranging from 52 – 95 µg/L. This study found that the load from industrial and domestic wastewater discharges was low but constant and widespread. The study also determined that the concentrations of 1,4-dioxane at a polluted groundwater sampling site were highly correlated with the concentrations of the legacy contaminant 1,1,1-TCA.

Since 90% of all dioxane produced in the United States was reportedly used as a stabilizer for 1,1,1-TCA, it is not surprising that 1,4-dioxane contamination in groundwater has been predominantly associated with the release of 1,1,1-TCA (Zenker *et al*., 2003; Mohr *et al*., 2010, Jackson & Dawarkanath, 1999). Although 1,1,1-TCA releases have declined over the last two decades, 1,4-dioxane has continued to persist in the environment. At sites contaminated with chlorinated solvents, analysis for 1,4-dioxane was either conducted late in the remediation process or not at all (Jackson & Dawarkanath, 1999). The prevalence of 1,4-dioxane in the environment is exacerbated by the physico-chemical properties of its cyclic ether structure. This molecular structure (Figure 2.1A) makes dioxane very stable and hydrophilic, allowing it to be extremely mobile in aquatic environments and resistant to many remediation strategies used to remove chlorinated solvents. Studies at solvent release sites consistently report that groundwater plumes of 1,4-dioxane in the subsurface are substantially larger than those of volatile organic compounds, such as 1,1,1-TCA, due to its lack of retardation (via sorption) and low volatility (Mohr, 2001). These plumes are poorly retarded by sorption to soil particles because dioxane does not partition well into the organic phase (log K_{ow} = -0.27) (ATSDR, 2007).

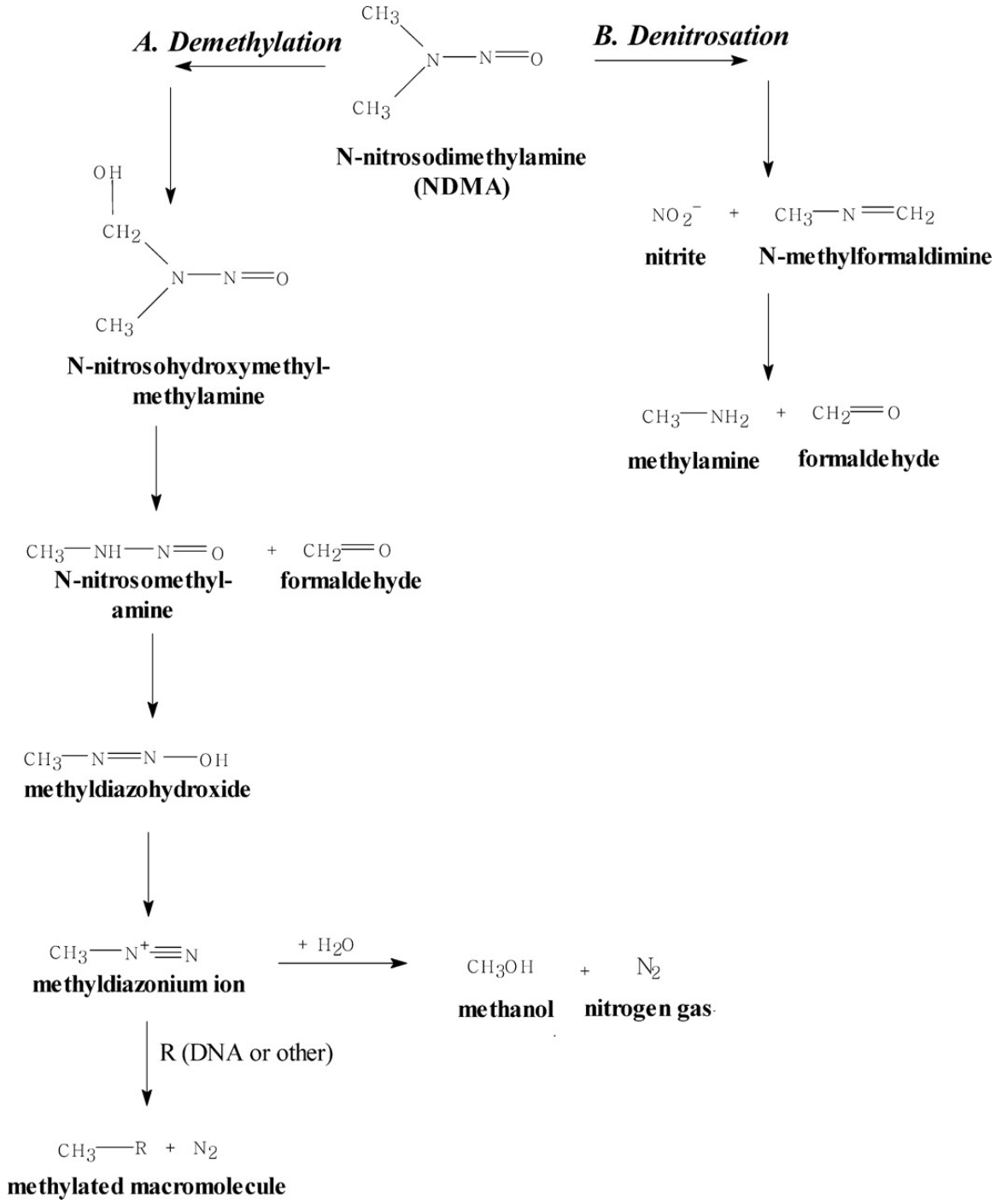


Figure 2.2. α-Hydroxylation (demethylation) and denitrosation pathways of NDMA metabolism in mammalian systems. From Fournier *et al*. (2006), adapted from Keefer *et al*. (1987) and Frei *et al*. (1999).

2.2.4. Drinking water regulations and health effects of NDMA.

Currently, a federal MCL for *N*-nitrosodimethylamine (NDMA) has not been established. However, in October 2009, NDMA was included in the Third CCL (USEPA, 2009) and was recently selected in June 2011 as one of the 32 constituents for further evaluation in its regulatory determination process (USEPA, 2011). Despite the lack of drinking water regulations, the USEPA has set a cleanup level of 0.7 ng/L at Superfund sites in California, based on a 1 in 10^{-6} lifetime excess cancer risk in drinking water (USEPA, 2001). The California Department of Public Health has published a drinking water NL of 10 ng/L for NDMA (CDPH, 2010).

The health effects of NDMA have been studied in laboratory animals since the 1950s. In 1954, Barnes and Magee (1954) became interested in studying the toxicology of NDMA in laboratory animals when two industrial laboratory workers developed cirrhosis of the liver after exposure to NDMA. Their initial study found that NDMA caused toxic liver injury in rats, mice, guinea pigs, rabbits, and dogs. Over the next two decades, further studies by Magee *et al.* (1956, 1958, 1962, 1964, and 1972) demonstrated the production of kidney and liver tumors in rats exposed to NDMA and other nitrosamines. In addition, several acute inhalation studies with animals resulted in death (ATSDR, 1989), while fatalities also resulted when human's drinks were found to be poisoned with NDMA (Cooper and Kimbrough, 1980). Small hemorrhages in the bronchi, trachea, gastrointestinal region, and subpericardial region were observed in one industrial worker, mentioned above, who died from accidental exposure to vapors of NDMA (Freund, 1937, qtd. in ATSDR, 1989). Additional acute studies, where laboratory animals were exposed to NDMA by gavage or by ingestion through drinking water, showed characteristic hepatotoxicity (*i.e.*, chemical-driven liver damage) (ATSDR, 1989), while chronic exposure to NDMA in laboratory animals induced tumor development in the liver, lungs, nasal cavity, and/or kidneys of mice, hamsters, rats, and minks (ATSDR, 1989). Oral doses of NDMA prior to mating or during pregnancy in mice resulted in increased perinatal death (ATSDR, 1989).

The genotoxicity of NDMA as a carcinogenic agent that reacts with DNA has been well studied in a number of *in vitro* and *in vivo* bioassays. Genotoxic effects include gene mutations, DNA fragmentation, chromosomal aberrations, DNA damage, micronuclei, DNA strand breaks and the formation of methylated DNA adducts (OEHHA, 2006). The mechanism for NDMA-induced carcinogenesis is believed to involve the formation of a highly reactive radical intermediate of NDMA metabolism that attacks DNA. Two pathways for NDMA metabolism have been characterized in animals, an α-hydroxylation pathway and a denitrosation pathway (Figure 2.3) (Keefer *et al*., 1987). While both pathways appear active in human and animal studies, α-hydroxylation is the predominant metabolic pathway that contributes to the carcinogenicity and genotoxicity of NDMA. The α-hydroxylation pathway is initiated by the hydroxylation of NDMA at the α carbon yielding α-hydroxymethyl nitrosamine, an unstable intermediate. This intermediate then breaks down into formaldehyde and, after a few steps, methyldiazonium ion. The methyldiazonium ion reacts with nucleophiles (*e.g.*, DNA, RNA, and proteins) by transferring a methyl group and releasing N_2. The cytochrome P450 enzyme (namely CYP2E1)

catalyzes both the α-hydroxylation and denitrosation pathways. Therefore, the occurrence of tumors in the liver appears to be related to the fact that cytochrome P450 enzymes are concentrated and induced primarily in the liver.

Despite the overwhelming evidence in animal studies, there remains a lack of epidemiological data to evaluate the carcinogenicity of NDMA in humans. For this reason, NDMA is classified as a Group B2 probable and confirmed animal carcinogen (USEPA, 2008).

2.2.5. Uses of NDMA.

NDMA is not produced for commercial or industrial use in the United States. Only small quantities of NDMA are prepared for research purposes. Prior to 1976, NDMA was formerly used as an intermediate in the production of 1,1-dimethyldrazine, a storable liquid rocket fuel (Fine *et al.*, 1977; Fine, 1979; Shapley, 1976). It has also been used historically as an additive for lubricants, a softener for polymers, and a rubber accelerator (ASTDR, 1989).

2.2.6. Occurrence and persistence of NDMA in the environment.

Although NDMA is not widely used, its occurrence in the environment is widespread. NDMA has been found in alcoholic beverages and various foods, especially cured and preserved meats, and it is detectable in cigarettes (OEHHA, 2006). Drinking water NDMA contamination can result from different industrial and commercial processes. NDMA can be formed in trace quantities as a by-product in tanneries, pesticide manufacturing plants, rubber and tire plants, and dye manufacturers (ATSDR, 1989). NDMA has also been found at wastewater treatment plants in association with metal plating and printed circuit board manufacturing plants (Sedlak *et al*., 2005). NDMA has also been reported to form in waters treated with ion-exchange resins having quaternary amine functional groups that have the potential to serve as NDMA precursors (Gough *et al*., 1977; Kimoto *et al*., 1980).

While the occurrence of NDMA in water can originate from the many sources mentioned above, the most significant sources of NDMA are from liquid rocket fuels and chlorinated wastewater. Unsymmetrical dimethylhydrazine (UDMH) is a typical component of liquid rocket fuels. When UDMH is oxidized at moderate pH levels, NDMA is formed as one of the products (Mitch, 2002). Therefore, NDMA has been frequently detected in groundwater at military sites where UDMH-based rocket fuels were used. Although UDMH-based rocket fuels are no longer used in the United States, NDMA contamination has persisted. As a highly hydrophilic compound, NDMA has exhibits significant mobility. For example, at the Baldwin Park Operable Unit in San Gabriel, CA, where concentrations have

exceeded 10,000 ng/L, NDMA was detectable in the groundwater as far as 8 miles down gradient (WateReuse, 2006). The plume from a rocket engine testing facility in Sacramento County, CA impacted drinking water wells so much that concentrations were as high as 400,000 ng/L on-site and 20,000 ng/L off-site, making it necessary to close the drinking water wells down gradient from this testing facility (CalDHS, 2002; WateRuse, 2006).

Another major source of NDMA in water is its formation during tertiary treatment involving chlorine based disinfection of wastewater. The primary mechanism of NDMA formation was believed to be a result of primary and secondary amines reacting with chloramine to form UDMH, which is then oxidized to NDMA (Choi and Valentine, 2002; Mitch and Sedlak, 2002). However, Schreiber and Mitch (2006) revisited the nitrosamine formation pathway and demonstrated that the presence of dichloramine ($NHCl_2$) and dissolved oxygen greatly enhanced NDMA formation. They also determined that chlorinated UDMH (UDMH-Cl) is formed from dimethylamine and $NHCl_2$, rather than UDMH itself. Laboratory tests by Mitch and Sedlak (2002) showed that secondary wastewater treated with chloramine could result in the formation of NDMA at concentrations between 20 and 100 ng/L. Samples taken after chloramine disinfection at the Santa Clara Water Pollution Plant and Orange County Water District had NDMA concentrations of 30 and 120 ng/L, respectively. With direct and indirect reuse of wastewater becoming more commonplace, the formation of NDMA during the treatment of wastewater has negatively impacted drinking water supplies. For instance, the West Basin Waster Recycling Program that used reclaimed wastewater to recharge the groundwater aquifer produced blend water with an average NDMA concentration of 60 ng/L and a high of 1,200 ng/L (WateReuse, 2006).

2.3. Abiotic treatment strategies.

Conventional abiotic treatment strategies, such as air stripping, activated carbon adsorption, and chemical oxidation, have not proven to be effective in removing either 1,4-dioxane or NDMA from water. The low volatility and hydrophilic nature of both compounds make them resistant to traditional remediation technologies. The dimensionless Henry's constants (H_c) for 1,4-dioxane and NDMA are 1.96×10^{-4} and 1.1×10^{-5}, respectively. These values are orders of magnitude smaller compared to 1,4-dioxane's volatile co-contaminant 1,1,1-TCA ($H_{c*} = 3.27 \times 10^{-1}$). Therefore, even though air stripping would be effective at removing chlorinated solvents, it is a poor strategy for remediating 1,4-dioxane and NDMA contamination. For instance, data from an air stripper designed to completely remove chlorinated solvents only decreased 1,4-dioxane concentrations from 610 µg/L to 430 µg/L (Bowman *et al*., 2001).

The hydrophilicity of 1,4-dioxane and NDMA is due to the presence of polar functional groups on each compound. The high water solubility of each compound is characterized by their small octanol/water partition coefficients (K_{ow}), $10^{-0.27}$ for 1,4-dioxane (Howard, 1990) and $10^{-0.57}$ for NDMA (ATSDR, 1989). This means that activated carbon treatment

systems are ineffective at removing either of these compounds. For example, a groundwater treatment facility that employed granular activated carbon was unable to reduce 1,4-dioxane concentrations from 14 µg/L to the treatment target of 3 µg/L (Bowman *et al*., 2001), while a study by Chung *et al*. (2009) showed that even at high concentrations of powdered activated carbon and long contact times, only 45% removal of NDMA was achieved.

In contrast to these traditional treatment strategies, advanced oxidation processes have shown to be effective at removing 1,4-dioxane from water. Multiple combinations including those of ozone (O_3) and hydrogen peroxide (H_2O_2) at neutral pH (Adams *et al*., 1994), H_2O_2 and ferrous iron (Fenton's reagent) (Klecka and Gonsior, 1986), UV and TiO_2 catalyst (Stefan and Bolton, 1998; Maurino *et al*., 1997), and UV and Fe(II)/H_2O_2 (Kim *et al*., 2006) were able to significantly degrade 1,4-dioxane. The major by-products of these advanced oxidation processes are ethylene glycol diformate, formic acid, acetic

Table 2.2. Bacterial Strains Capable of Aerobic Biodegradation of 1,4-dioxane

Bacterial strain	**Growth substrate(s)**	**Monooxygenase-expressed**	**Reference(s)**
Mycobacterium sp. PH-06	1,4-dioxane	Unknown	Kim *et al*. (2009)
Psuedonocardia dioxanivorans CB1190	1,4-dioxane	Unknown	Parales *et al*. (1994) Mahendra & Alvarez-Cohen (2006)
Pseudonocardia benzenivorans B5	1,4-dioxane	Unknown	Mahendra & Alvarez-Cohen (2006)
Rhodococcus ruber 219	1,4-dioxane	Unknown	Bernhardt & Diekmann (1991)
Burkholderia cepacia G4	Toluene	Toluene-2-MO	Mahendra & Alvarez-Cohen (2006)
Flavobacterium sp.	THF	Tetrahydrofuran MO	Sun *et al*., (2010)
Mycobacterium vaccae JOB5	Propane	Unknown	Mahendra & Alvarez-Cohen (2006)
Methylosinus trichosporium OB3b	Methane	Soluble methane MO	Mahendra & Alvarez-Cohen (2006)
Pseudomonas mendocina KR1	Toluene	Toluene-4-MO	Mahendra & Alvarez-Cohen (2006)
Pseudonocardia sp. ENV425	THF	Tetrahydrofuran MO	Mahendra & Alvarez-Cohen (2006)
Pseudonocardia tetrahydrofuranoxydans K1	THF	Tetrahydrofuran MO	Kohlweyer *et al*. (2000) Mahendra & Alvarez-Cohen (2006)
Ralstonia picketti PKO1	Toluene	Toluene-p-MO	Mahendra & Alvarez-Cohen (2006)
Rhodococcus sp RR1	Toluene	Unknown	Mahendra & Alvarez-Cohen (2006)

acid, methoxyacetic acid, and oxalic acid. Advanced oxidation processes using sonolytic degradation have also shown to be effective at removing 1,4-dioxane (Beckett and Hua, 2000 and 2003).

Unlike 1,4-dioxane, advanced oxidation processes are less desirable than direct UV photolysis for treatment of NDMA contamination. In a study assessing the possibility of using O_2/H_2O_2 to remove NDMA, relatively large doses of ozone were necessary to achieve >50% oxidation of NDMA (Lee *et al*., 2007). However, large doses of ozone are problematic because it causes the formation of bromate, which has a drinking water standard (MCL) of 10 µg/L. Therefore, advanced oxidation processes using ozone to treat NDMA contamination would negatively impact drinking waters by producing bromate. On the other hand, direct UV photolysis of NDMA is extremely effective. NDMA exhibits adsorption bands centered at 227 nm and 332 nm and UV absorption causes the cleavage of the N-N bond by hydrolysis, forming dimethylamine and nitrite as major products (Lee *et al*., 2005; Sharpless and Linden 2003). Minor products of UV photolysis include nitrate, formaldehyde, formate, and methylamine (Stefan and Bolton 2002). UV treatment has been used to remove NDMA at a drinking water plant in Ohsweken, Ontario (Jobb *et al*., 1994) and at the Water Factory 21 in Orange County, CA (OCWD, 2000).

Although advanced oxidation processes and UV photolysis systems are effective at removing 1,4-dioxane and NDMA, respectively, both are cost-inhibitive. Although the commercially available HiPOx Advanced Oxidation Process (Applied Process Technologies, Inc., Long Beach, CA) was effective at removing 1,4-dioxane at three groundwater remediation sites (APT, 2011ab), the additional cost for hydrogen peroxide and ozone to eliminate 1,4-dioxane drastically increased the capital and operating costs compared to pump-and-treat and air stripper system that can already achieve complete removal of 1,1,1-TCA (Mahendra, 2008; Mohr *et al*., 2010). For removal of NDMA from drinking water systems, the amount of UV required to reduce NDMA concentrations by an order of magnitude is ten times higher than that required for equivalent virus removal (Mitch *et al*., 2003). Thus, UV treatment of NDMA is more expensive than UV treatment for disinfection purposes.

2.4. Biodegradation of 1,4-dioxane

A promising alternative to the above mentioned strategies for removal of 1,4-dioxane is biological treatment. In 1991, the first report of 1,4-dioxane degradation by a bacterial strain was published (Bernhardt and Diekmann, 1991). Since then, a number of studies have shown that pure and mixed cultures of bacteria and fungi have the ability to degrade 1,4-dioxane under aerobic conditions. One study also reports anaerobic biodegradation by sludge enriched with iron-reducing microorganisms (Shen et al., 2008).

Aerobic biotransformation of 1,4-dioxane can occur either metabolically, with 1,4-dioxane serving as the sole source of carbon and energy, or co-metabolically, with 1,4-dioxane

degradation occurring after growth on an inducing substrate, such as propane or tetrahydrofuran (THF) (Burback and Perry, 1993; Roy *et al*., 1994; Raj *et al*., 1997; Parales *et al*., 1994; Kim *et al*., 2009; Kohlweyer *et al*., 2000; Zenker *et al*., 2000; Vainberg *et al*., 2006; Mahendra and Alvarez-Cohen, 2006). Bacterial degradation of 1,4-dioxane appears to be catalyzed by monooxygenase enzymes. Although the fungal species, *Cordyceps sinensis*, is capable of using dioxane as a sole carbon and energy source (Nakimaya *et al*., 2005) and the fungi, *Aureobasidium Pullmans* (Patt and Abebe, 1995) and *Graphium* sp. (ATCC 58400) (Skinner *et al*., 2009), can co-metabolize 1,4-dioxane, further review of 1,4-dioxane biodegradation will be limited to bacteria.

2.4.1. 1,4-Dioxane biodegradation by monooxygenase-expressing bacteria.

The Bernhardt and Diekmann (1991) study was the first to propose that the 1,4-dioxane degradation pathway was catalyzed by a hydroxylation step. Degradation of 1,4-dioxane by *Mycobacterium vaccae* following growth on propane along with the detection of hydroxylated cyclic compounds as intermediates, indicated the involvement of a propane-induced oxygenase in catalyzing the hydroxylation of 1,4-dioxane (Burback and Perry, 1993). The demonstration of 1,4-dioxane degradation by THF-grown *Pseudonocardia tetrahydrofuranoxydans* strain K1 (Mahendra and Alvarez-Cohen, 2006) and the eventual discovery of a THF-induced multicomponent monooxygenase (THFMO) in strain K1 (Thiemer *et al.*, 2003) led to the conclusion that a monooxygenase enzyme was responsible for catalyzing 1,4-dioxane biotransformation in strain K1 (Mahendra and Alvarez-Cohen, 2006; Mahendra *et al*., 2007). Two additional bacteria, isolated on THF, *Pseudonocardia* sp. strain ENV478 (Vainberg *et al*., 2006) and a *Flavobacterium* sp. (Sun *et al*., 2011), demonstrated the same ability to degrade 1,4-dioxane after growth on THF. An extensive study by Mahendra and Alvarez-Cohen (2006) studied the kinetics of 1,4-dioxane biodegradation by a large number of monooxygenase-expressing bacteria.

2.4.2. Bacterial strains capable of co-metabolic transformation of 1,4-dioxane.

Out of 20 bacterial isolates tested, Mahendra and Alvarez-Cohen (2006) found 13 strains capable of biodegradation of 1,4-dioxane with only two strains capable of growth on 1,4-dioxane. Co-metabolic transformation of 1,4-dioxane was observed for monooxygenase-expressing strains that were induced with methane, propane, THF, and toluene. Monooxygenase activity was confirmed by a colorimetric naphthalene assay on agar plates. Acetylene, a monooxygenase-specific inhibitor, was also used to determine whether a monooxygenase enzyme was responsible for 1,4-dioxane degradation.

The soluble methane monooxygenase (sMMO) in *Methylosinus trichosporum* OB3b was found to degrade 1,4-dioxane, when it was grown in the absence of copper. However, after induction of the particulate methane monooxygenase (pMMO) in the presence of

copper, strain OB3b was unable to degrade 1,4-dioxane. This study also confirmed the work of Burback and Perry (1993) by demonstrating 1,4-dioxane degradation by the propane-oxidizing *Mycobacterium vaccae* JOB5. In addition to verifying that THF-grown *Pseudonocardia tetrahydrofuranoxydans* K1 can degrade 1,4-dioxane, Mahendra and Alvarez-Cohen (2006) demonstrated the strain K1 can degrade 1,4-dioxane after growth on toluene. It was presumed that both toluene and THF induce for a THFMO in strain K1. A *Flavobacterium* sp. has also been reported to degrade 1,4-dioxane after growth on THF (Sun *et al.*, 2011).

The induction of different types of toluene monooxygenases in a number of strains resulted in the ability to degrade 1,4-dioxane. Biotransformation of 1,4-dioxane was observed after the induction of the toluene-2-MO (T2MO), toluene-*p*-MO (TPMO), and the toluene-4-MO (T4MO) in *Burkholderia cepacia* G4, *Ralstonia picketti* PKO1, and *Pseudomonas mendocina* KR1, respectively. The T2MO, TPMO, T4MO, and toluene-*o*-xylene MO (ToMO) were also homologously expressed in *Escherichia coli* TG1. 1,4-Dioxane degradation was present in all of these *E. coli* clones except for the ToMO. The toluene-side chain-MO enzyme in *Pseudomonas putida* mt-2 was also unable to degrade 1,4-dioxane. In addition to toluene monooxygenase, the toluene-2,3-dioxygenase (T23DO) was tested in *Pseudomonas putida* F1 and *Pseudomonas* sp. JS150 but neither exhibited the ability to degrade 1,4-dioxane. Growth on toluene led to 1,4-dioxane degradation by *Rhodococcus* sp. RR1 but not the MTBE degrader *Methylibium petroleiphilum* PM1. However, the specific toluene monooxygenase expressed in *Rhodococcus* sp. RR1 is unknown.

2.4.3. Bacterial strains capable of metabolic growth on 1,4-dioxane.

To date, only four bacterial strains have been described as capable of using 1,4-dioxane as a sole carbon and energy growth substrate. *Rhodococcus ruber* 219 was shown to grow on a variety of ethers, including tetrahydrofuran, tetrahydropyran, furan, 1,4-butanediol, dioxolane, and 1,4-dioxane (Bernhardt and Diekmann 1991). *Pseudonocadia dioxanivorans* strain CB1190, which was isolated from a dioxane-contaminated sludge, also grew on a number of cyclic and linear ethers, including 1,4-dioxane, which it could mineralize to CO_2 (Parales *et al.*, 1994). In the Mahendra and Alvarez-Cohen (2006) study, they confirmed that in addition to strain CB1190, *Pseudonocardia benzenivorans* strain B5 is capable of growth on 1,4-dioxane. More recently, *Mycobacterium* sp. PH-06 was isolated from river sediment in South Korea contaminated with 1,4-dioxane (Kim *et al*., 2009).

2.4.3.1. *Pseudonocardia dioxanivorans* strain CB1190.

In 2008, the Department of Energy's Joint Genome Institute (JGI) chose the genome of *Pseudonocardia dioxanivorans* strain CB1190 as a project for its Community Sequencing

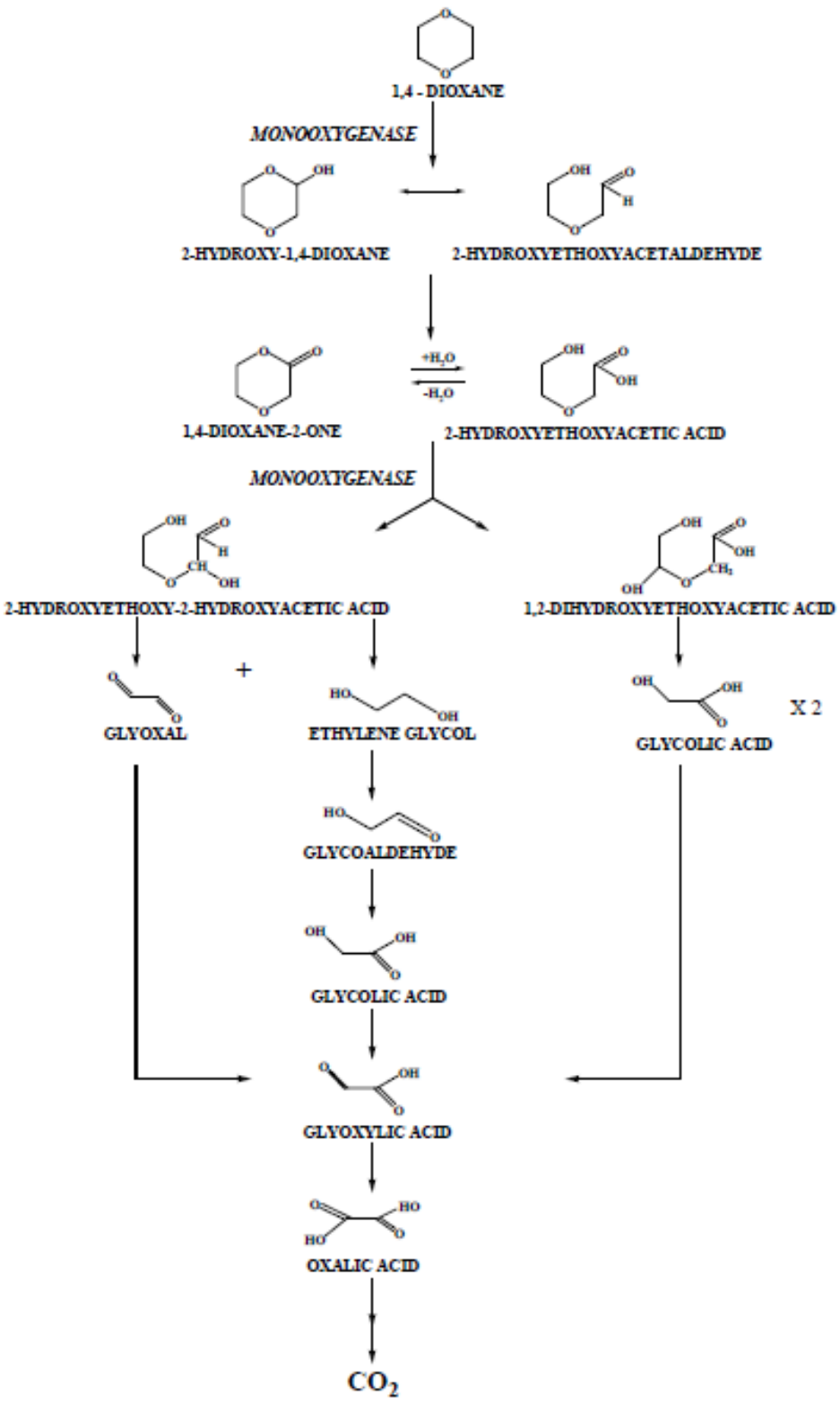


Figure 2.3. Proposed biodegradation pathway of 1,4-dioxane by monooxygenase-expressing bacteria. From Mahendra *et al*. (2007).

Program. Parales *et al*. (1994) initially enriched strain CB1190 on THF but was eventually able to maintain growth on 1,4-dioxane alone. In addition to aerobic growth on 1,4-dioxane and THF, growth was observed on the ethers 1,3-dioxane, tetrahydropyran, 2-methyl-1,3-dioxolane, butyl methyl ether, and diethyl ether. Strain CB1190 was also shown to grow autotrophically on hydrogen and carbon dioxide when a 60:25:10:5 ratio of H_2:N_2:CO_2:O_2 was maintained. Based on morphology and fatty acid analyses, strain CB1190 was described as a Gram-positive actinomycete belonging to the genera *Amycolata* and *Amycolatopsis*. Cultures of strain CB1190 were used in a phytoremediation study to determine the effect of bioaugmentation on planted and unplanted soil on the biodegradation of 1,4-dioxane (Kelley *et al*., 2001).

Subsequently, in-depth analysis led to the re-classification and renaming of strain CB1190 (Mahendra and Alvarez-Cohen, 2005). Analysis of the 16s rRNA gene sequence indicated the strain belonged to the genus *Pseudonocardia*, being closely related to *Pseudonocardia hydrocarbonyxdans*, *P. sulfidoxydans* and *P. halophobica*. None of these other *Pseudonocardia* species could grow on 1,4-dioxane, which led to classification of strain CB1190 as a novel species, *Pseudonocardia dioxanivorans*. Further biochemical characterization of strain CB1190, led to the discovery that it can utilize a variety of nitrogen sources, including NH_4^+, NO_3^-, and N_2. In addition to ethers, aerobic growth on the aromatic compounds, benzene and toluene, was observed.

Even though 1,4-dioxane biodegradation kinetics have been characterized for strain CB1190 (Mahendra and Alvarez-Cohen, 2006) and biodegradation intermediates have been identified (see section 2.4.2), little is known about the biochemistry and molecular biology of microorganisms that can degrade 1,4-dioxane. With the availability of genomic sequencing data for strain CB1190 produced in collaboration with JGI, part of the research described in this dissertation will enhance our understanding of the biochemical and molecular components and mechanisms that permit strain CB1190 to metabolize 1,4-dioxane.

2.4.4. Intermediates and products of aerobic biodegradation of 1,4-dioxane by bacteria

The majority of metabolites generated during bacterial biodegradation of 1,4-dioxane are similar to those discovered in toxicological studies of 1,4-dioxane in humans and laboratory animals. Even though they did not perform any tests to detect for specific metabolites, Bernhardt and Diekmann (1991) proposed that the catabolic pathway for THF and 1,4-dioxane in *Rhodococcus ruber* 219 was catalyzed by α-hydroxylation to form the primary intermediates of tetrahydrofuran-2-ol and dioxane-2-ol, respectively. Their study only quantified chemical oxygen demand (COD), which indicated that 1,4-dioxane was completely mineralized. Another strain capable of growth on 1,4-dioxane, *P. dioxanivorans* CB1190 was shown to convert 50% of degraded 1,4-dioxane to CO_2, with the rest presumably going to biomass (Parales *et al*., 1994).

For the 1,4-dioxane co-metabolizing *Pseudonocardia* sp. strain ENV478, 1,4-dioxane-2-ol and 2-hydroxyethoxyacetic acid (HEAA) were unambiguously identified as intermediates of 1,4-dioxane degradation (Vainberg *et al*., 2006). Culture liquor from [^{14}C]-1,4-dioxane-fed strain ENV478 co-eluted with HEAA and no other potential intermediates. Furthermore, radioactivity was not detected in the form of CO_2 or in the biomass, indicating that HEAA is a dead-end product of 1,4-dioxane degradation in strain ENV478.

Using fully deuterated 1,4-dioxane-*d8*, GC/MS analysis detected 1,4-dioxane-2-ol and ethylene glycol as the major metabolites of 1,4-dioxane degradation by *Mycobacterium* sp. PH-06 (Kim *et al*., 2008). Although this study did not detect any other products, they proposed that 1,4-dioxane is hydroxylated to 1,4-dioxane-2-ol, which is then converted to HEAA. The four-carbon HEAA is then transformed to two ethylene glycols. It is presumed that ethylene glycol then gets converted to oxalic acid, which enters the TCA cycle.

Mahendra *et al*. (2007) performed a more comprehensive examination of 1,4-dioxane biodegradation intermediates using monooxygenase-expressing bacteria. They examined the degradation products of monooxygenase-expressing bacteria shown to grow on or to co-metabolize 1,4-dioxane. Studies using uniformly labeled [^{14}C]-1,4-dioxane demonstrated that strain CB1190 converted over 50% of the 1,4-dioxane into CO_2, while 5% became associated with biomass. The remainder of the radio-labeled carbon was in the form of volatile organic acids and non-volatile compounds. Similar studies with bacteria capable of only co-metabolic transformation of 1,4-dioxane mineralized 50% of the 1,4-dioxane into CO_2. Fourier transform ion cyclotron resonance-mass spectrometry (FTICR-MS) analysis identified the following compounds as intermediates of 1,4-dioxane degradation by metabolic and co-metabolic strains: 2-hydroxy-1,4-dioxane (1,4-dioxane-2-ol), 2-hydroxyethoxyacetaldehyde, 1,4-dioxane-2-one, HEAA, 1,2-dihydroxyethoxyacetic acid, and 2-hydroxyethoxy-2-hydroxyacetic acid. Unlike the results for strain ENV478 (Vain-

Table 2.3. Bacterial Strains Capable of Aerobic Biodegradation of NDMA

Bacterial strain	Growth substrate(s)	Monooxygenase-expressed	Reference(s)
Mycobacterium vaccae JOB5	Propane	Unknown	Sharp *et al*. (2005)
Methylosinus trichosporium OB3b	Methane	Soluble methane MO	Sharp *et al*. (2005)
Pseudomonas mendocina KR1	Toluene	Toluene-4-MO	Sharp *et al*. (2005) Fournier *et al*. (2006)
Ralstonia picketti PKO1	Toluene	Toluene-p-MO	Sharp *et al*. (2005)
Rhodococcus sp RR1	Soy broth	Unknown	Sharp *et al*. (2005)
Rhodococcus ruber ENV425	Propane	Unknown	Fournier *et al*. (2009)

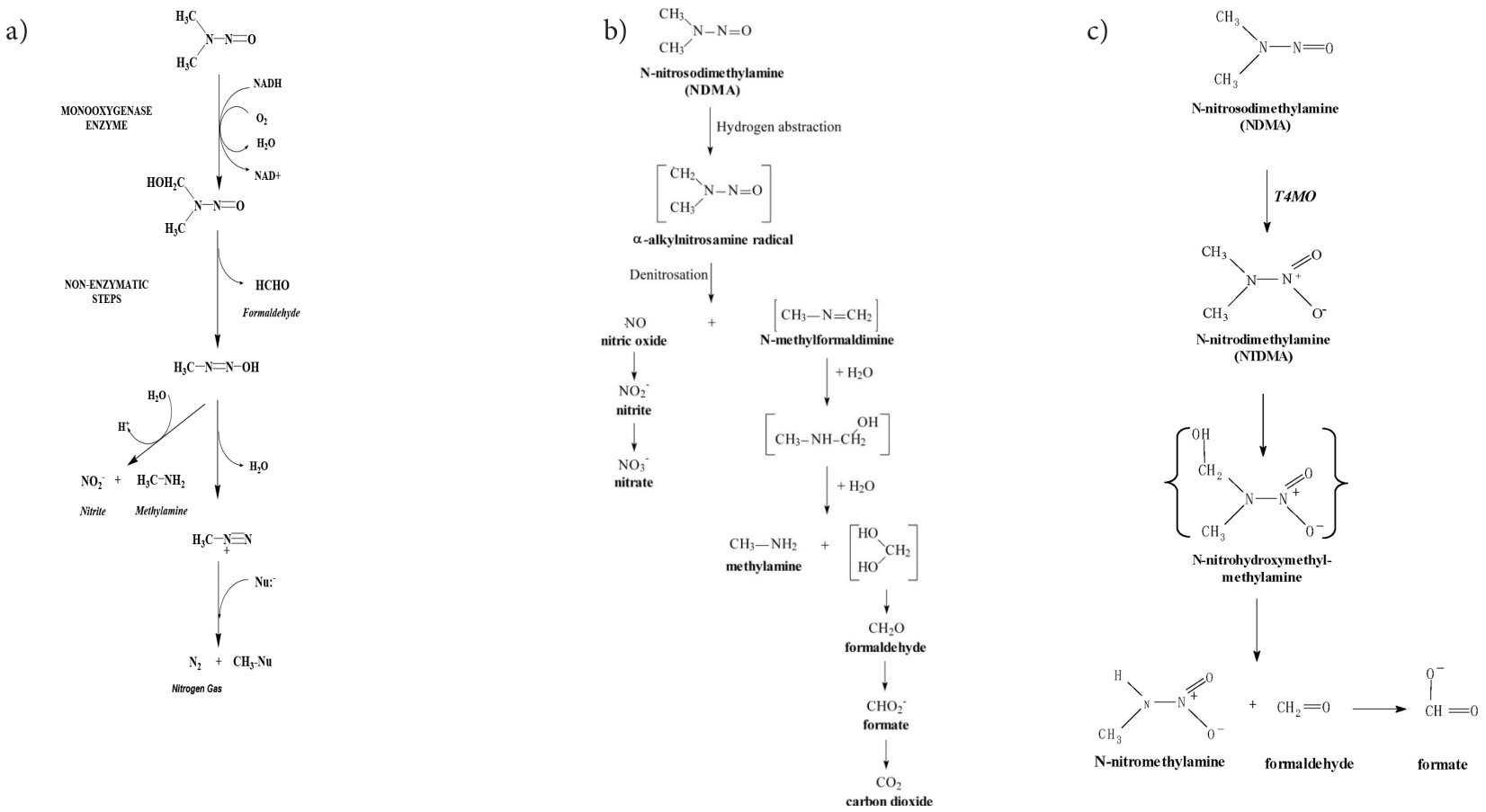


Figure 2.4. Potential pathways for aerobic biodegradation of NDMA. a) Proposed pathway for propane-grown *Rhodoccoccus* sp. RR1 (Sharp, 2006); b) Proposed pathway for propane-grown *Rhodococcus ruber* ENV425 (Fournier *et al*., 2009); c) Proposed pathway for T4MO-expressing *Pseudomonas mendocina* KR1 (Fournier *et al*., 2006).

berg *et al*., 2006), HEAA did not accumulate during either metabolic or co-metabolic 1,4-dioxane biodegradation. Direct injections into an electrospray ionization quadropole ion-trap mass spectrometer (ESI-Q-trap) identified the following intermediates: ethylene glycol, glycolic acid, glycoaldehyde, glyoxal, glyoxylic acid, and oxalic acid.

Based on these identified intermediates, Mahendra *et al*. (2007) proposed the biodegradation pathway of 1,4-dioxane by monooxygenase-expressing bacteria shown in Figure 2.3. In this biodegradation pathway, 1,4-dioxane is transformed by two monooxygenase catalyzed reactions to HEAA. This HEAA is then degraded into a mixture of linear dihydroxy-substituted ethoxyacetic acids, which are believed to be mineralized to CO_2 by common cellular metabolic pathways. With the genome sequence data being produced for *P. dioxanivorans* CB1190 (Chapter 3), we were able to gain a better understanding of the genes that encode for the enzymes involved in the 1,4-dioxane metabolism (Chapters 4 and 5).

2.5. Biodegradation of NDMA.

Bioremediation has also become an attractive option for treatment of NDMA contaminated water. Multiple consortia of soil microorganisms, in laboratory and field studies, have demonstrated the ability to degrade NDMA aerobically and anaerobically (Mallik and Tesfai, 1981; Kaplan and Kaplan, 1985; Gunnison *et al*., 2000; Bradley *et al*., 2005; Sedlak *et al*., 2006; Yang *et al*., 2005; Gan *et al*., 2006; Zhou *et al*., 2008; Padhye *et al*., 2009). Although biodegradation of NDMA is possible, the large plume of NDMA at the Rocky Mountain Arsenal in Commerce City, CO demonstrates that abiotic and biotic removal of NDMA from groundwater can be negligible (Gunnison *et al*., 2000). The inconsistency of NDMA biodegradation was also observed in the extent of degradation of NDMA during secondary biological treatment, which ranged from 0% to 75% at different municipal wastewater treatment plants (Sedlak *et al*., 2005). The low extent of NDMA degradation in the environment has been hypothesized to occur either because of the presence of competing substrates that inhibit NDMA degradation (Kaplan and Kaplan, 1985; Gunnison *et al*., 2005) or because of the lack of organic compounds to promote growth of bacteria capable of degrading NDMA (Yang *et al*., 2005; Gan *et al*., 2006; Padhye *et al*., 2009). The absence of knowledge regarding the agents and mechanisms involved in NDMA biodegradation led Sharp *et al*. (2005) to carry out an extensive study to characterize the ability of monooxygenase-expressing bacteria to aerobically degrade NDMA. They based their study on the fact that cytochrome P450 monooxygenases (P450) were identified to catalyze NDMA oxidation in mammalian systems.

2.5.1. Aerobic biodegradation of NDMA by monooxygenase-expressing bacteria.

Similar to P450 enzymes in Eukaryotes, bacterial multicomponent monooxygenases

(BMMs) have exhibited the ability to catalyze NDMA biodegradation. The first BMM observed to degrade NDMA was the methane-induced monooxygenase in *Methylosinus trichosporium* OB3b (Yoshinari and Shafer, 1990). Sharp *et al*. (2005) determined that the soluble form of the methane monooxygenase (sMMO), and not the particulate methane monooxygenase (pMMO), was capable of degrading NDMA in strain OB3b. The propane-induced monooxygenase in *Mycobacterium vaccae* JOB5 also degraded NDMA, as well as the T4MO enzymes expressed in *Ralstonia picketti* PKO1 and *Pseudomonas mendocina* KR1. *Escherichia coli* TG1/pBS(Kan) possessing recombinant plasmids containing the T4MO genes in strains PKO1 and KR1 were able to degrade NDMA as well. However, the T2MO and toluene side chain monooxygenase induced in toluene-grown *Burkholderia cepacia* G4 and *Pseudomonas putida* F1, respectively, were not capable of degrading NDMA. This study also demonstrated that toluene-2,3-dioxygenases expressed in a number of *Pseudomonas* species were not capable of NDMA degradation. In addition, all of the monooxygenase-expressing bacterial strains that were able to degrade NDMA did so co-metabolically. Although Yoshinari and Shafer (1985) noted that ^{14}C-labeled NDMA was mineralized to $^{14}CO2$ and taken up by the cell when strain OB3b was grown with methane, they noted that strain OB3b was unable to grow on NDMA alone. To date, no bacterial strains have demonstrated to grow on NDMA as a sole carbon and energy source. However, *Rhodococcus ruber* strain ENV425 demonstrated the ability to use nitrogen from NDMA, when grown on propane as a carbon source and in the absence of an alternative nitrogen source (*i.e.*, NH_4^+ or NO_3^-) (Fournier *et al*., 2009).

2.5.2. Intermediates and products of aerobic biodegradation of NDMA.

A number of degradation pathways have been proposed for the aerobic biodegradation of NDMA by monooxygenase-expressing bacteria. These pathways are very similar to the α-hydroxylation and denitrosation pathways (see Figure 2.2) described in the toxicology literature for NDMA (Keefer *et al*., 1987). The major metabolites in the denitrosation pathway are nitrite, N-methylformaldimine, methylamine, and formaldehyde, while the α-hydroxylation pathway produces the following stable metabolites: formaldehyde, methanol, and nitrogen gas. Formaldehyde and methylamine have been previously detected in soil consortia that degrade NDMA (Kaplan and Kaplan, 1985). In Jonathan O. Sharp's dissertation (2006), nitrogen gas, nitrite, methanol, and formaldehyde were detected during NDMA biodegradation by propane-grown *Rhodococcus* sp. RR1. It was discovered that at least 19±2% (mol/mol) of ^{15}N-labeled NDMA was transformed to nitrogen gas and that 13±2% (mol/mol) ends up as nitrite. Although attempts at measuring the short-lived methyldiazonium ion were futile, the production of nitrogen gas suggests the formation of the methyldiazonium ion during NDMA degradation by strain RR1. The pathway for aerobic NDMA degradation that Sharp (2006) proposed in his dissertation shows that a monooxygenase enzyme catalyzes the hydroxylation of one of the methyl groups on NDMA (see Figure 2.4a).

The intermediates produced by another *Rhodococcus* species shown to degrade NDMA co-metabolically, *Rhodococcus ruber* ENV425, were also studied (Fournier *et al*., 2009).

In that study, strain ENV425 was observed to mineralize more than 60% of [^{14}C]-NDMA to $^{14}CO_2$ after growth on propane. Other primary metabolites detected were methylamine, nitrate, nitrite, and formate. In contrast to Sharp's proposed pathway (2006), the propane-induced monooxygenase in this case is believed to abstract hydrogen from one of the methyl groups in NDMA, similar to the denitrosation pathway in mammalian systems (see Figure 2.4b).

The co-metabolism of NDMA by the toluene-grown *Pseudomonas mendocina* KR1 was found to be different from the degradation pathways proposed for propane-grown *Rhodococci*. The major metabolites detected during NDMA degradation by strain KR1 were *N*-nitrodimethylamine (NTDMA), *N*-nitromethylamine (NTMA), and formate. It was proposed that the T4MO enzyme expressed in strain KR1 added an oxygen atom to the nitroso group of NDMA creating *N*-nitrodimethylamine (NTDMA). The T4MO enzyme was then suspected to catalyze the subsequent α-hydroxylation of one of the methyl groups of NTDMA to form *N*-nitrohydroxymethylamine, which further breaks down to NTMA and formate (see Figure 2.4c).

2.5.3. Propane-enhanced degradation of NDMA.

The fastest observed rates of NDMA degradation occurred with strains grown on propane. The rate of NDMA degradation by *Mycobacterium vaccae* JOB5 (100 ng/mg/min) was 20 times higher than that exhibited by *Pseudomonas mendocina* KR1 (Sharp *et al*., 2005). In Chapters 6 and 7, the NDMA degradation rates for *Rhodococc*us sp. RR1 and *Rhodococcus jostii* RHA1 grown on propane were also found to be rapid. Interestingly, only the *Rhodococcus* species are capable of constitutive degradation of NDMA during growth on non-inducing substrates (*i.e.*, pyruvate) or growth media (*i.e.*, tripticase soy broth or Luria-Bertani (LB) broth). In addition, the dissertation of Sharp (2006) described enhanced degradation rates of soil consortia enriched on propane versus pyruvate. In the propane-enriched consortia, an increase in *Rhodococci* was measured by quantitative PCR of 16s rRNA sequences. These results indicate that propane-enhanced bioremediation strategies may be an option for treating NDMA contamination of water. In fact, a recent study reported that aerobic treatment of NDMA was achieved in a propane-fed membrane bioreactor (Hatzinger *et al*., 2011).

Although propane-enhanced biodegradation of NDMA appears to be a viable bioremediation strategy, very little is known about the propane-induced enzymes that appear to be involved in NDMA degradation. Biochemical tests have suggested that propane-induced degradation involves monooxygenase enzymes but no molecular information is known about these enzymes. Identifying the molecular sequences that encode for the propane-induced monooxygenases would provide a means to study the presence, expression, and activity in bacterial isolates or communities.

2.5.3.1. *Rhodococcus jostii* RHA1.

Despite being extensively studied for their ability to degrade a number of environmental contaminants, there is a lack of biological sequence information for the propanotrophs, *Mycobacterium vaccae* JOB5 and *Rhodococcus* sp. RR1, that are capable of rapid degradation of NDMA. Fortunately, the third propanotroph shown to degrade NDMA, the PCB degrader *Rhodococcus jostii* RHA1, has its complete genome sequenced (McLeod *et al*., 2007). The genome of strain RHA1 demonstrates that it has the potential to grow on a wide variety of aromatic compounds and carbohydrates and to degrade a number of xenobiotics and polychlorinated biphenyls. The genome is predicted to encode over 200 oxygenases, including a propane monooxygenase (PrMO) and an alkane monooxygenase (AlkMO), whose activity may be induced by propane. The PrMO found in strain RHA1 is homologous to the gene cluster encoding the multicomponent PrMO (*prmABCD*) in *Gordonia* sp. TY-5, *Mycobacterium* sp. TY-6 and *Pseudonocardia*.sp TY-7 (Kotani *et al*., 2003; Kotani *et al*., 2006). The genome sequence of strain RHA1, as described in Chapters 6 and 7, allowed for the identification of the propane-induced enzymes involved in NDMA degradation in strain RHA1 and two additional *Actinomycetales, Rhodococcus* sp. RR1 and *Mycobacterium vaccae* JOB5.

Chapter 3

The genome of *Pseudonocardia dioxanivorans* strain CB1190

3.1. Introduction.

The cyclic ether, 1,4-dioxane, has recently emerged as a pervasive water contaminant. Although a number of industrial and commercial processes use 1,4-dioxane directly as an ingredient or solvent, the primary source of 1,4-dioxane as an evironmental contaminant is due to its use as a solvent stabilizer in the widely used chlorinated solvent, 1,1,1-tricholorethane (TCA). 1,4-Dioxane is also found as a by-product in the production of surfactants commonly used in the formulation of personal care products, such as cosmetics and shampoos (Mohr *et al*., 2010). As a results of its widespread use, the occurrence of 1,4-dioxane in the environment is becoming prevalent. Furthermore, its hydrophilic nature and stable structure have allowed 1,4-dioxane to persist and spread with ease in aqueous environments. One possible method for removing 1,4-dioxane contamination from affected water supplies is bioremediation.

Although anaerobic biodegradation of 1,4-dioxane has been observed with sludge enriched with iron-reducing bacteria (Shen *et al*., 2009), most reports of biodegradation of 1,4-dioxane by fungi and bacteria have occurred under aerobic conditions. Over a dozen bacterial and fungal isolates have exhibited the ability to degrade 1,4-dioxane aerobically (Bernhardt and Diekmann, 1991; Parales *et al*., 1994; Patt and Abebe, 1995; Nakimaya *et al*., 2005; Mahendra and Alvarez-Cohen, 2006; Vainberg *et al*., 2006; Kim *et al*., 2009; Skinner *et al*., 2009; Sun *et al*., 2011). However, only five of those isolates are capable of growing on 1,4-dioxane as a sole carbon and energy source. These include four bacterial strains, *Rhodococcus ruber* 219 (Bernhardt and Diekmann, 1991), *Pseudonocardia dioxanivorans* CB1190 (Parales *et al*., 1994; Mahendra and Alvarez-Cohen, 2005), *Pseudonocardia benzenivorans* B5 (Mahendra and Alvarez-Cohen, 2006), and *Mycobacterium* sp. PH-06 (Kim *et al.*, 2009), and one fungal species *Cordyceps sinensis* (Nakimaya *et al*., 2005).

Described here is the genome sequence of the Gram-positive actinomycete *Pseudonocardia dioxanivorans* strain CB1190. The genome sequence reported here is the first closed genome sequence in the genus of *Pseudonocardia* that is publically available, and at 73.12%, is one of the highest G+C content strains ever sequenced. Although 454-pyrosequencing data is publically available for another *Pseudonocardia* strain, *Pseudonocardia* sp. P1 (Genbank accession ADUJ00000000), which was isolated from the microbial community associated with colonies of the leaf-cutting ant species *Acromyrmex octospinosus*, it has only been loosely assembled into 975 contigs (Barke *et al*., 2010). Members of the *Pseudonocardia* genus have been isolated from soils polluted with aromatic chlorinated compounds (Kampfer and Kroppenstedt, 2004) and from glacial soils in the McMurdo Dry Valley in Antartica (Prabahar *et al*., 2004). *Pseudonocardia* species have also been identified as endosymbionts living in the tissue of Chinese medicinal plants (Gu *et al*., 2006; Chen *et al*., 2009; Qin *et al*., 2010) and as mutualistic ectosymbionts with leaf-cutting ants and their fungal gardens (Cafaro and Currie, 2005; Poulsen *et al*., 2005). strains of *Pseudonocardia* have also exhibited industrial and environmental uses. For instance, strains of *P. autotrophica* have shown the potential to produce polyene antifungal antibiotics (Lee *et al*., 2006), while *P. tetrahydrofuranoxydans* and *P. chloroethenivorans* have demonstrated the ability to degrade the environmental pollutants tetrahydrofuran (THF)

and chloroethenes, respectively (Kohlweyer *et al.,* 2001; Kampfer *et al*., 2006; Lee *et al*., 2004).

P. dioxanivorans strain CB1190 was isolated from a 1,4-dioxane contaminated industrial sludge in Darlington, South Carolina (Parales *et al*., 1994). In addition to growth on 1,4-dioxane, strain CB1190 can grow on other ethers, including 1,3-dioxane, THF, tetrahydropyran, 2-methyl-1,3-dioxolane, butyl methyl ether, diethyl ether, and the aromatic compounds benzene and toluene (Parales *et al*., 1994; Mahendra and Alvarez-Cohen, 2005). strain CB1190 has also demonstrated the capability of autotrophic growth on hydrogen and carbon dioxide (Parales *et al*., 1994), as well as the ability to fix nitrogen gas (Mahendra and Alvarez-Cohen, 2005). Even though degradation intermediates have been identified and biochemical assays have suggested the involvement of monooxygenase enzymes (Mahendra *et al*., 2007), nothing is known about the genes that encode for enzymes in the 1,4-dioxane metabolic pathways of strain CB1190 or any other microorganisms that can degrade 1,4-dioxane. The genome sequence described here will allow us to better understand 1,4-dioxane metabolism, as well as explore the functional capabilities of strain CB1190 as a toxic pollutant degrader. Furthermore, the genome sequence will provide the first ever insight into the genetics of the genus *Pseudonocardia* in comparison to the genomes of medically and industrially important members of the family *Pseudonocardiaceae*, which include the erythromycin producing *Saccharopolyspora erythraea* NRRL2338 (Oliynyk *et al*., 2008), the natural pesiticide producing *Saccharopolyspora spinosa* NRRL18395 (Pan *et al*., 2011), the rifamycin producing *Amycolatopsis mediterranei* U32 (Zhao *et al*., 2010) and *Amycolatopsis mediterranei* S699 (Verma *et al*., 2011), and the respiratory disease-causing *Saccharomonospora viridis* DSM 43017 (Pati *et al*., 2009).

3.2. Materials and methods.

3.2.1. Chemicals.

All chemicals used in medium preparation were of ACS reagent grade or better. 1,4-Dioxane (99.8%) was obtained from Sigma-Aldrich (Milwuakee, WI). Deioinized (DI) water, produced from a Barnstead Nanopure II water purifying system, was used for preparation of medium.

3.2.2. Culture conditions.

Cells of *Pseudonocardia dioxanivorans* strain CB1190 used for genomic DNA extraction were grown in ammonium mineral salts (AMS) liquid medium (Parales *et al*., 1994). One liter of AMS contains 0.66 g of $(NH_4)_2SO_4$, 1.0 g of $MgSO_4 \cdot 7H_2O$, and 0.015 g of $CaCl_2 \cdot 2H_2O$, 1.0 mL of AMS trace elements, 1.0 mL of stock A, and 20 mL of 1.0 M phosphate buffer (added after autoclaved sterilization). The AMS trace elements contain (per liter) 0.5 g of $FeSO_4 \cdot 7H_2O$, 0.4 g of $ZnSO_4 \cdot 7H_2O$, 0.02 g of $MnSO_4 \cdot H_2O$, 0.015 g of H_3BO_3, 0.01 g of $NiCl_2 \cdot 6H_2O$, 0.25 g of EDTA, 0.05 g of $CoCl_2 \cdot 6H_2O$, and 0.005 g of $CuCl_2 \cdot 2H_2O$. The AMS stock A contains (per liter) 5.0 g of Fe-Na EDTA and 2.0 g of $NaMoO_4 \cdot 2H_2O$. The 1 M phosphate buffer contains 113.0 g of K_2HPO_4 and 47.0 g of KH_2PO_4 and is autoclaved prior to aseptic addition to the remaining components. Filter-sterilized 1,4-dioxane was added to the culture medium to achieve a final concentration ranging from 1 to 5 mM. Cultures were incubated aerobically while shaking at 150 rpm at 30ºC. The liquid medium was always less than 20% of the total volume of the culture bottles to prevent mass transfer limitation of oxygen. Cultures of strain CB1190 were also maintained on liquid medium and 1.5% Bacto agar plates containing R2A (Becton, Dickinson and Company, Franklin Lakes, NJ).

3.2.3. Genomic DNA Extraction.

Large quantities of high molecular weight genomic DNA from strain CB1190 were isolated by modifying the CTAB DNA isolation method from the Department of Energy Joint Genome Institute (JGI). Genomic DNA was extracted from cells of strain CB1190 collected by filtration onto several 0.22 µm PVDF Duarpore membrane filters (Millipore, Billerica, MA). The cells from each filter were rinsed off and resuspended in 740 µL of TE buffer (10 mM tris, 1 mM EDTA, pH 8.0) in microcentrifuge tubes. In order to lyse the cells, the cell suspensions were gently mixed with 20 µL of lysozyme (100 mg/mL)

and incubated at room temperature for 20 minutes. Then, 40 µL of 10% SDS (sodium dodecyl sulfate) and 8 µL of Proteinase K (10 mg/mL) were added to each tube and incubated for 12 hours at 37ºC. Next, 100 µL of 5 M NaCl and 100 µL of CTAB/NaCl heated to 65ºC were added to each tube and incubated at 65ºC for 10 minutes. The CTAB/NaCl mixture was prepared by dissolving 4.1 g of NaCl and 10 g of CTAB (hexadecyltrimethyl ammonium bromide) in 100 mL of DI water by constant mixing at 65ºC for 3 hours and then sterilized by autoclaving. Following incubation with CTAB, the resultant lysate was gently mixed with 0.5 mL of chloroform:isoamyl alcohol (24:1) and then centrifuged for 10 minutes at maximum speed (21,000 x *g*) at room temperature. The upper aqueous phase was transferred to a clean microcentrifuge tube and gently mixed with 0.5 mL of phenol:chloroform:isoamyl alcohol (25:24:1) (pH 8.0) and then centrifuged for 10 minutes at maximum speed for 10 minutes at room temperature. The upper aqueous phase was transferred to a clean microcentrifuge tube and then mixed with 0.6 volumes of cold isopropanol (-20ºC) and incubated at room temperature for 30 minutes to precipitate the genomic DNA. The genomic DNA was then pelleted by spinning each tube at maximum speed (21,000 x *g*) for 15 minutes. The pellets were washed with 500 µL of 70% ethanol. After the supernatant was discarded, the pellet was dried at room temperature for 10 minutes. Finally, each pellet was resuspended in 20 µL of TE buffer plus RNase (final concentration of 0.1 mg/mL). The size, quantity, and quality of the isolated high molecular weight genomic DNA were determined on a 1% agarose gel.

3.2.4. Genomic sequencing and assembly.

The draft genome of strain CB1190 was generated at the DOE Joint genome Institute (JGI) using a combination of Illumina (Bennett, 2004) and 454 sequencing technologies (Margulies *et al*., 2005). For this genome we constructed and sequenced an Illumina GAii shotgun library which generated 33,134,275 reads totaling 1,192 Mb, a 454 Titanium standard library which generated 1,211,248 reads and paired-end 454 libraries with an average insert sizes of 3, 10, and 18 kb which generated 210,350 reads for a total of 504.6 Mb of 454 data. All general aspects of library construction and sequencing performed at the JGI can be found at http://www.jgi.doe.gov/. The initial draft assembly contained 283 contigs in 8 scaffolds. The 454 Titanium standard data and the 454 paired end data were assembled together with Newbler [Version 2.3]. The Newbler consensus sequences were computationally shredded into 2 kb overlapping fake reads (shreds). Illumina sequencing data was assembled with VELVET [Version 0.7.63] (Zerbino, 2008), and the consensus sequence was computationally shredded into 1.5 kb overlapping shreds. We integrated the 454 Newbler consensus shreds, the Illumina VELVET consensus shreds and the read pairs in the 454 paired end library using parallel phrap [Version SPS - 4.24] (High Performance Software, LLC). The software Consed (Ewing and Green 1998; Ewing *et al*. 1998; Gordon *et al*. 1998) was used in the following finishing process. Illumina data was used to correct potential base errors and increase consensus quality using the software Polisher developed at JGI (Alla Lapidus, unpublished). Possible mis-assemblies were corrected using gapResolution (Cliff Han, unpublished), Dupfinisher (Han and Chain, 2006), or sequencing cloned bridging PCR fragments with subcloning. Gaps between contigs were

closed by editing in Consed, by PCR and by Bubble PCR (J-F Cheng, unpublished) primer walks. A total of 809 additional reactions and 6 shatter libraries were necessary to close gaps and to raise the quality of the finished sequence. The total size of the genome is 7,440,794 bp and the final assembly is based on 285 Mb of 454 draft data which provides an average 38.1x coverage of the genome and 1,011 Mb of Illumina draft data which provides an average 134.8x coverage of the genome. The finished chromosome and plasmid sequences have been deposited to RefSeq and GenBank databases with accession numbers NC_015312-4 and CP002593-8.

3.2.5. Genome Annotation and Analysis.

Annotation of the genome of strain strain CB1190 was accomplished using the Oak Ridge National Laboratory genome annotation pipeline. Genes were identified using Prodigal (Hyatt *et al*., 2010), followed by a round of manual curation using the JGI GenePRIMP pipeline (Pati *et al*., 2010). The predicted coding sequences (CDSs) were translated and used to search the National Center for Biotechnology Information (NCBI) non-redundant database, UniProt, TIGRFam, Pfam, PRIAM, KEGG, COG, and InterPro databases. These data sources were combined to assert a protential description for each predicted protein. Non-coding genes and miscellaneous features were predicted using tRNAscan-SE (Lowe *et al*., 1997), RNAMMer (Lagesen *et al*., 2007), Rfam (Griffiths-Jones *et al*., 2003), TMHMM (Krogh *et al*., 2001), and signalP (Bendtsen *et al*., 2004).

Genes involved in transport systems were identified by analyzing putative protein sequences against the Transport Classification Database (http://ww.tcdb.org) (Tran, 2004), using the basic local alignment search tool for proteins (blastp) (e-value < 1e-5) (Altschul *et al*., 1990). Signal transduction proteins were found using COG assignments of CDSs and the Microbial Signal Transduction database MiST2.1 (http://mistdb.com) (Ulrich and Zhulin, 2010). Protein domains were identified using the NCBI Conserved Domain Database (CDD) (http://www.ncbi.nlm.nih.gov/Structure/cdd/cdd.shtmL) or MiST2.1. Genomic islands were identified by SIGI-HMM analysis and visualized using Islandviewer (http://www.pathogenomics.sfu.ca/islandviewer) (Langille and Brinkman, 2009).

3.3. Results.

3.3.1. Genome properties and features of *P. dioxanivorans* strain CB1190.

The genome of strain CB1190 has a total size of 7,440,794 bp and consists of four replicons: a circular chromosome (7,096,571 bp), a circular plasmid pPSED01 (192,355 bp), an unclosed circular plasmid pPSED02 (136,805 bp), and a linear plasmid pPSED03 (15,063 bp), see Figures 3.1-3.3 for schematic representations of all closed replicons. The unclosed circular plasmid pPSED02 is comprised of three contigs, with sizes of 24,346 bp, 45,552 bp, and 66,907 bp (GenBank Accession numbers CP002595-7), respectively. The average G+C content of the entire genome is 73.12%. The G+C contents of the plasmids are lower than that of the chromosome, with values of 73.41%, 71.15%, 68.38%, and 61.83%, for the chromosomes and plasmids, pPSED01, pPSED02, and pPSED03, respectively.

The genome of strain CB1190 contains 6,797 protein-coding sequences (CDS) and 226 pseudo genes. The distribution of CDS, pseudo genes, and hypothetical proteins for each replicon is shown in Table 3.1. The average length of CDSs across the genome is 963 bp. The chromosome contains 6,495 CDS and 194 pseudo genes, while plasmid pPSED01 contains 172 CDS and 20 pseudo genes, plasmid pPSED02 contains 116 CDS and 11 pseudo genes, plasmid pPSED03 contains 14 CDS and no pseudo genes. Of all predicted proteins in the genome, 4,955 (72.90%) have a putative function ascribed to them. 1,692 genes (26.05%) on the chromosome are annotated as hypothetical proteins. Interestingly, only 3 of the 14 genes (22.4%) on pPSED03 have a putative function ascribed to them.

A total of 59 RNA sequences were identified in the genome and all are located on the chromosome. Strain CB1190 contains three sets of 16S, 23S, and 5S ribosomal RNA (rRNA) genes are located on the chromosome, with one set located on the forward strand and the other two on the reverse strand. Out of the structural RNA sequences, 47 encode for transfer RNA (tRNA) genes, while the three remaining RNAs encode for a putative catalytic subunit of the RNaseP *rnpB*, a 4.5S RNA component of the signal recognition particle protein translocation system *ffs*, and a small stable RNA *ssrA*, which is involved in a process called *trans*-translation (Keiler *et al*., 1996; Karzai *et al*., 2000).

A slight coding bias exists in favor of the reverse strand on the chromosome (50.62%). The putative origin of replication, *oriC,* is localized between the 50s ribosomal subunit L34 *rpmH* (Psed_6692) and the chromosomal replication initiator protein *dnaA* (Psed_0001). The GC content in the region following *oriC* is visibly lower than the average for the rest of the chromosome. Although not pronounced, a slight inversion in the GC skew is seen in the forward strand near the *oriC*. The GC skew on the forward strand is markedly greater in the first half of the chromosome (Figure 3.1). The GC skew drops at an inflection point on the side opposite of the *oriC* on the circular chromosome, which represents the putative replication terminus if the chromosome is replicated in a bidirec-

tional manner.

3.3.2. Comparative genomics of *Pseudonocardiaceae* family.

To date, there are eleven bacterial strains, representing ten species from five different genera, in the family *Pseudonocardiaceae* with publically available genome sequencing data: *Amycolatopsis mediterranei* S699, *Amycolatopsis mediterranei* U32, *Pseudonocardia dioxanivorans* CB1190 (this study), *Pseudonocardia* sp. P1, *Pseudonocardia* sp. P2, *Saccharomonospora azurea* NA-128, *Saccharomonospora paurometabolica* YIM 9007, *Saccharomonaspora viridis* DSM43017, *Saccharopolyspora eryhtraea* NRRL2338, *Saccharopolyspora spinosa* NRRL18395, and *Thermobispora bispora* DSM43833. However, recent phylogenetic analysis of the 16S rRNA sequences in the genome of *T. bispora* DSM43833 shows that it belongs to the suborder *Streptosporangineae* (Liolios *et al*., 2010). Both the suborder *Streptosporangineae* and the family *Pseudonocardiaceae* fall within the order *Actinomycetales*. Further comparative genomic studies, reported herein, perpetuate the concept that *T. bispora* does not belong to the family *Pseudonocardiaceae*. While most members of the family *Pseudonocardiaceae* are Gram-positive, *S. viridis* is a Gram-negative bacterium (Pati *et al.,* 2009). Of further note, since two groups have sequenced the genome of *S. erythraea* NRRL2338 – here the genome sequenced by Oliynyk *et al*. (2008) will be referred to as *S. erythraea* NRRL2338-1 and the genome sequenced by the Italian Ministry of Research (Genbank ABFV00000000) as *S. erythraea* NRRL2338-2. The genome of *S. erythraea* NRRL2338-1 is closed, while the genome of *S. erythraea* NRRL2338-2 is comprised of 241 contigs.

Table 3.2 contains a detailed list comparing all genome features of each of the sequenced *Pseudonocardiaceae* strains, including T. bispora as an out-group (*Pseudonocardia* sp. P2 is left out because of the lack of annotation data). The genomes of *A. mediterranei* strains were originally sequenced because they are known to produce the antibiotic rifamycin. The two sequenced strains differ by the type of rifamycin that they produce. *A. mediterranei* S699 produces rifamycin B, while strain U32 produces rifamycin SV (Verma *et al*., 2011). The complete genome of *A. mediterranei* S699 is the largest in the family *Pseudonocardiaceae*, consisting of a circular chromosome with a total length of 10,236,779 bp The genome of *A. mediterranei* S699 was assembled by mapping sequencing reads to the genome of *A. mediterranei* U32 as a reference (Verma *et al*., 2011). The genome of *A. mediterranei* U32 also contains a single circular chromosome. The smallest of the sequenced genomes in the family *Pseudonocardiaceae* is that of *S. viridis*, with a total length of 4,308,349 bp. Although only draft sequences are available, the other two *Saccharomonospora* species have similar genome sizes (4,770,125 bp and 4,592,308 bp, respectively). The properties of the two genome sequences for *S. erythraea* NRRL2338 vary because the genome of *S. eythraea* NRRL2338-1 is closed, while the genome of *S, erythraea* NRRL2338-2 is a draft. As, shown in Table 3.2, the draft genome of *S. spinosa* is larger than the genome sequences for *S. erythraea*. In addition to strain CB1190, the only *Pseudonocardia* species with genome sequencing data are *Pseudonocardia* sp. P1 and *Pseudonocardia* sp. P2; however, their genomes are loosely assembled.

Although whole genome shotgun sequencing data is available for *Pseudonocardi*a sp. P2 (GenBank AEGE00000000), it has not been annotated and contains an extremely large number of contigs. Currently, the genome of Pseudonocardia sp. P2 has been assembled into 1,778 contigs consisting of 8.7 Mb.

Calculation of the number of reciprocal best protein blast hits between each pair of genomes (Table 3.3 and 3.4) reflects the phylogeny, classification, genome sizes, and completeness of the genome assembly of the strains within the family of *Pseudonocardiaceae*. Using a blastp cutoff e-value of 1e-5 and a minimum coverage of 70% to determine reciprocal blast hits between a pair of genomes, strain CB1190 is shown to share the most CDSs with the other *Pseudonocardia* species. Even though *Pseudonocardia* sp. P1 has 1,518 more predicted protein encoding genes than *Pseudonocardia* sp. P2, they have nearly identical numbers of reciprocal blast hits with strain CB1190 (3,251 and 3,242, respectively). Among all three *Pseudonocardia* species, *Pseudonocardia* sp. P1 and *Pseudonocardia* sp. P2 are more closely related to each other, sharing 4,210 common proteins. Among other genera, *Pseudonocardia* genomes are most related to *Amycolatopsis* genomes, with 39-48% similarity. In agreement with its 16S rRNA phylogenetic relationship, *T. bispora* shares the least number of protein-coding genes with members of the family *Pseudonocardiaceae* (1,840 to 2,136). As expected, a high degree of similarity is seen between genomes from the same species (95% -99% between *A. mediterranei*). Surprisingly, the two genomes from the same strain of *S. erythraea* appear to be less similar (93% -96%), but this may be due to the fact that the genome of *S. erythraea* NRRL2338-2 is unfinished while the genome of *S. erythraea* NRRL2338-1 is closed. A comparison of total CDS counts and genome sizes in Table 3.2 indicates that within a genus, draft genomes tend to have more commonality. Therefore, since the percentages calculated in Table 3.4 are based on genome sizes, the values listed for unfinished genomes may be underestimations.

Although some of the genomes analyzed are not finished, it appears that circular chromosomes are common among *Pseudonocardiaceae*. Among the finished genomes, only strain CB1190 contains free-replicating plasmids. The presence of the plasmid sequences pSE101 and pSE211 as integrated elements in *Saccharopolyspora erythraea* NRRL2338 has been known for some time (Brown *et al*., 1988; Brown *et al*., 1990; Brown *et al*., 1994). Analysis of the genome sequence of *S. erythraea* NRRL2338-1 confirmed the presence of pSE101 and pSE211, with lengths of 20.4 kb and 11.7 kb, respectively (Oliynyk *et al*., 2007). In a more recent effort to characterize integrative and conjugative elements in *Amycolatopsis* and *Saccharopolyspora*, two novel integrated elements were identified in the genome of *S. erythaea* NRRL2338-1 (te Poele *et al*., 2008), named pSE222 and pSE102. The novel element pSE102 has high sequence similarity to pSE101. All of the integrated elements in *S. erythraea* are homologues of the plasmid pMEA100, which has been isolated from *A. mediterranei* strains (te Poele *et al*., 2007). In the genome of *A. mediterranei* U32, two putative integrated plasmids were found (Zhao *et al*., 2010). After performing blastn searches using the pMEA100 sequence (Genbank EU149765), I discovered two integrated plasmids in the genome of *A. mediterranei S699*, one with a length of 18.8 kbp (6,810,279 – 6,829,104 bp) and the other with a length of 23.3 kbp (367,545 – 390,887 bp). Coincidentally, the genomic coordinates of the two integrated plasmids in *A. mediterranei* U32 are nearly identical (367,542 – 390,874 bp and

6,808,937–6,829 319 bp) (Zhao *et al*., 2010). Similar blastn searches against the genome of strain CB1190 did not yield a significant hit for pMEA100 homologues.

3.3.3. Mobile genetic elements.

The genome of strain CB1190 contains a large number of mobile genetic elements (MGEs) located on the chromosome and two of the plasmids, pPSED01 and pPSED02 (Table 3.5). A total of 129 open reading frames (ORFs) encode for transposases and integrases, belonging to the insertion sequence families of IS3, IS4, IS21, IS111A and IS116. The majority of MGEs, 106 of them, are located on the chromosome, while 13 and 9 of them are located on plasmids pPSED01 and pPSED02, respectively.

Using SIGI-HMM, a number of genomic islands (GIs) were predicted on the chromosome of strain CB1190. Two clusters of predicted GIs were located in the regions of 1.30-1.38 Mbp (Psed_1228 to Psed_1324) and 3.68-3.74 bp (Psed_3442 to Psed_3501). These regions have a G+C content of 68.7% and 68.3%, respectively, which is lower than the G+C content of the chromosome (73.41%). Both regions contain a number of transposase and integrase genes, so they are likely a result of a horizontal gene transfer event. The first cluster contains a number of genes encoded for heavy metal resistance of mercuric compounds. The second cluster contains a number of transporter and chaperone genes that may help the cell to overcome toxicity. The inclusion of a plasmid partitioning gene *ParB* (Psed_3493) in the second cluster may suggest this GI originated from a plasmid.

3.3.4. General carbon metabolism.

The ability of strain CB1190 to grow on mineral medium indicates it can synthesize all of its cellular components, including fatty acids, nucleotides, and amino acids, and *in silico* analysis of the genome supports this notion. Complete pathways are present for the synthesis of 22 amino acids, including the selenoamino acids: selenocysteine and selenomethionine. Both the glycolysis and pentose phosphate (PP) pathway are complete, suggesting that it has at least two ways to metabolize glucose. The primary role of the PP pathway is anabolic and it is broken up into two distinct phases. The first phase is the oxidative phase, which produces NADPH, and the second is involved in the synthesis of 5-carbon sugars. In addition, the entire citric acid or tricarboxylic acid (TCA) cycle is present. Open reading frames (ORFs) encoding for a malate synthase (Psed_4782) and isocitrate lyase (Psed_4635) are present, suggesting that strain CB1190 may convert isocitrate to succinate and malate via the glyoxylate shunt pathway.

The genome also suggests that *strain CB1190* can metabolize xylose using the isomerase pathway. The genome contains 11 putative xylose isomerase genes (Psed_0624,

Psed_1104, Psed_2264, Psed_2595, Psed_3711, Psed_3726, Psed_5635, Psed_6921, Psed_6931, Psed_6943, and Psed_6944), which encode proteins to convert D-xylose into D-xylulose. Also present are two genes for xylulokinases (Psed_1102 and Psed_3672) that can phosphorylate D-xylulose into D-xylulose-5-phosphate, an intermediate in the PP pathway.

3.3.5. Carbon-fixation pathways.

Strain CB1190 has previously shown the ability to grow autotrophically by fixing CO_2 in the presence of H_2 (Parales *et al*., 1994). There are six primary CO_2 fixation pathways: 1) the Calvin cycle or Calvin-Benson-Bassham (CBB) pathway or Reductive pentose pathway, 2) the Reductive TCA cycle or reverse citric acid cycle, 3) the reductive acetyl Co-A pathway or Wood-Ljundahl pathway, 4) the 3-hydroxypropionate pathway/malyl-CoA pathway (3-HP), 5) the 3-hydroxypropinate/4-hydroxybutyrate cycle, and 6) the dicarboxylate/4-hydroxybutyrate cycle (Kumar *et al*., 2011). Below is an examination of the completeness of these pathways in the genome of strain CB1190.

The genome of strain CB1190 has a number of CDSs that are predicted to be involved in the CBB pathway (Figure 3.4), including three genes annotated as ribulose-1,5-bisphosphate carboxylase oxygenase (RubisCO) (Psed_1692, Psed_6249, and Psed_6250), which carboxylates ribulose-1,5-bisphosphate (Ru1,5BP) to 3-phosphoglycerate (PGA). Two phospholgycerate kinase genes (Psed_3418 and Psed_6236) were identified, which catalyze the phosphorylation of PGA to 1,3-bisphosphoglycerate (1,3BPGA). Three copies of glyceralde-3-phosphate (G3P) dehydrogenase gene (Psed_3419, Psed_4844, and Psed_6237), which reduces 1,3BPGA to G3P using NADPH, have also been found. A triosephosphate isomerase gene is present (Psed_3417), which converts all of the G3P to dihydroxyacetone phosphate (DHAP). Four fructose-bisphosphate aldolase genes (Psed_6239, Psed_6242, Psed_6248, and Psed_6523) and two fructose-1,6-bisphosphatase genes (Psed_0960 and Psed_1418) are present, which convert G3P and DHAP into fructose-6-phosphate (F6P). Two transketolase genes (Psed_3406 and Psed_6244) are present, which can remove two carbons from F6P giving eyrthrose-4-phosphate (E4P) and use the two carbons to produce xylulose-5-phosphate (Xu5P). E4P and DHAP are converted to sedoheptulose-1,7-bisphosphate (S1,7BP) by one of the fructose-bisphosphate aldolases listed above. Like other bacteria known to fix carbon through the CBB pathway, genes encoding a homologue to the plant sedoheptulose-1,7-bisphosphatase, which converts S1,7BP into sedoheptulose-7-phosphate (S7P), are missing. Instead, strain CB1190 likely uses one of the fructose-1,6-bisphosphatases to produce S7P. Similarly, based on analogous biochemical reactions, the transketolases that can act on F6P, could also act on S7P, removing two carbons to generate ribose-5-phosphate (R5P) and transferring the two carbons to a 3GP to produce Xu5P. The R5P could then be converted into ribulose-5-phosphate (Ru5P) by the R5P isomerase (encoded by Psed_1910), while the Xu5P could be converted into Ru5P by a ribulose-phohsphate 3-epimerase (encoded by Psed_1246 and Psed_3431). Finally, phosphoribulokinase (PRK) genes were identified (Psed_1417 and Psed_6243), which encode proteins for the conversion of Ru5P into

Ru1,5BP, completing the CBB cycle.

The strain CB1190 genome is missing a few key enzymes for the reductive (or reverse) TCA cycle pathway to be complete (Figure 3.5). While many of the enzymes in this pathway are identical to the oxidative TCA cycle, different enzymes are needed to overcome some of the reverse reactions that are energetically unfavorable. These unfavorable reverse reactions include the regeneration of acetyl-CoA from citrate, the conversion of fumarate to succinate, and the CO_2-fixing step to convert succinyl-CoA to α-ketoglutarate (Saini *et al.*, 2011). Strain CB1190 has seven genes that encode for fumarate reductase/ succinate dehydrogenase (Psed_1464, Psed_1474, Psed_2567, Psed_2608, Psed_4589, Psed_4592, and Psed_4593), which converts fumarate to succinate. Also present in the genome are two genes that are homologues to α-ketoglutarate:ferredoxin oxidoreductase (Psed_5549 and Psed_5550), which catalyzes the conversion of succinyl-CoA to α-ketoglutarate. In some bacterial and mammalian systems, the enzyme involved in the cleavage of citrate to acetyl-CoA and oxaloacetate is an ATP-citrate lyase (ACL), but a gene encoding for an ACL is not present in the genome of strain CB1190. The combination of citryl-CoA synthase (CCS) and citryl-CoA lyase catalyzes the cleavage of citrate in hydrogen thermophiles (Aashima *et al*., 2004a; Aashima *et al.*, 2004b). While strain CB1190 has four genes encoding for citryl-CoA lyases (Psed_1046, Psed_2057, Psed_4168, and Psed_5276), it is missing a gene encoding for a citryl-CoA synthase.

The third CO_2 fixation pathway is the reductive acetyl-CoA or Wood-Ljundahl pathway, first discovered in acetogenic bacteria. Although strain CB1190 has not been characterized as an acetogen, it contains a number of genes associated with the Wood-Ljungdahl pathway. The genes for the Wood-Ljungdahl pathway have only been thoroughly characterized in the recently sequenced genome of the acetogen *Moorella thermoacetica* ATCC 39073 (Ragsdale and Pierce, 2008). The Wood-Ljungdahl pathway is broken into two branches (Figure 3.6). The Eastern branch of the pathway involves the stepwise reduction of CO_2 to a methyl group initiated by reduction of CO_2 to formate by a formate dehydrogenase. The genome of strain CB1190 contains two genes predicted to encode for formate dehydrogenases (Psed_2418 and Psed_3043) but is missing a gene for 10-formyl-H_4folate synthetase, which catalyzes the ATP-condensation of formate and H_4folate into 10-formyl-H_4folate. The gene encoding for the bifunctional protein *bifolD*, which acts as a 5,10-methenyl-H_4folate cyclohydrolase and a 5,10-methylene-H_4folate dehydrogenase, is present in strain CB1190 (Psed_2098, Psed_4021, Psed_4923, and Psed_5314). The cyclohydrolase cyclizes 10-formyl-H_4folate into 5,10-methenyl-H_4folate, while the dehydrogenase reduces 5,10-methenyl-H_4folate to 5,10-methylene-H_4folate. Also present in the strain CB1190 genome are putative 5,10-methylene-H_4folate reductase genes (Psed_2104, Psed_2420, Psed_2785, and Psed_5345), whose proteins catalyze the last step in the Eastern branch to produce 5-methyl-H_4folate from 5,10-methylene-H_4folate.

The genes encoding the Western branch of the Wood-Ljungdahl pathway in *M. thermoacetica* are co-localized in the *acs* gene cluster, which include genes for a carbon monoxide dehydrogenase (CODH), an acetyl-CoA synthase (ACS), and two subunits of the corrinoid iron-sulfur protein (CFeSP), and a methyltransferase. This cluster is not present in the genome of strain CB1190 and homologues to the individual components ACS and CFeSP are missing. Therefore, strain CB1190 is not expected to produce acetyl-CoA us-

ing the Wood-Ljungdahl pathway. However, a total of 46 CODH genes have been identified in strain CB1190, which may enable for the oxidation of CO to CO_2. The CODH genes are usually found in clusters of three in the genome, containing a large subunit (~800 bp) and two small subunits (~150 bp and ~300 bp).

The fourth pathway for CO_2 fixation is the bicyclic 3-hydroxypropionate pathway or malyl-CoA pathway (Figure 3.7). Based upon the 3-hydroxypropionate pathway described for *Chloroflexus auatniacus* OK-70, the first cycle begins with the carboxylation of acetyl-CoA to malonyl-CoA by acetyl-CoA carboxylase (Zarzycki *et al*., 2009). Three genes encoding for acetyl-CoA carboxylase have been identified in strain CB1190 (Psed_1545, Psed_2963, and Psed_5260). However, it is missing the genes for the second step, malonate-semialdehyde dehydrogenase and 3-hydroxypropionate dehydrogenase or the bifunctional enzyme malonyl-CoA reductase, which catalyze the reduction of malonyl-CoA to 3-hydroxypropionate via malonate-semialdehyde. In *C. aurantiacus* OK-70, a single enzyme, propionyl-CoA synthase, catalyzes the reductive conversion of 3-hydroxypropionate to propionyl-CoA. A propionyl-CoA synthase gene was not found in strain CB1190, but three propionyl-CoA carboxylase genes were found (Psed_0517, Psed_5261, and Psed_5277) that putatively encode for the protein that catalyzes the carboxylation of propionyl-CoA to methylmalonyl-CoA. Genes encoding for methylmalonyl-CoA epimerase (Psed_1714) and methylmalonyl-CoA mutase (Psed_1691, Psed_3124, Psed_3125, and Psed_4409), which convert methylmalonyl-CoA to succinyl-CoA, have also been found. The genome of strain CB1190 contains 23 ORFs annotated to encode formyl-CoA transferase, which may act as a succinyl-CoA:malate-CoA transferase to transform succinyl-CoA to malyl-CoA in conjunction with succinate dehydrogenase and fumarate hydratase. Of note, strain CB1190 has 11 genes annotated to encode succinate dehydrogenases and one gene as a fumarate hydratase (Psed_0958). Following transformation of succinyl-CoA to malyl-CoA, malyl-CoA may then be split into acetyl-CoA and glyoxylate by a lyase. In *C. aurantiacus*, a trifunctioal lyase was found to act on multiple CoA-containing substrates, including (*S*)-maly-CoA, (2*R*,3*S*)-β-methylmalyl-CoA, and (*S*)-citramalyl-CoA lyase. Strain CB1190 has four genes encoded as putative citryl-CoA lyases (Psed_1046, Psed_2057, Psed_4168, and Psed_5276), one or more of which may act as a tri-functional lyase.

The second cycle of the 3-hydroxypropionate pathway combines propionyl-CoA and glyoxylate into (2*R*,3*S*)-β-methylmalyl-CoA using a lyase. The (2*R*,3*S*)-β-methylmalonyl-CoA is then converted to mesaconyl-C1-CoA by a hydratase. One of the 34 enoyl-CoA hydratases found in the genome of strain CB1190 could catalyze this reaction to produce mesaconyl-C1-CoA. One of the formyl-CoA transferases mentioned in the previous paragraph may be capable of catalyzing the conversion of mesaconyl-C1-CoA to mesaconyl-C4-CoA. Then, one of the enoyl-CoA hydratases, mentioned earlier, may be able to convert mesaconyl-C4-CoA to citramalyl-CoA. Finally, one of the four putative citryl-CoA lyases may convert citramalyl-CoA to pyruvate and acetyl-CoA, thus closing the second cycle. Therefore, although strain CB1190 is missing key genes for converting malonyl-CoA to propionyl-CoA, which are important for connecting the two cycles of the 3-hydroxypropionate pathway, it contains a number of genes for the rest of the pathway, including the two carboxylases involved in the only CO_2-fixing steps of the pathway (Figure 3.7).

The fifth pathway for CO_2 fixation is the 3-hydroxypropionate/4-hydroxybutyrate cycle that has only been described in aerobic autotrophic members of the archaeal order *Sulfobales*. According to *Metallosphaera sedula*, the model organism for this pathway, the cycle starts with the carboxylation of acetyl-CoA to malonyl-CoA by acetyl-CoA carboxylase (Berg *et al.*, 2007) (Figure 3.8). In our analysis of the 3-hydroxypropionte pathway genes in strain CB1190, three acetyl-CoA carboxylases were found. Malonyl-CoA is converted to malonic semialdehyde by malonyl-CoA reductase, which is missing in the strain CB1190 genome. Further missing genes are those encoding malonic semialdehyde reductase, which reduces malonic semialdehyde to 3-hydroxypropionate, 3-hydroxypropionyl-CoA synthase, which converts 3-hydroxypropionate to 3-hydroxypropionyl-CoA, 3-hydroxypropionyl-CoA dehydratase, which dehydrates 3-hydroxypropionyl-CoA to acronyl-CoA, and acryloyl-CoA reductase, which reduces acronyl-CoA to propionyl-CoA using NADPH. As was reported earlier, strain CB1190 has multiple genes encoding for propionyl-CoA carboxylase, which carboxylates propionyl-CoA to methylmalonyl-CoA. Methylmalonyl-CoA epimerase and mutase genes are also present, which encode for the protein that rearranges methylmalonyl-CoA to succinyl-CoA. The remaining part of the cycle regenerates acetyl-CoA from succinyl-CoA via the intermediates succinic semialdehyde, 4-hydroxybutyrate, 4-hydroxybutyryl-CoA, crotonyl-CoA, 3-hydroxybutyryl-CoA, and acetacetyl-CoA. The enzymes catalyzing these steps are succinyl-CoA reductase, succinate semialdehyde reductase, 4-hydroxybutyryl-CoA synthetase, 4-hydroxybutyryl-CoA dehydratase, crotonyl-CoA hydratase, 3-hydroxybutyryl-CoA dehydrogenase and acetoacetyl-CoA β-ketothiolase. While strain CB1190 has three ORFs that encode for thiolases (Psed_0627, Psed_ 2234, and Psed_2494) that could convert acetoacetyl-CoA into two acetyl-CoA molecules, it is missing the rest of the genes in the latter part of the 3-hydroxypropionate/4-hydroxybutyrate cycle (Figure 3.8).

The sixth autotrophic CO_2 fixation pathway is the dicarboxylate/4-hydroxybutyrate cycle (Figure 3.9). Using the proposed dicarboxylate pathway for the archaea *Ignicoccus hospitalis* as a reference (Huber *et al*., 2008), the first step involves a pyruvate synthase, which uses the reducing power from a ferredoxin to carboxylate acetyl-CoA into pyruvate. The strain CB1190 genome is missing a pyruvate synthase homologue. Next, conversion of pyruvate to phosphoenolpyruvate is catalyzed by a pyruvate:water dikinase (Psed_2515 and Psed_2944), with CO_2 subsequently being fixed during the carboxylation of phosphenolpyruvate to oxaloacetate. In strain CB1190, an ORF encoding a phosphoenolpyruvate carboxylase involved in catalyzing this reaction is present (Psed_6164). Three malate dehydrogenase genes are also present (Psed_6328, Psed_2085, and Psed_2875), which encode for proteins that convert oxaloacetate to malate. Just as in the 3-hydroxypropionate cycle, fumarate hydratase and fumarate reductase (succinate dehydrogenase) genes are present that encode for proteins that convert malate to succinate via fumarate. In *I. hospitalis*, succinate is converted to succinyl-CoA by a succinate thiokinase, which is missing in strain CB1190. Rather strain CB1190 likely uses a succinyl-CoA ligase to convert succinate to succinyl-CoA, as it does in the Reductive TCA cycle. Genes encoding for the enzymes that would close the dicarboxylate/4-hydroxybutyrate cycle by converting succinyl-CoA all the way to acetyl-CoA are missing (Figure 3.9).

3.3.6. Nitrogen metabolism.

Strain CB1190 has demonstrated the ability to utilize multiple nitrogen sources (Mahendra and Alvarez-Cohen, 2005). Four CDS are predicted to be for glutamine synthetases (Psed_2546, Psed_3968, Psed_4574, and Psed_4917), which allows for ammonia (NH_3) uptake by condensation with glutamate to form the amino acid glutamine. In addition, strain CB1190 can grow on minimal media with nitrate (NO_3^-) as its only nitrogen source. A total of eight ORFs encode for nitrate reductases, which convert NO_3^- to nitrite (NO_2^-). One of these nitrate reductase genes (Psed_1088) is in a cluster of genes that encode for subunits of a nitrite reductase (Psed_1089 to Psed_1091), a nitrite transporter (Psed_1087), and an uroporphyrinogen-III synthase HEM4 (Psed_1092). Based on blastp analyses, the reductases in this gene cluster are most similar to assimilatory NAD(P)H-dependent nitrate and nitrite reductases in *Amycolatopsis mediterranei* U32, which function to reduce nitrate to NH_3 via NO_2^- and hydroxylamine. In strain CB1190, the uroporphyrinogen-III synthase catalyzes the synthesis of uroporphyrinogen-III, a precursor for siroheme, which is a cofactor for nitrite reductase (Shao *et al*., 2011). In addition to being able to assimilate NO_3^-, strain CB1190 also appears capable of dissimilatory nitrate reduction. The presence of a cluster of four ORFs encoding for subunits of a respiratory nitrate reductase (Psed_4300 to Psed_4303) suggests the ability to use NO_3^- as a terminal electron acceptor under anaerobic conditions. However, genes for respiration of NO_2^- are absent, such as *nrfA*-encoded cytochrome c nitrite reductase, which reduces NO_2^- to ammonia (NH_4^+), or the *nirS*-encoded cytochrome cd1 nitrite reductase or the *nirK*-encoded copper nitrite reductase, which can each reduce NO_2 to nitric oxide (NO). Rather, the NO_2^- is reduced to NH_4^+ by either assimilatory or disimilatory mechanisms without producing a proton-motive force (*i.e.*, during NO_2^- respiration). Furthermore, nitric oxide and nitrous oxide reductases are missing in the genome, meaning NO_3^- cannot be reduced all the way to dinitrogen (N_2). However, genes are present for nitric oxide dioxygenases (Psed_4325, Psed_5584, and Psed_6551), which may allow strain CB1190 to cope with nitric oxide stress by oxidizing it to nitrate.

A couple of methods were previously used to demonstrate that *strain CB1190* has the capability to fix dinitrogen (N_2) (Mahendra and Alvarez-Cohen, 2005). Nitrogenase activity was observed by the ability of strain CB1190 cells to convert acetylene to ethylene in the presence of glucose as an external energy source. Ammonia production was detected in strain CB1190 cells growing in mineral media void of nitrogen sources (*i.e.*, NH_3, NO_3^-, or NO_2^-). Presently, no other *Pseudonocardia* species are reported to fix N_2. No homologues to nitrogenase genes are found in the genome of strain CB1190, except for two genes annotarted as *Nif*U-like proteins (Psed_3394 and Psed_6367) and one as a *Nif*C-like porter (Psed_5656). Many organisms which are unable to fix dinitrogen contain *Nif*U-like proteins because they can assist in the formation of metalloclusters, typically FeS clusters, for proteins other than nitrogenases. Similarly, *Nif*C-like porters, which are involved in the transport of molybdenum for the molybdenum-containing nitrogenase enzymes, could transport molybdenum for other enzymes or co-factors. While a unique system called superoxide-dependent N_2 (sdn) fixation was described in *Streptomyces thermoautotrophicus*, in which N_2 reduction to ammonium is coupled to oxidation of superoxide produced from O_2 and CODH, the short N-teminal polypeptide sequences for the CODH, the superoxide oxidoreductase, and the novel oxygen-insensitive nitrogenase from *S.*

thermoautotrophicus are not sufficient for homology searches (Ribbe *et al*., 1997). Although Hoffmann-Findeklee *et al.* (2002) describe cloning and sequencing of *sdn* genes from *S. thermoautorophicus* and suggest that the nitrogenase belongs to the molybdenum-hydroxylase family, neither nucleotide or amino acid sequences are publically available. Therefore, the N_2 fixation system used by strain CB1190 remains unknown.

2.3.7. Signal transduction systems

A total of 657 ORFs were determined to be signal transduction proteins. These signal transduction proteins allow cells to respond to environmental changes. According to the Microbial Signal Transduction Database (MiST2.1) analysis, 51 ORFs were predicted to encode putative histidine kinases and 57 were predicted to encode putative response regulators. Based on co-localization, 37 two-component regulatory systems were identified (listed in Table 3.6). These two-component systems consist of a histidine kinase (HK) and a response regulator (RR). The HK senses specific environmental stimuli, while the RR controls the cellular response by the differential expression of certain genes. Signal transduction occurs through the transfer of phosphoryl groups among adenosine triphosphate (ATP), HK, and RR. When the HK senses a stimulus, it transfers a phosphate group it received from ATP to an aspartic acid residue on the RR. This phosphorylation causes the conformation of the RR to change, leading to a change in the regulation of the expression of certain genes. Using the Conserved Domain Database (CDD) and MiST2.1, most of the RR genes in two-component systems contain a RR domain followed by an output domain (usually, GerE or trans_reg_c), as seen in Table 3.6. The RR domain consists of a phosphoacceptor site that is phosphorylated by its associated histidine kinase. The GerE and trans_reg_c domains are usually located in the C-terminal end of the RR and are believed to play a role in DNA-binding, as activators or repressors of gene expression. The HK genes in two-component systems consistently contain a HAMP domain, whose function is poorly understood, and a C-terminal located transmitter domain (*i.e.*, HK_CA or HisKA), which is involved in phosphotransfer (Inouye and Dutta, 2002). In addition to the pairs that make up the two-component systems, a co-located set of three genes (Psed_0434 to Psed-0436) were found with a gene arrangement of RR-HK-RR. Interestingly, rather than the output domain functioning in DNA binding, the RR protein encoded by Psed_0434 contains a conserved Stage II sporulation protein E domain, which functions as a phosphatase. Furthermore, a gene encoding a two-component system containing a hybrid HK (Psed_6430) was found containing two RR domains.

As is common in bacteria, one-component signal transduction systems are more abundant in strain CB1190 than two-component systems. One-component systems consist of a single protein that contains input (sensory) and output (regulatory) domains but lack phosphotransfer domains (*i.e.*, receiver (RR) and transmitter (HK) domains) typical of two-component systems (Ulrich *et al*., 2005). A total of 502 genes encoding one-component signal transduction proteins were identified that contain at least one conserved output domain (Appendix 1). The output domains in one-component systems are significantly more diverse than those in two components, containing mostly DNA-binding

domains but also RNA-binding domains, phosphohydrolase (HD) domains, phosphatase domains, protein kinase (Pkinase) domains, and nucleotide (adenylate and di-guanylate) cyclase domains. Even among the DNA-binding domains, there is a higher degree of variety in one-component systems than two-component systems. Similar to DNA-binding domains, the RNA-binding domains also serve to regulate gene expression. For instance, the ANTAR RNA-binding domain acts as an anti-terminator of transcription (Shu and Zhulin, 2002), while the *CsrA* in *E. coli* is known to bind to RNA molecules to effect the regulation of glycogen biosynthesis, glyconeogenensis, and glycolysis (Liu *et al*., 1997). The di-guanylate cyclase domains, GGDEF and EAL, are involved, respectively, in the synthesis and degradation of cyclic di-guanlyate monophosphate (c-di-GMP), which is an intracellular signaling molecule that is involved in the regulation of enzyme activities or gene expression. Also known as a second messenger, c-di-GMP relays signals from receptors on the cell surface (*i.e*., first messengers) to target molecules inside the cell, often leading to amplification of the original signal (Tamayo *et al*., 2005). Proteins with the phosphohydrolase HD domain have been shown to degrade phosphodiester bonds in second messenger molecules (Aravind and Koonin, 1998). The protein kinase (Pkinase) domain has the catalytic function to phosphorylate proteins, which results in conformational changes that regulate the function of the protein.

A limited number (82) of the one-component systems listed in Appendix 1 have input domains. The input domains identified in one-component systems have either an enzymatic or small-molecule binding function. An example of a one-component system with an enzymatic function is the protein encoded by Psed_1132, which has a shikimate kinase (SKI) domain and GntR DNA-binding output domain. Therefore, when this enzyme catalyzes the phosphorylation of shikimate, it simultaneously regulates the expression of other genes via the GntR domain. Other enzymatic input domains in the strain CB1190 genome include: HEM4 (uroporphyrinogen III synthase), which is involved in nitrate assimilation, and Rhodanese, which aids in the detoxification of cyanide by converting it to thiocyanate. More predominant as one-component system input domains in strain CB1190 are small-molecule binding domains. The putative substrates for these small-molecule binding domains are largely uncharacterized. However, there appears to be consistent input and output domain pairings, such as IclR and HTH_IclR, PAS and EAL/GGDEF, Amino_tran_1_2 and GntR, and LysR_substrate and HTH_1. In addition to one-component and two-component proteins, genes encoding a total of 25 signal transduction proteins with only input domains were identified in the genome (Appendix 2).

A third important class of bacterial signal transduction proteins are extracytoplasmic function (ECF) σ factors. All bacteria have a primary σ factor that controls basal level expression of most genes. Bacterial σ factors assist RNA polymerase in initiating transcription by allowing them to bind to specific gene promoters (Helmann and Chamberlin, 1988). Alternative σ factors, such as ECF σ factors, are activated in the presence of certain environmental stimuli (Helmann, 2002). In the absence of a stimulus, alternative σ-factors are kept inactive through protein-protein binding with anti-σ factors (Brown and Hughes, 1995). As shown in Appendix 3 a total of 50 ORFs encode for putative RNA polymerase σ factors and 34 of these contain conserved ECF σ factor domains recognized by MiST2.1. Despite being unrecognized by Mist2.1, the remaining σ factors were found to have output domains homologous to the common σ^{70} factor domains. Although anti-σ

factors are believed to be co-expressed and located next to their corresponding ECF σ factors, only 18 anti-σ factors genes were identified and only three pairs of co-located σ-factor/anti-σ-factor genes were found (Psed_0659/Psed_0660, Psed_2039/Psed_2040, and Psed_5156/Psed_5155). In general, anti-σ factors are difficult to identify because, thus far, they seem poorly conserved and their σ interaction domains are short in length (Staron *et al*., 2009). Furthermore, a total of four ORFs annotated to encode σ^{54} interaction domain-containing proteins harboring DNA-binding domains were discovered.

Compared to other *Pseudonocardiaceae*, strain CB1190 has fewer signal transduction proteins than *A. mediterranei* U32 (1,141), *S. erythraea* NRRL2338-1 (738), and *S. erythraea* NRRL2338-2 (727), but more than *S. viridis* P101 (323) and *Pseudonocardia* sp. P1 (585). According to the MiST2.1 database, one-component systems in all *Pseudonocardiaceae* make up approximately 70-80% of all signal transduction proteins. In addition, 84 proteins with conserved ECF σ factors in *A. mediterranei* U32, 32 in *S. erythraea* NRRL2338-1, 17 in *Pseudonocardia* sp. P1, and 13 in *S. viridis* were discovered.

3.3.8. Transport systems.

Strain CB1190 contains a large number of genes associated with transport. A total of 961 genes (13.7% of all CDS) encoding a broad range of transport functions were identified according to blastp searches (e-value < 10e-5) against the Transport Classification Database. The transport genes are listed in Appendix 4, along with their respective Transport Classification number and putative substrate/function. The majority of the predicted transport genes were located on the chromosome, while only 21, 15, and 1 were identified on the plasmids pPSED1, pPSED2, and pPSED3, respectively. Twenty six genes were predicted to encode for transmembrane channels (TC# 1.A), including those involved in the transport of potassium, ammonia, urea-amide, and magnesium-cobalt-nickel. A total of 230 genes were homologous to carriers (TC #2) that include specificity for sugars, aromatic compounds, drugs, heavy metals, nitrate, nitrite, sulfate, amino acids, and nucleosides. Ninety one of these potential carriers were members of the Major Facilitator Superfamily (MFS) (TC# 2.A.1). The largest group of transporters was the ATP-binding Cassette (ABC) Superfamily (TC# 3.A.1), consisting of 283 genes or 29.4% of all transport associated genes in the strain CB1190 genome. These ABC tranporters were predicted to be involved in P-P hydrolysis driven transport of substrates such as carbohydrates, heavy metals, drugs, liposaccharides, lipoproteins, nitrate, nitrite, bicarbonate, organic acids, cholesterol, amino acids, urea, and oligopeptides.

A significant number of ABC and MFS transporters, 44 and 45, respectively, were predicted to be involved in drug-resistance or export of drugs in the genome of strain CB1190. This accounts for 15% of ABC transporters and 35% of MFS transporters. Sixteen of the ABC transporter genes were found to be members of the Drug Exporter-1 (DrugE1) Family (TC# 3.A.1.105). Three of the MFS transporter genes(Psed_0339, Psed_4022, and Psed_6185) were identified as members of the Drug:H+ Antiporter-1 (DHA1) Family (TC# 2.A.1.2), while 35% of all MFS transporter genes were members

of the Drug:H+ Antiporter-2 (DHA2) Family (TC# 2.A.1.3).

The genome of strain CB1190 contains a number of genes involved in heavy metal resistance. A cluster of genes (Psed_6718 to Psed_6725) is homologous to the mercury resistance operon of *Streptomyces lividans* (Sedlmeier and Altenbuchner, 1992). The genome of strain CB1190 also contains two alkylmercury lyases (Psed_1313 and Psed_1314) and a mercuric reductase (Psed_1317), which are involved in the detoxification of mercury. An arsenate resistance operon, containing genes for a putative arsenate reductase (Psed_2407), an arsenical resistance protein (Psed_2408), and an arsenic-related regulatory protein ArsR (Psed_2409), was also identified.

3.3.9. Secretion systems.

Many types of secretion systems have been identified in bacteria (also in Appendix 4). Since strain CB1190 is Gram-positive, no genes belonging to the Type I protein secretion system (common in Gram-negative bacteria) were identified. However, genes for the general secretory pathway (the Sec of Type II protein secretion system, TC #3.A.5) were discovered. Unlike *E. coli* but similar to *Comamonas testosteroni* CNB-2 (Ma *et al.,* 2009), the Sec genes identified in strain CB1190 were not clustered, except for *SecF*, *SecD*, and *YajC* (Psed_3580 to Psed_3582), which encode a putative protein complex. The strain CB1190 genome also contains genes predicted to be members of the Type III (IIISP) and Type IV (IVSP) secretory pathways, which are typically involved in the transport of virulence factors. Three type IV secretory pathway genes are located on plasmid pPSED2 (Psed_6900, Psed_6901, and Psed_6994).

3.3.10. Chaperone proteins.

The genome of strain CB1190 contains 51 chaperone genes. Two genes (Psed_6730 and Psed_6374) were annotated as *hypC*/*hupF* chaperones involved in the assembly of hydrogenases. A gene annotated as a nitrite reductase molybdenum cofactor assembly chaperone (Psed_4302) was also identified. A number of genes were also identified to encode genes for heat shock and cold shock proteins (see Table 3.7). Similar to *Rhodococcus jostii* RHA1 (Sharp *et al.,* 2007) and *Gordonia* sp. TY-5 (Kotani *et al* 2003), a chaperonin *GroEL* (Psed_0633) is found within a cluster of genes that encode for a putative propane monooxygenase.

3.3.11. Monooxygenases.

The first step in 1,4-dioxane biodegradation by strain CB1190 is catalyzed by a monooxygenase reaction (Mahendra and Alvarez-Cohen, 2006; Mahendra *et al*., 2007). Monooxygenases are enzymes that use the energy from NAD(P)H to reduce dioxygen (O_2) into water and a hydroxyl group, which is subsequently incorporated onto a substrate. A total of 84 ORFs are predicted to encode for monooxygenases. Since monooxygenase enzymes catalyze a hydroxylation reaction, they are often referred to and contain proteins encoded as hydroxylases. The genome of strain CB1190 contains 14 ORFs annotated as hydroxylases. In addition, 11 ORFs are predicted to encode for cytochrome P450 (CYP) enzymes, which are known to catalyze monooxygenase reactions of a variety of organic compounds.

Further analysis of these monooxygenase related ORFs indicates the presence of eight gene clusters of bacterial multicomponent monooxygenases (BMMs). These BMMs contain two to six co-located genes that contain at least a monooxygenase (or hydroxylase) protein and a reductase protein. The reductase protein supplies electrons to the active site of the hydroxylase component by oxidizing NAD(P)H. These electrons allow the hydroxylase component to catalyze the hydroxylation of the substrate. The hydroxylase component of BMMs may consist of two proteins: a large α subunit and smaller β subunit. Classification of BMMs is based upon their substrate specificity. The subunit composition and arrangement of coding sequences differs for each type of BMM. Therefore, the order of co-localized genes and their similarity to sequences of known BMM components were used to categorize and deduce the function of the eight BMM clusters in strain CB1190 (see Figure 3.10).

Seven of the BMM clusters are located on the chromosome. The first is a propane monooxygenase (*prm*) gene cluster (Psed_0629 to Psed_0632), consisting of four subunits *prmABCD*. The subunits *prmA* (Psed_0629) and *prmC* (Psed_0630) encode for the α and β subunits of the hydroxylase, respectively. The subunit *prmB* is a ferredoxin-NAD (+) reductase, while *prmD* is a coupling protein. The coupling (or regulatory) protein, which is often found in other BMM clusters, is believed to enhance the monooxygenase reaction by improving the coupling between the hydroxylase and reductase subunits (Notomista *et al*., 2003; Leahy *et al*., 2003). The second BMM contains components homologous to the multicomponent phenol hydroxylases *dmpKLMNOPQ* first sequenced in *Pseudomonas* sp. strain CF600 (Nordlund *et al*., 1990), and *poxRABCDEFG* from *Ralstonia eutropha* strain E2 (Hino *et al*., 1998). Only five of the eight subunits, homologous to *dmpLMNOP* and *poxBCDEF*, are found in strain CB1190 (Psed_0770 to Psed_0766), as shown in Figure 3.10.

Four of the BMMs located on the chromosome are similar to aromatic monooxygenases that target benzene, toluene, ethylbenzene, and xylene. The clusters denoted as aromatic monooxygenase 1 (Psed_0815 to Psed_0810) and aromatic monooxygenase 3 (Psed_1436 to Psed_1441) in Figure 3.10 have identical *tmoABDEC* gene structures. The sixth gene in each cluster, located at Psed_0810 and Psed_1441, encode for ferredoxin-NAD(+) reductases. Furthermore, Table 3.8 indicates that the corresponding proteins in aromatic monooxygenase 1 and aromatic monooxygenase 3 share 46.6% to 81.6% identity. Similarly, the order of the genes in aromatic monooxygenase 2 (Psed_1155 to Psed_1159) and aromatic monooxygenase 4 (Psed_6062 to Psed_6058) are identical to

one another and are similar to that of the toluene/o-xylene monooxygenase *touABCDEF* gene cluster found in *Pseudomonas stutzeri* OX1 (Bertoni *et al*., 1998) and the toluene 4-monooxygenase *tmoABCDEF* gene cluster found in *Pseudomonas mendocina* KR1 (Yen *et al*., 1991; Yen *et al*., 1992). However, neither aromatic monooxygenase 2 nor aromatic monooxygenase 4 contain a gene encoding for *tmoF*, a ferredoxin-reductase. The protein sequences in aromatic monooxygenase 2 and 4 are 37.8% to 50.0% identical.

The simplest BMM found is a two-component 4-hydroxyphenylacetate 3- monooxygenase encoded by Psed_6067 and Psed_6066. This monooxygenase is a flavin adenine dinucleotide (FAD)-dependent monooxygenase. The large component *HpaB* (Psed_6066) utilizes reduced $FADH_2$ to oxidize 4-hydroxyphenylacetate (HPA) (Prieto *et al*., 1994). The $FADH_2$ required for hydroxylation is produced by the small subunit *HpaC* (Psed_6066), a flavin:NAD(P)H reductase, which oxidizes NAD(P)H in order to reduce FAD (Galan *et al*., 2000).

The only BMM not found on the chromosome is a tetrahydrofuran (THF) monooxygenase (Psed_6976 to Psed_6979), which is located on plasmid pPSED02 and is in a cluster of nine genes (Psed_6974 to Psed_6982). Eight of the genes are highly homologous to tetrahydrofuran monooxygenase (*thm*) gene clusters in *Pseudonocardia* sp. ENV478, *P. tetrahydrofuranoxydans* K1, and *Rhodococcus* sp. YYL (Genbank HQ99619.1, AJ296087.1, and EU732588.2, respectively). The eight common *thm* genes are *orfY*, *sad*, *thmA* , *thmD*, *thmB*, *thmC*, *orfZ*, and *aldH*. The one gene only found in strain CB1190 encodes for a NRAMP family Mn^{2+}/Fe^{2+} transporter (Psed_6982). The existence of this Fe^{2+} transporter makes sense because of the high similarity of THF monooxygenases to binuclear-iron-containing multicomponent monooxygenases (Thiemer *et al.,* 2001; Thiemer *et al.,* 2003). Sequence analysis indicates that the *thm* cluster in strain CB1190, excluding the transporter gene, shares 96%, 94%, and 87% identity at the nucleotide level to *Pseudonocardia* sp. ENV478, *P. tetrahydrofuranoxydans*, and *Rhodoccocus* sp. YYL, respectively. The discovery of the *thm* cluster on plasmid pPSED2 in strain CB1190 is consistent with previous findings that the *thm* gene sequences in *P. tetrahydrofuranoxydans* had a characteristically low G+C content and was shown to be located on a large plasmid (pPSK50) (Thiemer *et al.,* 2003). The *thm* proteins encoded on these plasmids are nearly identical, sharing 91.4 to 99.6% similarity (Figure 3.11). Furthermore, the GC content of the *thm* cluster in strain CB1190 is 61.3%, while the GC content of the entire plasmid pPSED2 is 68.4%.

3.3.12. Dioxygenases.

The strain CB1190 genome contains 92 genes annotated as putative dioxygenases that catalyze the incorporation of both atoms of dioxygen into substrates as hydroxyl groups. Many of these dioxygenases appear to be involved in the degradation of aromatic compounds. Two of the dioxygenase genes are located on plasmid pPSED02, an extradiol ring-cleave dioxygenase class III protein subunit B (Psed_6935) and a phthalate 3,4-dioxygenase ferredoxin subunit (Psed_6938). Half of the putative dioxygenases (46) are

annotated as glyoxylase/bleomycin resistance protein/dioxygenases and are distributed throughout the chromosome. Some of the dioxygenases appear to be involved in the metabolism of aromatic amino acids, such as phenylalanine with 3-carboxyethylcatechol 2,3-dioxygenase (Psed_0244) and tyrosine with gentisate 1,2-dioxygenase protein (Psed _1337) and 4-hydroxyphenylpyruvate dioxygenase (Psed_1410). Genes annotated to be involved in the degradation of the following compounds were identified in the genome of strain CB1190: protocatechuate (Psed_1566 to Psed_1564), catechol (Psed_3093 to Psed_3095); vanillate (Psed_2465 to Psed_2466). A number of genes suggesting that assimilation of benzoate may occur via benzoyl-CoA were discovered, such as those annoated as benzoate-CoA ligase (Psed_1491), benzoyl-CoA-dihydrodiol lyase (Psed_1490), and benzoyl-CoA oxygenase component B (Psed_1489).

A few ORFs encoded as dioxygenases are found co-localized with BMM cluster genes involved in hydroxylation of aromatic compounds. A catechol 1,2-dioxygenase (Psed_0764) is found downstream of the phenol monooxygenase cluster (Psed_0770 to Psed_0766). Another ORF annotated as a catechol 1,2-dioxygenase (Psed_6065) is located immediately downstream of the HPA monooxygenase gene cluster (Psed_6067 to Psed_6066) and a few genes upstream of the aromatic monooxygenase 4 gene cluster (Psed_6062 to Psed_6058). The co-location of these BMMs and the catechol 1,2-dioxygenase genes is consistent with the fact that the metabolism of aromatic compounds (such as toluene, phenol, and HPA) typically involves monooxygenase reactions that produce dihydroxy aromatic compounds (such as catechol, benzoate, and dihydroxyphenylacetate). These dihydroxy aromatic compounds are substrates of ring-cleaving dioxygenase reactions, which produce intermediates that can enter the TCA cycle (Cooper and Skinner, 1980; Diaz, 2004).

Table 3.1. Genome feature of *P. dioxanivorans* strain CB1190

Feature	Genome	Chromosome	Plasmid pPSED01	Plasmid pPSED02	Plasmid pPSED03
Topology		Circular	Circular	Circular	Linear
Length	7,440,794 bp	7,096,571 bp	192,355 bp	136,805 bp	15,603 bp
G+C Content	73.12%	73.41%	71.15%	68.38%	61.83%
Coding Density	87.2%	88.5%	76.1%	80.0%	69.2%
Coding Sequences	6,797	6,495	172	116	14
Pseudo genes	226	194	20	11	0
Average CDS length	963 bp	967 bp	946 bp	851 bp	744 bp
rRNAs	3	3			
tRNAs	47	47			
Hypothetical proteins	1,842	1,692	88	51	11

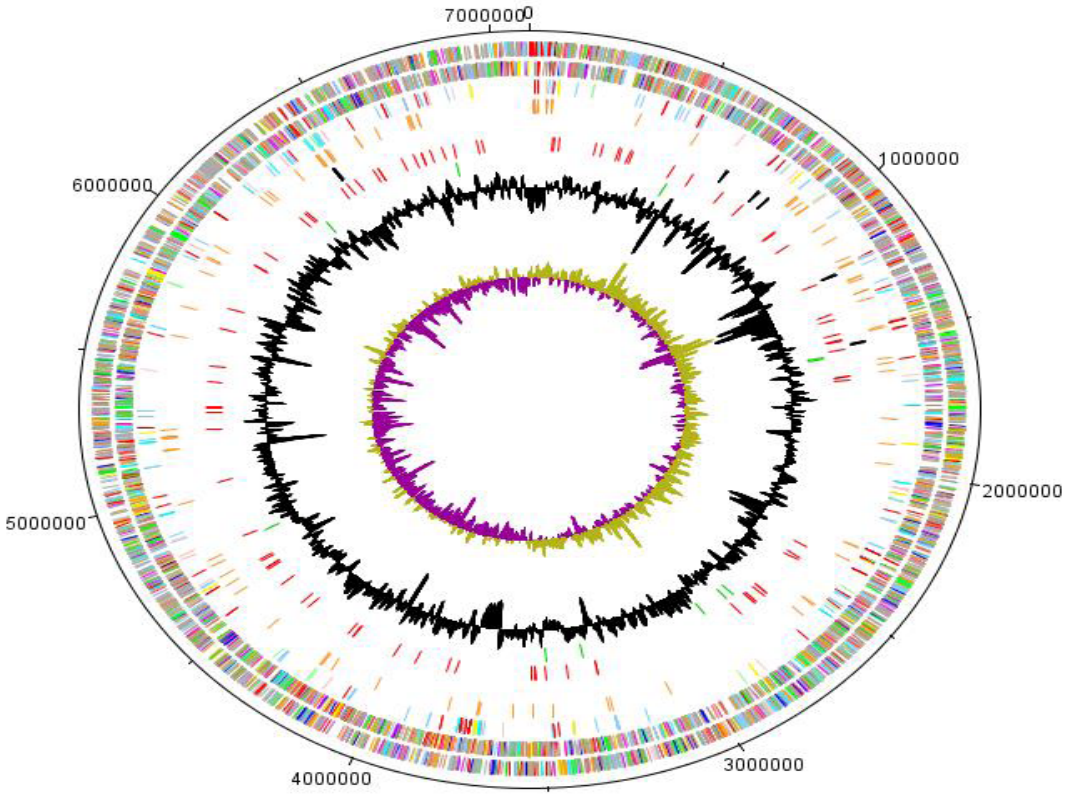


Figure 3.1. Schematic representation of the strain CB1190 chromosome. The outer scale is numbered in bases, starting from the origin of replication (*orf*). Circles 1 and 2 (from the outside in) are all coding sequences (CDS), for reverse and forward strands, respectively, color-coded by their COG (Cluster of Orthologous Groups) associated function (red – chromatin structure and dynamics, DNA replication, recombination; and repair; green – energy production and conversion; dark blue – carbohydrate transport and metabolism, magenta – transcription, RNA processing and modification; yellow – translation, ribosomal structure and biogenesis; pastel green – RNAs; light blue – cellular processes; orange – amino acid transport and metabolism; brown – general function prediction only; pink – coenzyme metabolism; gray – hypothetical; black – membrane transport; magenta – signal transduction; light red – nucleotide transport and metabolism); circle 3, pseudo genes; circle 4, mobile genetic elements (insertion sequences, integrases, and transposases) (orange); circle 5, monooxygenases [excluding P450 monooxygenase] (black); circle 6, dioxygenases (red); circle 7, cytochrome P450 enzymes (green); circle 8, GC content; circle 9, GC-skew ((G-C)/(G+C)), khaki indicates value >1, purple values <1).

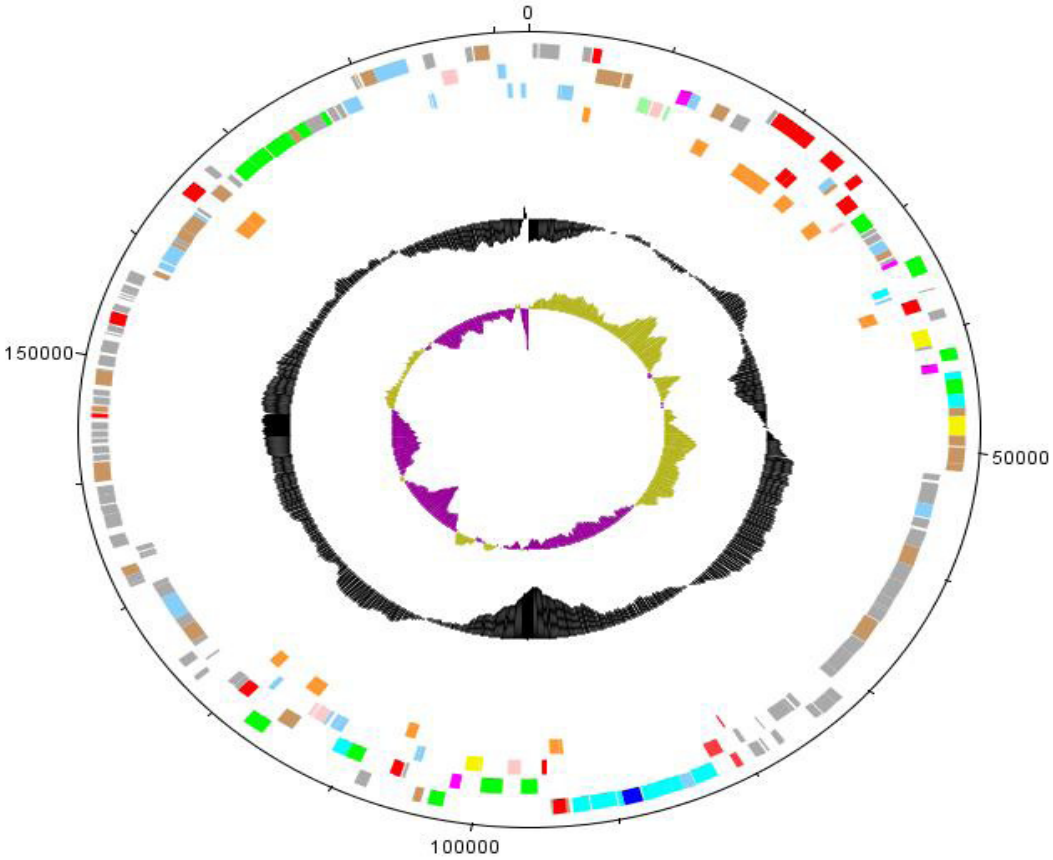


Figure 3.2. Schematic representation of the circular strain CB1190 plasmid pPSED01. The outer scale is numbered in bases. Circles 1 and 2 (from the outside in) are all coding sequences (CDS), for reverse and forward strands, respectively, color-coded by their COG (Cluster of Orthologous Groups) associated function (red – chromatin structure and dynamics, DNA replication, recombination, and repair; green – energy production and conversion; dark blue – carbohydrate transport and metabolism; magenta – transcription, RNA processing and modification; yellow – translation, ribosomal structure and biogenesis; pastel green – RNAs; light blue – cellular processes; orange – amino acid transport and metabolism; brown – general function prediction only; pink – coenzyme metabolism; gray – hypothetical; black – membrane transport; magenta – signal transduction; light red – nucleotide transport and metabolism); circle 3, pseudo genes; circle 4, mobile genetic elements (insertion sequences, integrases, and transposases) (orange); circle 5, monooxygenases [excluding P450 monooxygenases] (black); circle 6, dioxygenases (red); circle 7, cytochrome P450 enzymes (green); circle 8, GC content; circle 9, GC-skew ((G-C)/(G+C)), khaki indicates value >1, purple values <1).

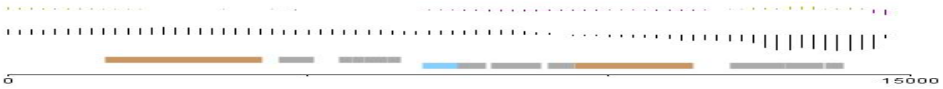


Figure 3.3. Schematic representation of the linear *P. dioxanivorans* plasmid pPSED03. The outer scale is numbered in bases. Line 1 and 2 (from bottom to top) are all coding sequences (CDS), for reverse and forward strands, respectively, color-coded by their COG (Cluster of Orthologous Groups) associated function (light blue – cellular processes; brown – general function prediction only; gray – hypothetical); line 3, GC content; line 4, GC-skew ((G-C)/(G+C)), khaki indicates value >1, purple values <1).

Table 3.2. Features of sequenced *Pseudonocardiaceae* genomes[a]

Features	*P. dioxanivorans*	*A. mediterranei* S699	*A. mediterranei* U32	*Pseudonocardia.* sp. P1	*S. azurea*	*S. paurometabolica*	*S. viridis*	*S. erythraea* NRRL2338-1	*S. erythraea* NRRL2338-2	*S. spinosa*	*T. bispora*
Status	Finished	Finished	Finished	Draft	Draft	Draft	Finished	Finished	Draft	Draft	Finished
Topology	Circular	Circular	Circular	Unknown	Unknown	Unknown	Circular	Circular	Unknown	Unknown	Circular
Genome size	7,096,571 bp	10,236,779 bp	10,236,715 bp	6,388,771 bp	4,770,125 bp	4,592,308 bp	4,308,349 bp	8,212,805 bp	8,079,083 bp	8,527,776 bp	4,189,976 bp
GC content	73%	71%	71%	73%	70%	71%	67%	71%	71%	68%	72%
Coding Density	88%	90%	89%	88%	90%	89%	86%	85%	83%	80%	83%
CDSs[c]	6,797	9.575	9,228	6,620	4,543	4,876	3,828	7,197	7,305	8,215	3,546
Pseudo genes	226	ND[d]	ND	ND	ND	ND	78	1	ND	ND	50
Hypothetical genes	1,842 (27.1%)	3,173 (33.1%)	2,854 (30.9%)	2,206 (33.3%)	1,127 (24.9%)	1,271 (26.0%)	980 (25.6%)	2,204 (30.6%)	2,326 (31.8%)	3,339 (40.6%)	980 (27.6%)
Plasmids[b]	3, free replicons	1, integrated	2, integrated	Unknown	Unknown	Unknown	0	4, integrated	Unknown	Unknown	0
rRNA operons	3	4	4	1	1	1	4	4	1	1	3
tRNA genes	47	52	52	50	48	47	53	50	50	50	53
Contigs	NA[e]	NA	NA	875	187	878	NA	NA	241	118	NA

All information is from GenBank and Refseq databases (*P. dioxanivorans*, NC_015312-4 and CP002593-8; *A. mediterranei* S699, CP002896; *A. mediterranei* U32, NC_014318; *Pseudonocardia* sp. P1, NZ_ADUJ00000000; *S. azurea*, NZ_AGIU00000000; *S. paurometabolica*, NZ_AGIT00000000; *S. viridis*, NC_013159; *S. erythraea* NRRL2338-1, NC_009142; *S. erythraea* NRRL2338-2, NZ_ABFV00000000; *S. spinosa*, NZ-AEYC00000000; *T. bispora*, NC_014165).

[a] *T. bispora* does not belong in the family *Pseudonocardiaceae*, it belongs to the suborder *Streptosporangineae*

[b] Information regarding integrated plasmids in *S. erythraea* NRRL2338-1 and *A. mediterranei* U32 are from te Poele *et al*. (2008) and Zhao *et al*. (2008), respectively.

[c] CDS – Protein coding sequence; [d] ND – Not determined; [e] NA – Not applicable

Table 3.3. Reciprocal Best Blast Hits between *Pseudonocardiaceae*. The values indicate the number of shared proteins between a pair of genomes, based on a count of all reciprocal best blastp hits between the pairs, using a minimum e-value = 1e-5 and a minimum coverage = 70%.

	P. dioxanivorans CB1190	*A. mediterranei* S699	*A.mediterranei* U32	*Pseudonocardia* sp. P1	*Pseudonocardia* sp. P2	*S. azurea*	*S. paurometabolica*	*S. viridis*	*S. erythreaa* NRRL2338-1	*S. erythraea* NRRL2338-2	*S.spinosa*	*T. bispora*
P. dioxanivorans CB1190	-	3219	3209	3251	3242	2392	2368	2210	2842	2845	2789	1890
A. mediterranei S699	3219	-		3035	3138	3109	2943	2846	3666	3665	3358	2136
A. mediterranei U32	3209	9137	-	3033	3135	3106	2939	2846	3657	3657	3346	2135
Pseudonocardia sp. P1	3251	3035	3033	-	4210	2408	2387	2241	2989	2990	2815	1840
Pseudonocardia sp. P2	3242	3138	3135	4210	-	2399	2387	2252	2950	2936	2813	1872
S. azurea	2392	3109	3106	2408	2399	-	3043	3195	2901	2893	2660	1849
S. paurometabolica	2368	2943	2939	2387	2387	3043	-	2849	2738	2719	2568	1807
S. viridis	2210	2846	2846	2241	2252	3195	2849	-	2662	2656	2472	1785
S. erythraea NRRL2338-1	2842	3666	3657	2989	2950	2901	2738	2662	-	6901	4091	2024
S. erythraea NRRL2338-2	2845	3665	3657	2990	2936	2893	2719	2656	6901	-	4063	2022
S. spinosa	2789	3358	3346	2815	2813	2660	2568	2472	4091	4063	-	1955
T. bispora	1890	2136	2135	1840	1872	1849	1807	1785	2024	2022	1955	-

Table 3.4 Heat map of percentages of total protein-encoding genes shared between genomes. The percentage of homologous protein coding sequences in genomes listed in rows and columns according to the shared protein cutoff described for Table 3.3. The total number of CDSs in *Pseudonocardia* sp. P2 is 8,138, as determined by Glimmer (http://www.ncbi.nlm.nih.gov/genomes/MICROBES/glimmer_3.cgi).

	A.mediterranei S699	*A. mediterranei* U32	*P. dioxanivorans* CB1190	*Pseudonocardia* sp. P1	*Pseudonocardia* sp. P2	*S. azurea*	*S. paurometabolica*	*S. viridis*	*S. erythraea* NRRL2338-1	*S. erythraea* NRRL2338-2	*S. spinosa*	*T. bispora*
A.mediterranei S699		95%	34%	32%	33%	32%	31%	30%	38%	38%	35%	22%
A. mediterranei U32	99%		35%	33%	34%	34%	32%	31%	40%	40%	36%	23%
P. dioxanivorans CB1190	48%	48%		49%	49%	36%	35%	33%	43%	43%	42%	28%
Pseudonocardia sp. P1	46%	46%	49%		64%	36%	36%	34%	45%	45%	43%	28%
Pseudonocardia sp. P2	39%	39%	40%	52%		29%	29%	28%	36%	36%	35%	23%
S. azurea	68%	68%	53%	53%	53%		67%	70%	64%	64%	59%	41%
S. paurometabolica	60%	60%	49%	49%	49%	62%		58%	56%	56%	53%	37%
S. viridis	74%	74%	58%	59%	59%	83%	74%		70%	69%	65%	47%
S. erythraea NRRL2338-1	50%	49%	38%	40%	40%	39%	37%	36%		93%	55%	27%
S. erythraea NRRL2338-2	51%	51%	40%	42%	41%	40%	38%	37%	96%		56%	28%
S. spinosa	41%	41%	34%	34%	34%	32%	31%	30%	50%	49%		24%
T. bispora	60%	60%	53%	52%	53%	52%	51%	50%	57%	57%	55%	

Table 3.5 Mobile genetic elements in *P. dioxanivorans* CB1190

Family	Chromosome	pPSED01	pPSED02
Integrases	Psed_0009,Psed_0016,Psed_0028,Psed_0061,Psed_0867,Psed_0874, Psed_0909,Psed_1055,Psed_1123,Psed_1146,Psed_1256,Psed_1394, Psed_1398,Psed_1720,Psed_2156,Psed_3250,Psed_3368,Psed_3467, Psed_3468,Psed_3944,Psed_3946,Psed_3948,Psed_4393,Psed_4812, Psed_4813,Psed_4853,Psed_4855,Psed_4857,Psed_4859,Psed_4885,- Psed_5234,Psed_5339,Psed_5482,Psed_5534,Psed_5820,Psed_5842, Psed_5961,Psed_6042,Psed_6045,Psed_6047,Psed_6077,Psed_6078, Psed_6086,Psed_6183,Psed_6247,Psed_6344	Psed_6704, Psed_6713, Psed_6726, Psed_6779, Psed_6802, Psed_6859,	Psed_6967, Psed_6987
IS111A/IS328/IS533	Psed_1145,Psed_1211,Psed_2453,Psed_2931,Psed_3915,Psed_4336, Psed_5853,Psed_6039,Psed_6080,Psed_6179		
IS116/IS110/IS902	Psed_0062,Psed_0498,Psed_1201,Psed_1289,Psed_1560,Psed_1804, Psed_2401,Psed_3313,Psed_6354		Psed_6985, Psed_6990, Psed_6991
IS21/IS1162	Psed_0017,Psed_0027,Psed_0866,Psed_0910,Psed_1147,Psed_1257, Psed_2155,Psed_3464,Psed_4814,Psed_4858	Psed_6697, Psed_6791	Psed_6988
IS3/IS911		Psed_6778	
IS4	Psed_0014,Psed_0888,Psed_0993,Psed_1197,Psed_1514,Psed_1535, Psed_1864,Psed_2205,Psed_3080,Psed_3090,Psed_3553,Psed_4121, Psed_4267,Psed_4304,Psed_4415,Psed_4634,Psed_5538,Psed_5583, Psed_6351,Psed_6381,Psed_6383	Psed_6799	
Other	Psed_0018,Psed_0048,Psed_0049,Psed_0050,Psed_0304,Psed_0865, Psed_2406,Psed_3781,Psed_6049,Psed_6353	Psed_6709, Psed_6858	Psed_6948, Psed_6964, Psed_6968
Tn3		Psed_6707	

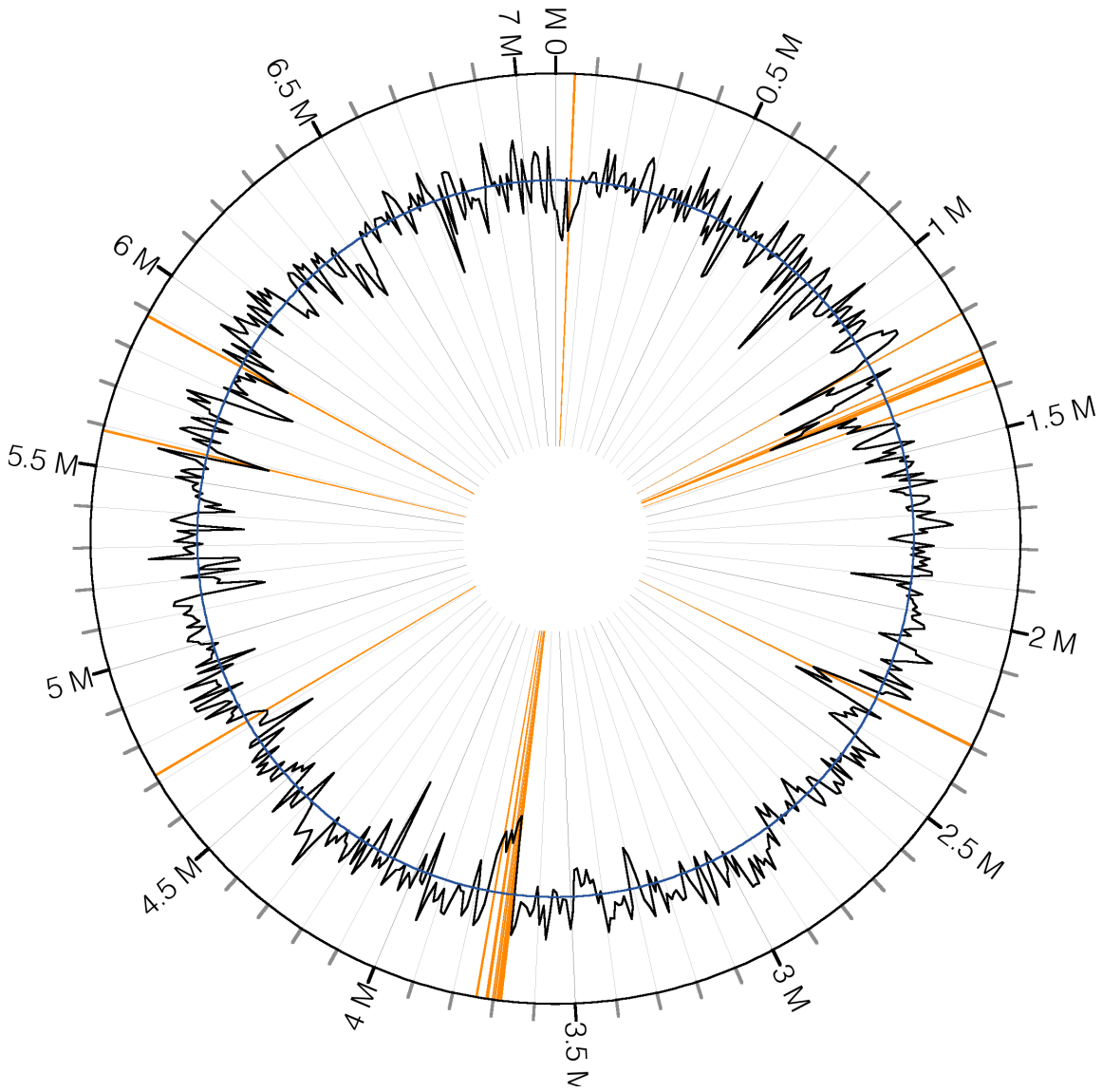


Figure 3.3. Genomic islands map of the strain CB1190 chromosome. This figure was produced using IslandViewer (Langille and Brinkmann, 2009). The outer scale is numbered in bases, the inner circle shows GC-content. The orange lines indicate genomic islands (GIs) found using SGI-HMM (Waack *et al*., 2006). A total of 14 GIs were identified at these coordinates: 44,373 - 48,400 bp; 1,199,207 - 1,203,551 bp; 1,300,847 - 1,305,076 bp; 1,318,802 - 1,323,133 bp; 1,325,885 - 1,337,782 bp; 1,378,990 - 1,383,727 bp; 2,291,903 - 2,298,725 bp; 3,680,116 - 3,693,309 bp; 3,695,403 - 3,703,768 bp; 3,712,621 - 3,719,786 bp; 3,739,174 - 3,744,337 bp; 4,716,784 - 4,722,873 bp; 5,585,339 - 5,590,927 bp; 5,884,036 - 5,889,729 bp.

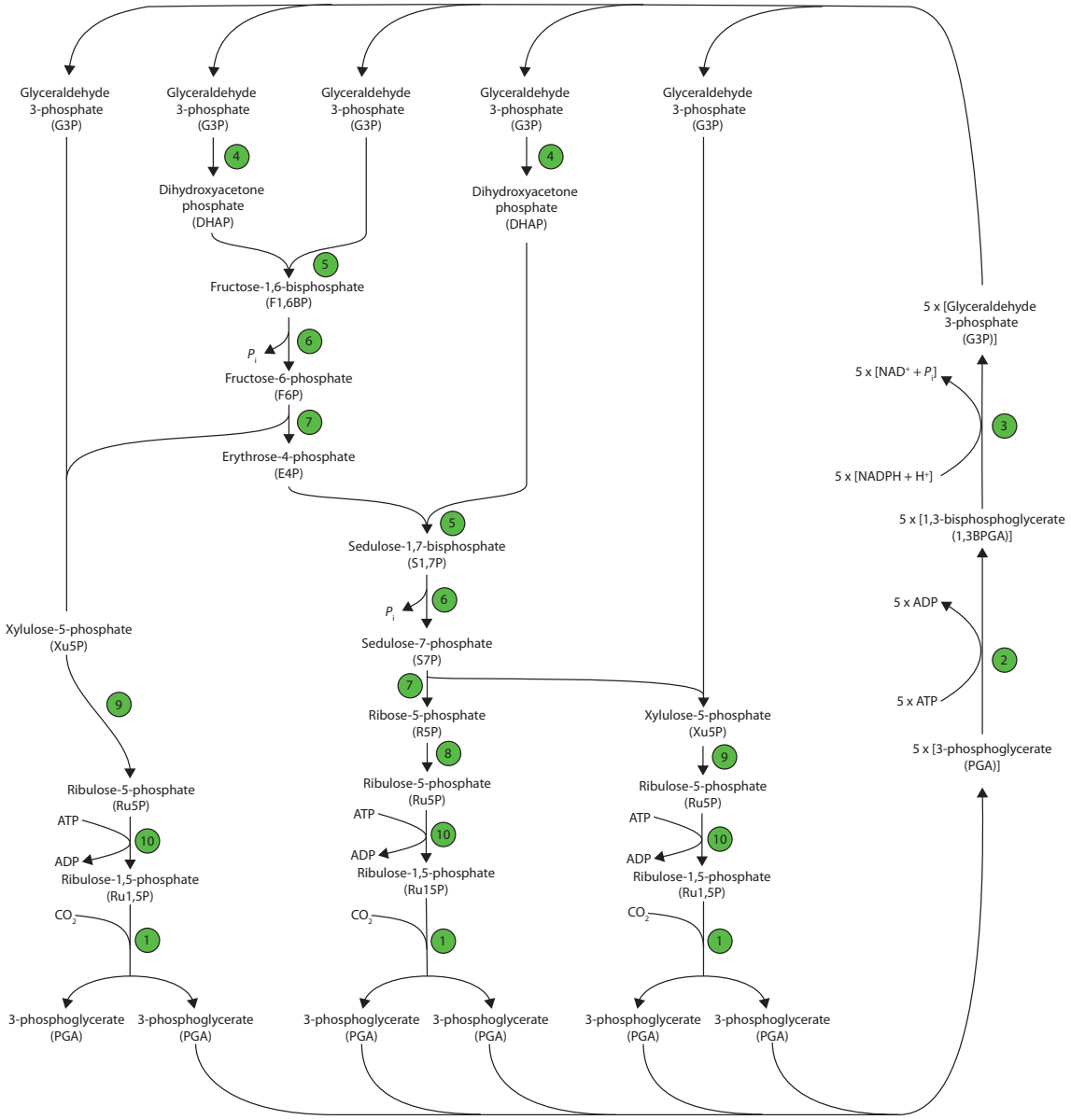


Figure 3.4. The complete Calvin-Benson-Bassham carbon fixation pathway in strain CB1190. Numbered circles specify enzymes catalyzing individual reactions: 1, ribulose-1,5-bisphosphate carboxylase oxygenase; 2, phosphoglycerate kinase; 3, glycerate-3-phosphate dehydrogenase; 4, trios-ephoshpate isomerae; 5, fructose-bisphophate aldolase; 6, fructose-1,6-bisphosphatase; 7, transketolase; 8, ribulose-5-phosphate isomerase; 9, ribulose-phosphate 3-epimerase; 10, phosphoribulokinase. Green indicates the presence of homologues in the genome of strain CB1190 encoding for that enzyme. Figure is adapted from http://users.humboldt.edu/rpaselk/BiochSupp/PathwayDiagrams/CalFlwDiag.gif.

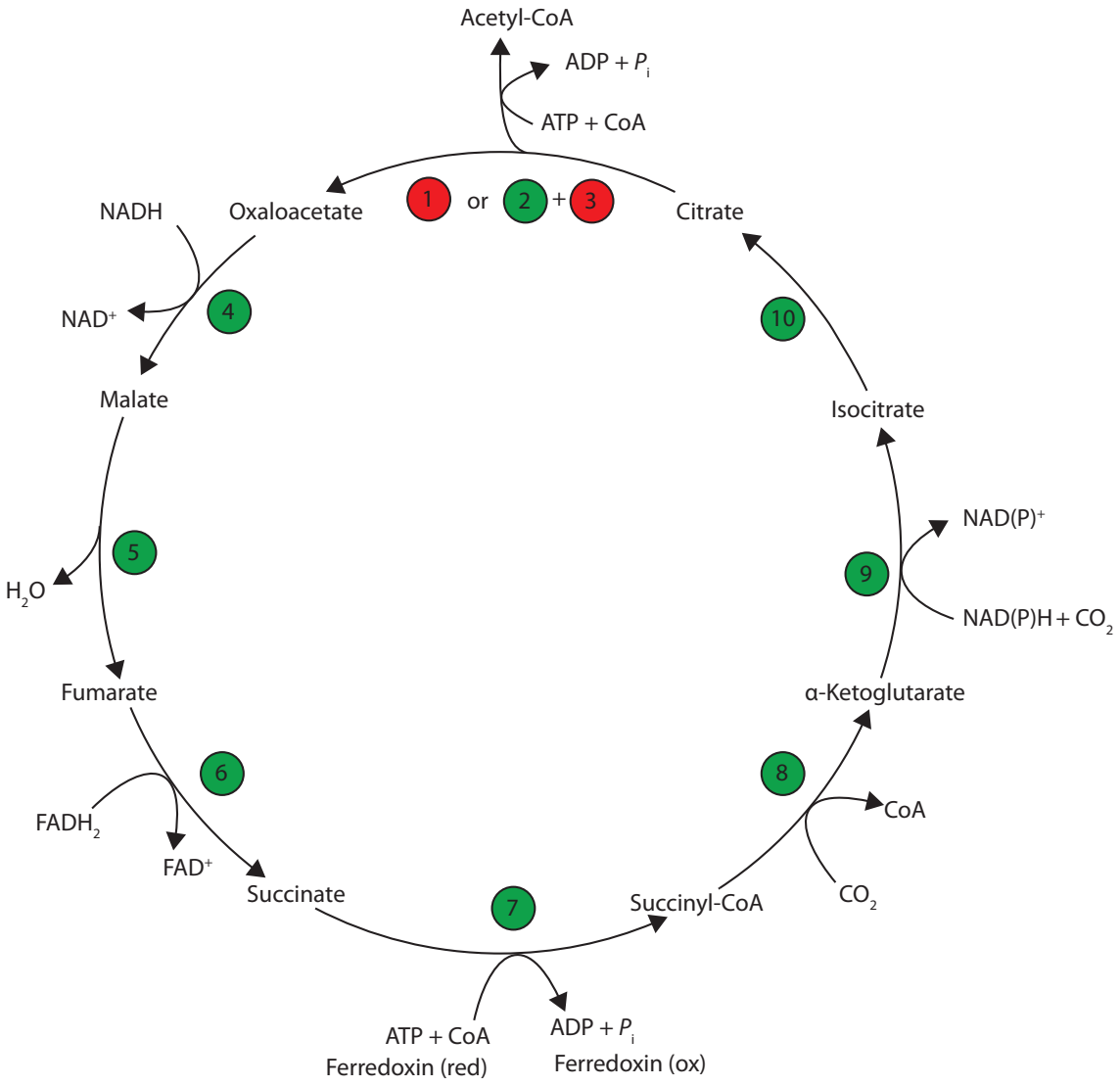


Figure 3.5. The incomplete Reductive TCA cycle carbon fixation pathway in strain CB1190. Numbered circles specify the enzyme that catalyzes a certain reaction: 1, ATP-citrate lyase; 2, Citryl-CoA lyase; 3, Citryl-CoA synthase; 4, malate dehydrogenase; 5, fumarate hydratase; 6, succinate dehydrogenase; 7, succinate-CoA ligase; 8, a-ketoglutarate:ferredoxin oxideoreductase; 9, isocitrate dehydrogenase; 10, aconitate hydratase. Green indicates the presence and red indicates the absence of homologues in the genome of strain CB1190 encoding for that enzyme.

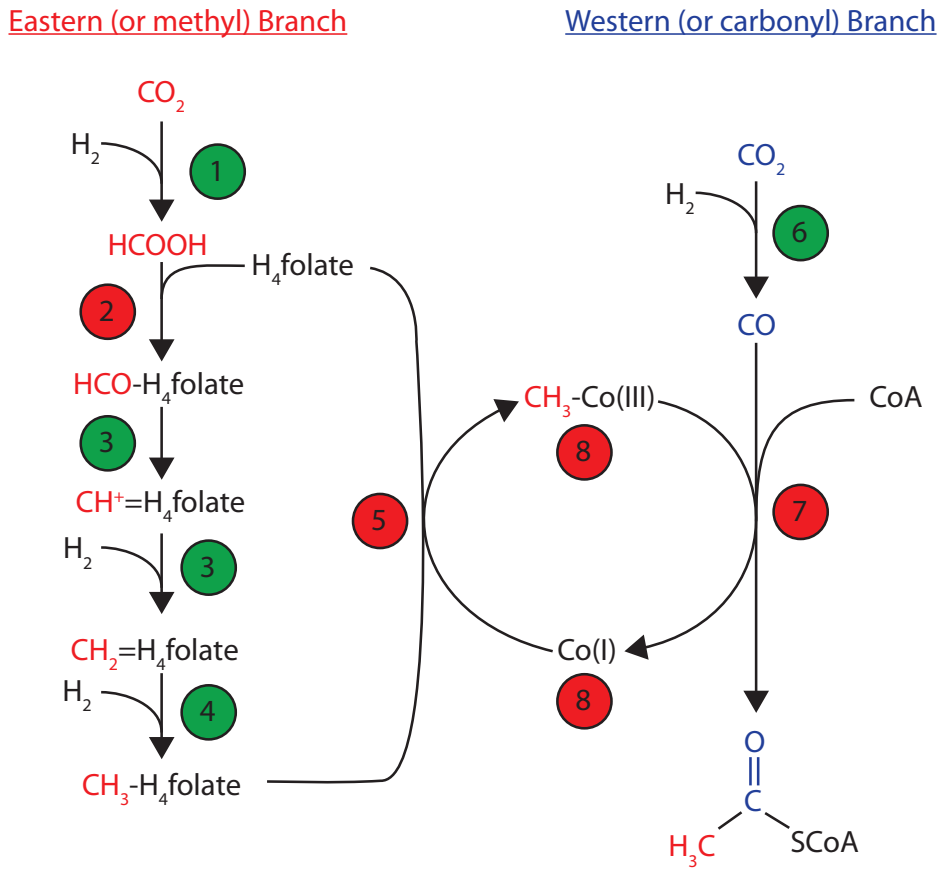


Figure 3.6. The Woods-Ljungdahl carbon fixation pathway in strain CB1190. Numbered circles specify the enzyme that catalyzes a certain reaction: 1, formate dehydrogenase; 2, 10-formyl-H_4folate synthetase; 3, bifunction protein (5,10-methenyl-H_4folate cyclohydrolase and 5,10-methylene-H_4folate dehydrogenase); 4, 5,10-methylene-H_4folate; 5, methyltransferase; 6, carbon monooxide dehydrogenase (CODH); 7, acetyl-CoA synthase (ACS); 8, corrinoid iron-sulfur protein (CFeSP). Green indicates the presence and red indicates the absence of homologues in the genome of strain CB1190 encoding for that enzyme. Adapted from Ragsdale and Pierce (2008).

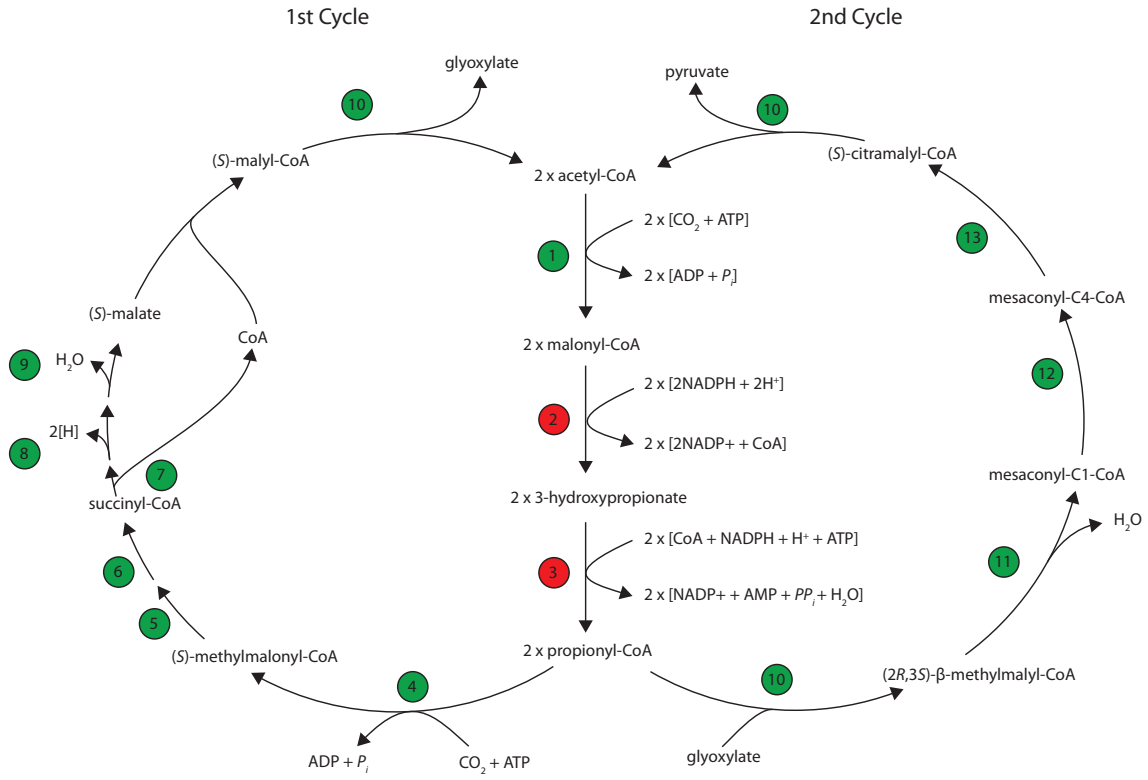


Figure 3.7. The incomplete 3-hydroxypropionate carbon fixation pathway in strain CB1190. Numbered circles specify the enzyme that catalyzes a certain reaction: 1, acetyl-CoA carboxylase; 2, malonate-semialdehyde dehydrogenase and 3-hydroxypropionate or malonyl-CoA reductase; 3, propionyl-CoA synthase; 4, propionyl-CoA carboxylase; 5, methylmalonyl-CoA epimerase; 6, methylmalonyl-CoA mutase; 7, succinyl-CoA:malate-CoA transferase; 8, succinate dehydrogenase; 9, fumarate hydratase; 10, (*S*)-malyl-CoA/(2*R*,3*S*)-β-methylmalyl-CoA/(*S*)-citramalyl-CoA lyase; 11, mesaconyl-C1-CoA hydratase (β-methylmalyl-CoA dehydratase); 12, mesaconyl-CoA C1-C4 CoA transferase; 13, mesaconyl-C4-CoA hydratase. Green indicates the presence and red indicates the absence of homologues in the genome of strain CB1190 encoding for that enzyme. Adapted from Zarzycki *et al*. (2008).

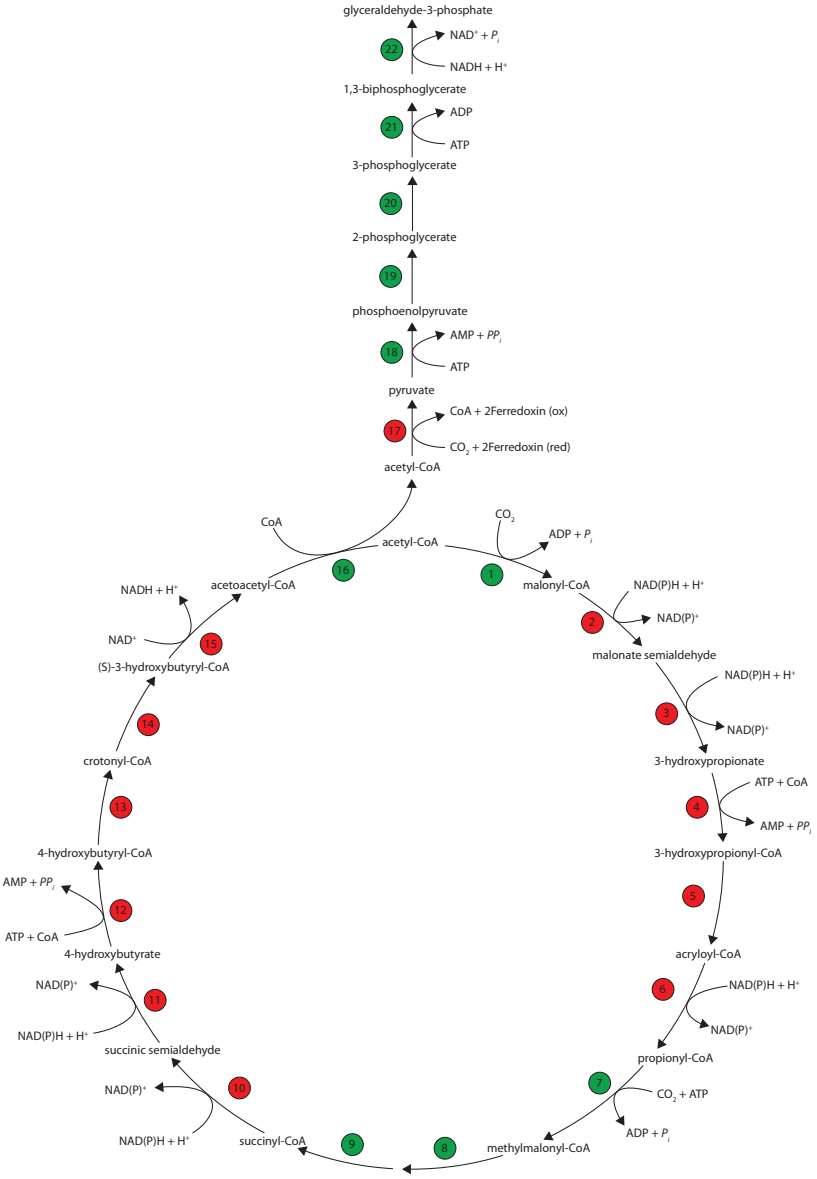


Figure 3.8. The incomplete 3-hydroxypropionate/4-hydroxybutyrate carbon fixation pathway in strain CB1190. Numbered circles specify the enzyme that catalyzes a certain reaction in 3-hydroxypropionate/4-hydroxybutyrate cycle: 1, acetyl-CoA carboxylase; 2, malonyl-CoA reductase; 3, malonate semialdehyde reductase; 4, 3-hydroxypropionyl-CoA synthetase; 5, 3-hydroxypropionyl-CoA dehydratase; 6, acryloyl-CoA reductase; 7, propionyl-CoA carboxylase; 8, methylmalonyl-CoA epimerase; 9, methylmalonyl-CoA mutase; 10, succinyl-CoA reductase; 11, succinate semialdehyde reductase; 12, 4-hydroxybutyryl-CoA synthetase; 13, 4-hydroxybutyryl-CoA dehydratase; 14, crotonyl-CoA hydratase; 15, 3-hydroxybutyryl-CoA dehydrogenase; 16, acetoacetyl-CoA b-ketothiolase. The following are enzymes involved in the conversion of CO_2 and acetyl-CoA to glyceraldehyde-3-phosphate via pyruvate: 17, pyruvate synthase; 18, pyruvate, water:dikinase [phosphoenolpyruvate (PEP) synthase]; 19, enolase; 20, phosphoglycerate mutase; 21, 3-phosphoglycerate kinase; 22, glyceraldehyde 3-phosphate dehydrogenase. Green indicates the presence and red indicates the absence of homologues in the genome of *P. dioxanivorans* encoding for that enzyme. Adapted from Berg *et al*. (2007).

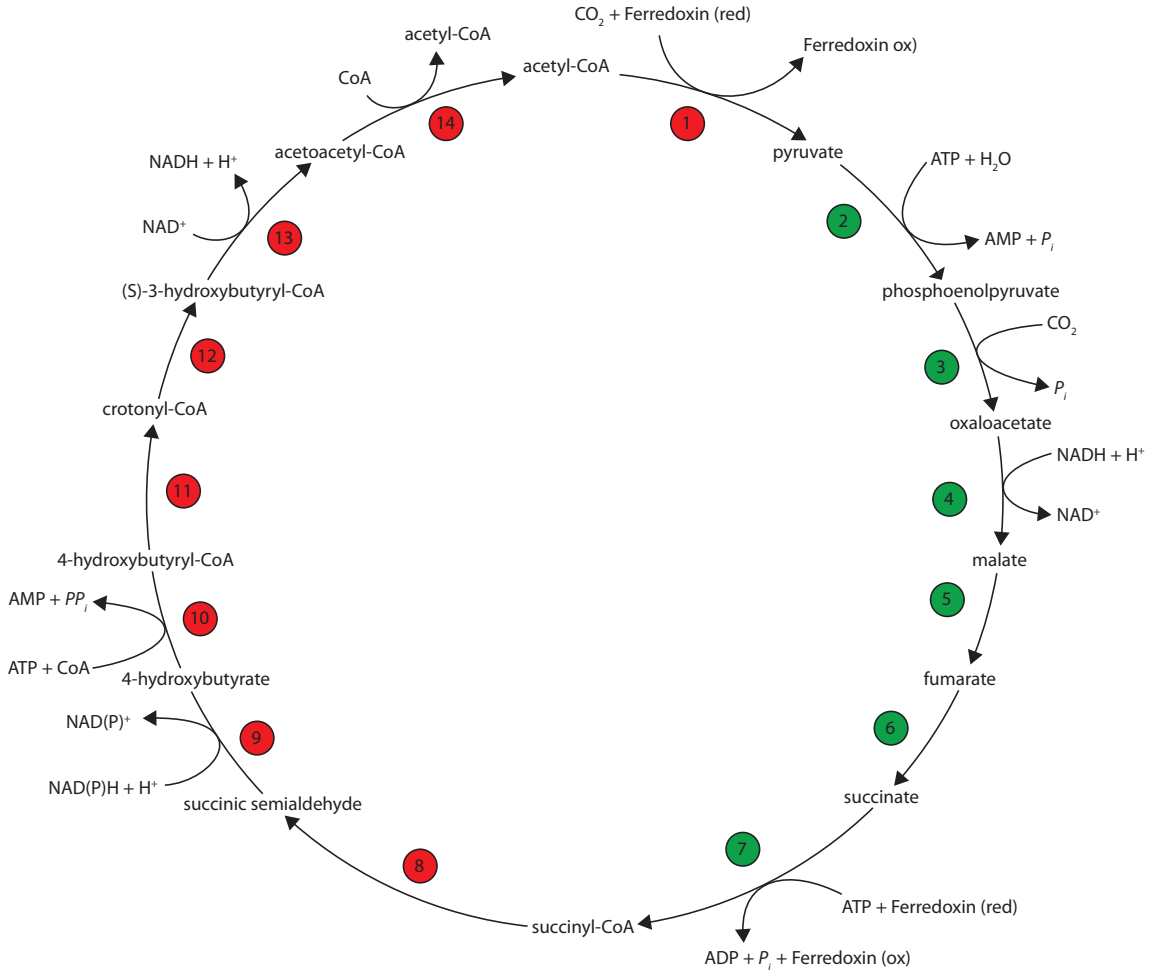


Figure 3.9. The incomplete dicarboxylate/4-hydroxybutyrate carbon fixation pathway in *P. dioxanivorans*. Numbered circles specify the enzyme that catalyzes a certain reaction: 1, pyruvate synthase; 2, pyruvate:water dikinase; 3,Phosphoenolpyruvate carboxylase; 4, malate dehydrogenase; 5, fumarate hydratase; 6, fumarate reductase; 7, succinyl-CoA ligase; 8, succinyl-CoA reductase; 9, succinate semialdehyde reductase; 10, 4-hydroxybutyryl-CoA synthetase; 11, 4-hydroxybutyryl-CoA dehydratase; 12, crotonyl-CoA hydratase; 13, 3-hydroxybutyryl-CoA dehydrogenase; 14, acetoacetyl-CoA-ketothiolase. Green indicates the presence and red indicates the absence of homologues in the genome of *P. dioxanivorans* encoding for that enzyme. Adapted from Huber *et al*. (2008).

Table 3.6. Two-component signal transduction systems in strain CB1190.

Response Regulator			**Histidine Kinase**			
Gene	Domains		Gene	Domains		
Psed_0153	RR	LytTR	Psed_0151	HK_CA:6		
Psed_0316	RR	GerE	Psed_0317	HisKA_3	HK_CA:8	
Psed_0747	RR	trans_reg_C	Psed_0748	HisKA	HK_CA:2	
Psed_0785	RR	trans_reg_C	Psed_0786	HAMP	HisKA	HK_CA:2
Psed_0994	RR	trans_reg_C	Psed_0995	HAMP	HisKA	HK_CA:7
Psed_1738	RR	GerE	Psed_1737	HK_CA:8		
Psed_1867	RR	trans_reg_C	Psed_1868	HAMP	HisKA	HK_CA:2
Psed_2821	RR	GerE	Psed_2822	HisKA_3	HK_CA:8	
Psed_2860	RR	GerE	Psed_2859	HisKA_3	HK_CA:8	
Psed_2889	RR	trans_reg_C	Psed_2890	HAMP	HisKA	HK_CA:2
Psed_3007	RR	GerE	Psed_3009	HisKA_3	HK_CA:8	
Psed_3179	RR	GerE	Psed_3180	HisKA_3	HK_CA:8	
Psed_3959	RR	trans_reg_C	Psed_3958	HAMP	HisKA	HK_CA:11
Psed_4017	RR	pyr_redox_2	Psed_4018	HK_CA:3		
Psed_4462	RR	GerE	Psed_4463	HisKA_3	HK_CA:8	
Psed_4535	RR	GerE	Psed_4536	HisKA_3	HK_CA:8	
Psed_4615	RR	GerE	Psed_4616	HisKA_3	HK_CA:8	
Psed_4823	RR	trans_reg_C	Psed_4822	HAMP	HisKA	HK_CA:2
Psed_4830	RR	trans_reg_C	Psed_4829	HAMP	HisKA	HK_CA:2
Psed_5028	RR	trans_reg_C	Psed_5029	HAMP	HisKA	HK_CA:2
Psed_5141	RR	trans_reg_C	Psed_5142	HAMP	HisKA	HK_CA:2
Psed_5184	RR	trans_reg_C	Psed_5183	HAMP	HisKA	HK_CA:2
Psed_5242	RR	trans_reg_C	Psed_5241	HAMP	HisKA	HK_CA:2

Psed_5341	RR	GerE	Psed_5342	PspC	HK_CA:8	
Psed_5392	RR	GerE	Psed_5393	HisKA	HK_CA:8	
Psed_5455	RR	GerE	Psed_5454	HK_CA:2		
Psed_5638	RR	trans_reg_C	Psed_5639	HisKA	HK_CA:2	
Psed_5665	RR	trans_reg_C	Psed_5666	HAMP	HK_CA	
Psed_5690	RR	GerE	Psed_5689	HK_CA:8		
Psed_5706	RR	GerE	Psed_5705	HisKA_3	HK_CA:8	
Psed_5946	RR	trans_reg_C	Psed_5945	HisKA	HK_CA:2	
Psed_6020	RR	trans_reg_C	Psed_6021	HAMP	HisKA	HK_CA:7
Psed_6227	RR	GerE	Psed_6228	HisKA_3	HK_CA:8	
Psed_6245	RR	GerE	Psed_6246	GAF	HisKA_3	HK_CA:8
Psed_6411	RR	trans_reg_C	Psed_6412	HAMP	HisKA	HK_CA:2
Psed_6567	RR	GerE	Psed_6568	HisKA_3	HK_CA:8	
Psed_6639	RR	GerE	Psed_6638	HisKA_3	HK_CA:8	

Receiver domains: RR, Response regulator domain.
Output (regulatory) domains: GerE, LuxR-type DNA-binding HTH (helix-turn-helix) domain; LytTr, DNA-binding domain; trans_reg_C, Transcriptional regulatory protein, C terminal; pyr_redox_2, Pyrudine nucleotide-disulfide oxidoreductase; PspC, phage shock protein C.
Transmitter domains (histidine kinases): HisKA, HK_CA, HK_CA:2, HK_CA:3, HK_CA:7, HK_CA:8, HK_CA:11,
Input (sensory) domains: GAF, cGMP-specific phosphodesterases, adenylyl cyclases, and FhlA domain.

Table 3.7. Chaperone genes in strain CB1190.

Locus Tag	Gene	Type	Protein
Psed_0131	*GroES*	Chaperonin GroES	Alcohol dehydrogenase *GroES* domain protein
Psed_0476	*DnaK*	Molecular chaperone	suppressor protein *DnaK*
Psed_0581		Molecular chaperone	Cold-shock protein DNA-binding
Psed_0633	*GroEL*	Chaperonin GroEL	chaperonin *GroEL*
Psed_1506	*ClpS*	Protease	ATP-dependent *Clp* protease adapter protein clpS
Psed_1645	*clp*	Protease	ATP-dependent *Clp* protease proteolytic subunit
Psed_1786		Protease	*Clp* domain protein
Psed_1830	*Hsp70*	Molecular chaperone	Heat shock protein 70
Psed_1963		Protease	ATP-dependent *Clp* protease proteolytic subunit
Psed_1964		Protease	ATP-dependent *Clp* protease proteolytic subunit
Psed_1965	*ClpX*	Protease	ATP-dependent *Clp* protease ATP-binding subunit *clpX*
Psed_2084	*GroES*	Chaperonin GroES	Alcohol dehydrogenase *GroES* domain protein
Psed_2140		Molecular chaperone	Cold-shock protein DNA-binding
Psed_2209	*DnaK*	Molecular chaperone	Chaperone protein *dnaK*
Psed_2210	*grpE*	Molecular chaperone	Protein *grpE*
Psed_2211	*DnaJ*	Chaperone	Chaperone *DnaJ* domain protein
Psed_2213	*ClpB*	Chaperone	ATP-dependent chaperone *ClpB*
Psed_2293	*DnaJ*	Chaperone	Heat shock protein *DnaJ* domain protein
Psed_3481	*ClpB*	Chaperone	ATP-dependent chaperone *ClpB*
Psed_3483	*DnaJ*	Chaperone	chaperone *DnaJ* domain protein
Psed_3484	*grpE*	Molecular chaperone	Protein *grpE*
Psed_3485	*DnaK*	Molecular chaperone	Chaperone protein *dnaK*
Psed_3487	*Hsp20*	Molecular chaperone	heat shock protein *Hsp20*
Psed_3632	*DnaJ*	Chaperone	*DnaJ*-like, subfamily C, domain-containing protein
Psed_4302	*NarJ*		nitrate reductase molybdenum cofactor assembly chaperone
Psed_4305		Protease	*Clp* domain protein
Psed_4493	*DnaJ*	Chaperone	Chaperone protein *dnaJ*
Psed_4577		Molecular chaperone	Cold-shock protein DNA-binding
Psed_4683		Molecular chaperone	Cold-shock protein DNA-binding
Psed_4742		Protease	*Clp* domain protein
Psed_5359		Chaperon	60 kDa chaperonin
Psed_5453	*GroES*	Chaperonin GroES	Alcohol dehydrogenase *GroES* domain protein
Psed_5546	*HtpX*	Peptidase	Protease *htpX*
Psed_5646	*Hsp90*	Molecular chaperone	HSP90 family heat shock protein
Psed_5651		Molecular chaperone	Cold-shock protein DNA-binding
Psed_5715		Molecular chaperone	Cold-shock protein DNA-binding
Psed_5716			60 kDa chaperonin

Psed_5788	*ClpP*	Protease	Peptidase S14 *ClpP*
Psed_6057	*GroES*	Chaperonin GroES	Alcohol dehydrogenase *GroES* domain protein
Psed_6199	*DnaJ*	Chaperone	Heat shock protein *DnaJ* domain-containing protein
Psed_6347	*Hsp20*	Molecular chaperone	Heat shock protein *Hsp20*
Psed_6370	*hypC/hupF*		Hydrogenase assembly chaperone *hypC*/*hupF*
Psed_6374	*hypC/hupF*		Hydrogenase assembly chaperone *hypC*/*hupF*
Psed_6547	*ClpB*	Chaperone	ATP-dependent chaperone *ClpB*
Psed_6553	*DnaJ*	Chaperone	Chaperone protein *dnaJ*
Psed_6554	*grpE*	Molecular chaperone	Protein *grpE*
Psed_6555	*DnaK*	Molecular chaperone	Chaperone protein *dnaK*
Psed_6591	*Hsp70*	Molecular chaperone	Heat shock protein 70
Psed_6603	*cpn10*	Chaperone	Chaperonin *Cpn10*

Figure 3.10. Bacterial multicomponent monooxygenases (BMMs) in strain CB1190. Seven of the BMMs (A-G) are located on the chromosome, while the tetrahydrofuran monooxygenase (THFMO) is located on the plasmid pPSED02. *prmA*, propane monooxygenase (PrMO) hydroxylase large subunit; *prmB*, PrMO reductase; *prmC*, PrMO hydroxylase small subunit; *prmD*, PrMO coupling protein; *poxB*/*dpmL*, phenol hydroxylase P1 protein; *poxC*/*dpmM*, phenol hydroxylase P2 protein; *poxD*/*dpmN*, phenol hydroxylase P3 protein; *poxE*/*dpmO*, phenol hydroxylase P4 protein; *poxF*/*dmpP*, phenol hydroxylase P5 protein; *tmoA*/*touA*, toluene monooxygenase (TMO) hydroxylase α subunit; *tmoB*/*touB*, TMO hydroxylase β subunit; *tmoC*/*touC*, TMO effector protein; *tmoD*/*touD*,TMO protein D; *tmoE*/*touE*, TMO protein E; *HpaB*, 4-hydroxyphenylacetate-3-hydroxylase reductase component, *HpaC*, 4-hydroxyphenylacetate-3-hydroxyalse oxygenase component; *thmA*, THFMO oxygenase component α subunit; *thmB*, THFMO oxygenase component β subunit; *thmC*, THFMO coupling protein; *thmD*, THFMO reductase component. Note Psed_6976* is annotated as a pseudogene (GenBank CP002597).

Table 3.8. Comparison of bacterial multicomponent monooxygenase (BMM) clusters in strain CB1190. Smith-Waterman algorithm was used for local pairwise alignments. Psed_0810, ferredoxin-NAD(+) reductase; Psed_0809, hypothetical protein; Psed_0808, antibiotic synthesis monooxygenase; Psed_1441, ferredoxin-NAD(+) reductase; Psed_1442, 50S ribosomal protein L22/L17; Psed_1443, hypothetical protein; Psed_1444, antibiotic synthesis monooxygenase.

Gene	Locus Tag	Nucleotide Length	Protein Length	Gene	Locus Tag	Nucleotide Length	Protein Length	Nucleotide Pairwise Alignment	Protein Pairwise Alignment	
		bp	aa			bp	aa	% Identity	% Identity	(% Similarity)
propane MO				***THF MO***						
prmA	Psed_0629	1653	550	*thmA*	Psed_6976	1638	546	58.5%	40.5%	(56.8%)
prmB	Psed_0630	1056	351	*thmD*	Psed_6977	1089	362	56.0%	45.3%	(62.3%)
prmC	Psed_0631	1188	395	*thmB*	Psed_6978	1041	346	49.7%	28.0%	(47.0%)
prmD	Psed_0632	378	125	*thmC*	Psed_6979	354	117	53.1%	26.6%	(44.7%)
Aromatic MO 1				***Aromatic MO 3***						
tmoA	Psed_0815	1533	510	*tmoA*	Psed_1436	1527	508	80.2%	81.6%	(89.6%)
tmoB	Psed_0814	309	102	*tmoB*	Psed_1437	279	92	67.6%	64.3%	(75.5%)
tmoD	Psed_0813	318	105	*tmoD*	Psed_1438	318	105	71.2%	57.1%	(77.1%)
tmoE	Psed_0812	1053	350	*tmoE*	Psed_1439	1008	335	69.2%	56.4%	(73.0%)
tmoC	Psed_0811	327	108	*tmoC*	Psed_1440	357	118	64.9%	63.7%	(73.5%)
	Psed_0810	1242	413		Psed_1441	1233	410	62.4%	46.6%	(62.7%)
	Psed_0809	762	253		Psed_1442	378	125	59.5%	44.5%	(55.5%)
	Psed_0809	762	253		Psed_1443	330	109	64.9%	63.1%	(70.9%)
	Psed_0808	291	96		Psed_1444	297	98	66.4%	58.3%	(75.0%)
Aromatic MO 2				***Aromatic MO 4***						
tmoA	Psed_1155	1518	505	*tmoA*	Psed_6062	1518	505	61.4%	50.0%	(66.5%)
tmoB	Psed_1156	273	90	*tmoB*	Psed_6061	246	81	45.7%	25.9%	(42.4%)
tmoC	Psed_1157	354	117	*tmoC*	Psed_6060	399	132	54.9%	47.8%	(57.8%)
tmoD	Psed_1158	303	100	*tmoD*	Psed_6059	303	100	57.6%	33.0%	(58.5%)
tmoE	Psed_1159	1020	339	*tmoE*	Psed_6058	1023	340	56.0%	37.8%	(57.2%)

Figure 3.11 THF MO gene clusters from *P. dioxanivorans* CB1190 and *P. tetrahydrofuranoxydans* K1. *orfY*, unknown function; *sad*, succinate semialdehyde dehydrogenase; *thmA*, THF MO α subunit; *thmB*, THFMO β-subunit; *thmC,* THFMO reductase; *thmD*, THFMO coupling protein; *orfZ*, unknown function; *aldH*, aldehyde dehydrogenase. Yellow indicates monooxygenase large subunit, red indicates reductase, green indicates monooxygenase small subunit, and blue indicates monooxygenase coupling protein.

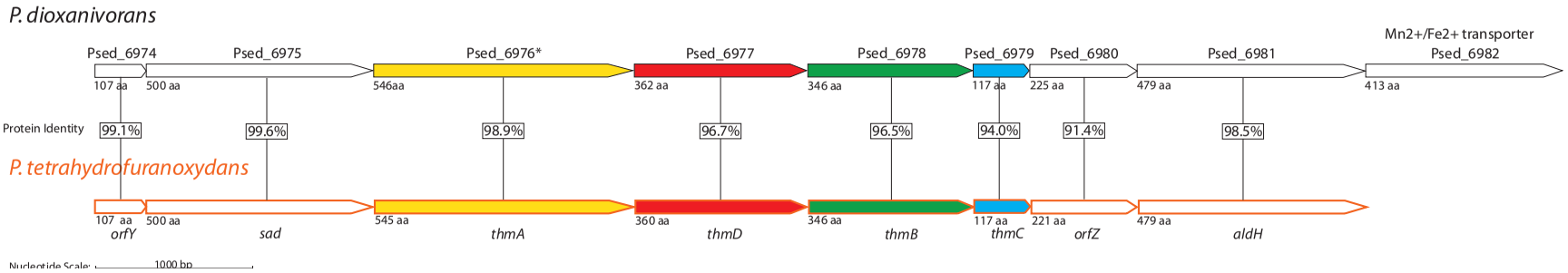
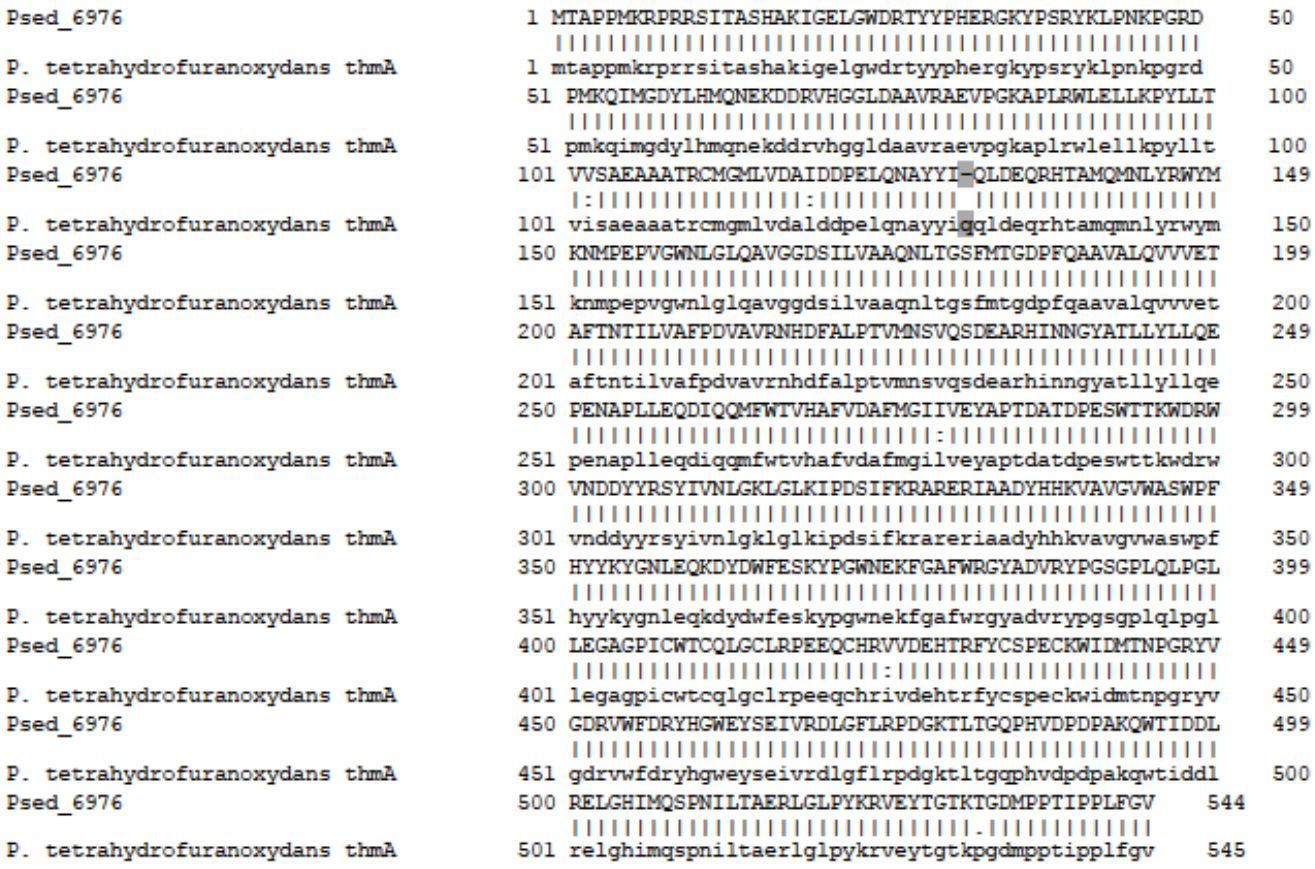


Figure 3.12 Pairwise alignment of *thmA* genes from *P. dioxanivorans* CB1190 (Psed_6976) and *P. tetrahydrofuranoxydans* K1. Highlighted amino acids show internal stop codon "-" for *P. dioxanivorans* CB1190 aligned with glutamine "q" in *P. tetrahydrofuranoxydans* K1. Identical residues are shown by "|", similar residues are marked by ":", mismatch residues are indicated by ".".



3.4. Discussion.

P. dioxanivorans strain CB1190 is the first bacterium with a finished genome in the genus *Pseudonocardia*. In general, members of the family *Pseudonocardiaceae* are characterized with a high GC-content (67-73%) and large genome sizes (4.3-10.2 Mb). Among the *Pseudonocardiaceae* genomes, strain CB1190 (along with *Pseudonocardia* sp. P1) has the highest GC content (73%) but is medial in terms of its total genome size (7.1 Mb) and CDS count (6,797). Although an extrachromosomal element, plasmid pMEA100, was previously isolated from *A. mediterranei* (formerly *Nocardia mediterranei*) strain LBG A3136 (Moretti *et al*., 1985) and a free replicating plasmid was isolated from *Amycolatopsis* sp. GY027 (te Poele *et al*., 2007), to date, the only free replicating plasmids found in the *Pseudonocardiaceae* sequenced genomes belong to strain CB1190. Despite the fact that they were isolated from disparate geographic locations and environments, strain CB1190 from contaminated activated sludge in North America (Parales *et al*., 1994) and *Pseudonocardia* sp. P1 from a mutualistic mixed community associated with a leaf cutter ant colony in Trinidad and Tobago (Barke *et al*., 2010), they share in common 3,251 CDSs or approximately 49% of their total genomes (Tables 3.3 and 3.4). The *Pseudonocardia* genomes, in general, share a large number of protein encoding genes with the genomes of *A. mediterranei* (3,035-3,219 CDS).

The majority of the genomes sequenced thus far in the family of *Pseudonocardiaceae* belong to organisms with industrial and medical importance, with the primary purpose to determine the proteins involved in the secondary metabolism of antibiotic production in these organisms. *A. meditteranei* U32 is used for industrial scale production of rifamycin-SV and its complete genome sequence revealed 26 clusters for the biosynthesis of polyketides, nonribosomal peptides, hybrid nonribosomal peptide-polyketides, and terpenoids, which potentially play an important role in rifamycin production (Zhao *et al*., 2010). Since the genome sequences of *A. mediterranei* S699 and *A. mediterranei* U32 share high similarity with respect to nucleotide identity and gene order (Verma *et al*., 2011), it is not surprising that polyketide synthase (PKS), nonribosomal peptide synthases (NRPS), and clusters required for rifamycin-B biosynthesis (*rfm*) are present. *S. erythraea* is best known for its industrial-scale production of the macrolide polyketide erythromycin A. While the cluster for erythromycin (*ery*) was previously analyzed, the genome sequence of *S. erythraea* revealed 22 more clusters containing PKS, NRPS, and terpenoid synthesis genes (Oliynyk *et al*., 2008). *Pseudonocardia* spp. discovered in mutualism with fungus-growing,leaf cutter ants, have been found to produce a selective antibiotic, dentigerumycin, which defends the ants from fungal parasites (Oh *et al*., 2009). The draft genome of *Pseudonocardia* sp. P1, which provides antifungal compounds as part of its mutualism with leaf-cutting ants, contains several PKS gene fragments with significant similarity to genes encoding proteins involved in the biosynthesis of an antifungal compound named nystatin-like *Psuedonocardia* polyene (NPP) that is produced by *Pseudonocardia autotrophica* (Kim *et al*., 2009; Barke *et al*., 2010; Jeon *et al*., 2011). On the other hand, the draft genome sequence of *Pseudonocardia* sp. P2 was previously reported to be void of biosynthetic genes for nystatin-like antifungal (Barke *et al*., 2010). Although genes encoding for antibiotic biosynthesis monooxygenases, beta-lactamases, polyketide cyclase/dehydrases, and proteins necessary for the production of precursors (*e.g.*, malonyl-CoA and methylmalonyl-CoA) used in secondary metabolism are present,

the genome of strain CB1190 is absent of homologues for PKS, NPRS, and terpenoid genes in known secondary metabolite biosynthesis pathways.

Although genes essential for secondary metabolism of antibiotic production appear to be absent, a large number of genes for antibiotic resistance are present in the genome, suggesting that strain CB1190 evolved in a complex and competitive microbial environment with antibiotic producing organisms. Genes encoding a wide variety of drug efflux proteins, including multidrug transport proteins and those putatively targeting antibiotics such as acriflavin, actinorhodin, camphor, cephamycin, chloramphenicol, landomycin, methicillin, and rifamycin, were identified. In addition to transporting mechanisms for coping with antimicrobial metabolites, half of the putative dioxygenase genes spread across the genome are related to the bleomycin resistance protein, which could degrade antibiotics into less toxic products. A number of signal transduction proteins contain tetracycline resistance (TetR) or multiple antibiotic resistance (MarR) domains, potentially giving strain CB1190 the ability to sense and subsequently respond to antibiotics in its environment. The genome also harbors genes for transport and signal transduction mechanisms to cope with heavy metal toxicity. Therefore, in addition to dealing with a competitive microbial community, strain CB1190 appears to be well-suited to deal with a hostile environment, as would be expected of its isolation source—1,4-dioxane contaminated sludge. However, the nature and extent of heavy metal toxicity that strain CB1190 can withstand has yet to be studied. Characterizing the effect of heavy metals on contaminant degradation by strain CB1190 could determine the strategy and feasibility of bioremediation at 1,4-dioxane sites contaminated with heavy metals.

Previous studies have implicated the role of a monooxygenase in catalyzing the first step of 1,4-dioxane degradation in strain CB1190 (Mahendra *et al*., 2007). Monooxygenase-specific acetylene inhibition of THF and 1,4-dioxane degradation by THF-grown strain CB1190 and *P. tetrahydrofuranoxydans* had demonstrated that the characterized THF monooxygenase is involved in the degradation of 1,4-dioxane by *P. tetrahydrofuranoxydans* and simultaneously suggested that strain CB1190 possesses a homologous monooxygenase enzyme (Mahendra *et al*., 2007). The THF monooxygenase genes *thmADBC* characterized on a plasmid in *P. tetrahydrofuranoxydans* encode for a four subunit enzyme homologous to different binuclear-iron-containing BMMs (Thiemer *et al*., 2003). Although a nearly identical *thm* gene cluster was found on plasmid pPSED2, strain CB1190 contains seven additional BMMs that could potentially be involved in 1,4-dioxane degradation. The aromatic monooxygenases 1-4 (Figure 3.11) share a high degree of similarity with toluene monooxygenases. Specifically, toluene-2-monooxygenase (T2MO), toluene-*p*-monooxygenase (T*p*MO), and toluene-4-monoxygenase (T4MO) were found to degrade 1,4-dioxane, but not toluene-*o*-xylene-monooxygenase (T*o*MO) (Mahendra and Alvarez-Cohen, 2006). Interestingly, aromatic monooxygenases 2 and 4 are similar, in sequence similarity and gene order (*tmoABCDE*), to both T4MO in *Pseudomonas mendocina* KR1, which can degrade 1,4-dioxane, and ToMO in *Pseudomonas strutzeri* OX1, which cannot degrade 1,4-dioxane. The gene clusters of aromatic monooxygenases 1 and 3 also contain T4MO and T*o*MO homologous genes but the arrangement of genes in these clusters is unique, *tmoABDEC*. In fact, the gene neighborhoods downstream of aromatic monooxygenases 1 and 3 are highly identical (Table 3.8), suggesting the likelihood of a large duplication event in the genome of at least 5.9 kb. Toluene-grown *P.*

tetrahydrofuranoxydans could also degrade 1,4-dioxane, but the type or sequence of the toluene-induced monooxygenase was not determined (Mahendra and Alvarez-Cohen, 2006). Although a propane-induced monooxygenase degrades 1,4-dioxane in *Mycobacterium vaccae* JOB5 (Burback and Perry, 1993) suggesting that the propane monooxygenase identified in strain CB1190 could oxidize 1,4-dioxane, a study (discussed in Chapter 7) using degenerate oligonucleotide PCR primers did not detect a propane monooxygenase in *M. vaccae* JOB5. Therefore, it remains unknown if propane monooxygenase genes homologous to the one found in strain CB1190 could degrade 1,4-dioxane. Sequence analysis and the arrangement of their genes indicate that the *prm* and *thm* clusters in strain CB1190 are closely related.

Although the THF monooxygenase gene on plasmid pPSED2 is probably the monooxygenase involved in 1,4-dioxane degradation, further studies examining the gene expression and function of the THF monooxygenase (discussed in Chapters 4 and 5) are needed for confirmation. The intermediate 2-hydroxyethoxyacetic acid (HEAA) of the 1,4-dioxane degradation pathway has been hypothesized to be hydroxylated by the same THF monooxygenase into 2-carbon metabolites that can be assimilated or used for energy by strain CB1190. Interestingly, *Pseudonocardia* sp. ENV478 and *P. tetrahydrofuranoxydans* both contain a THF monooxygenase, but only strain CB1190 is able to convert 1,4-dioxane past the intermediate HEAA and use 1,4-dioxane as a sole carbon and energy source (Vainberg *et al*., 2006; Mahendra and Alvarez-Cohen, 2006). Examination of global transcription by strain CB1190 using whole-genome microarrays (Chapters 4 and 5) could help pinpoint the genes involved in the degradation and assimilation of 1,4-dioxane. Furthermore, functional genomics studies guided by the available genome sequence could elucidate the network and the circumstances under which different pathways are used for CO_2 fixation, as well as lead to the discovery of the unknown system responsible for N_2 fixation.

Chapter 4

Transcriptional analysis of bacterial 1,4-dioxane metabolism reveals carbon assimilation and energy generation through glyoxylate

4.1. Introduction.

The cyclic ether 1,4-dioxane (dioxane) is part of the growing class of emerging contaminants, whose wide distribution in the environment has only recently been recognized. 1,4-Dioxane is highly soluble in water and thus is easily transported in groundwater systems from point source contamination (Zenker *et al.*, 2003). Although the long-term effects of 1,4-dioxane exposure on human health are not well understood, animal studies indicate the compound is a potential carcinogen (Stickney *et al.*, 2003). Consequently, 1,4-dioxane contamination has become the focus of remediation research, and microbial systems capable of 1,4-dioxane degradation have been investigated (Zenker *et al*., 2003). The degradation of 1,4-dioxane under co-metabolic conditions (where other substrates provide carbon and energy for growth) appears to be somewhat common (Sun *et al*., 2011; Mahendra and Alvarez-Cohen, 2006; Vainberg *et al.*, 2006; Burback and Perry, 1993; Skinner *et al*., 2009), but an increasing number of microorganisms have also been identified that can use 1,4-dioxane as a sole source of carbon and energy (Bernhardt and Diekmann, 1991; Parales *et al.*, 1994; Patt and Abebe, 1995; Kim *et al.,*, 2009; Mahendra and Alvarez-Cohen, 2005; Nakimaya *et al*., 2005).

Initial investigations of biological 1,4-dioxane transformation were performed as part of toxicological studies, which identified hydroxyethoxyacetic acid (HEAA) and 1,4-dioxane-2-one as the major metabolites in humans (Young *et al*., 1976) and rats (Woo *et al*., 1977), respectively. Recently, the pathways of 1,4-dioxane biotransformation have been studied in a number of metabolizing and co-metabolizing bacteria and fungi in an attempt to understand the fate of environmentally-released 1,4-dioxane. Similar to the findings of the toxicological studies, a common feature of 1,4-dioxane transformation is the initial the hydroxylation of the 1,4-dioxane ring by either monooxygenase or cytochrome P450 enzyme systems (Mahendra and Alvarez-Cohen, 2006; Vainberg *et al*., 2006; Skinner *et al*., 2009). The hydroxylated metabolite then undergoes uncharacterized biotic or abiotic processing, leading to an opening of the ring structure. Researchers have used a number of techniques to detect post-hydroxylation metabolites in order to elucidate the 1,4-dioxane catabolic pathway. In the 1,4-dioxane co-metabolizing fungus *Cordyceps sinesis*, deuterated 1,4-dioxane-d_8 and GC-MS analysis were used to identify ethylene glycol, glycolate and oxalic acid as metabolites (Nakamiya *et al.*, 2005). In *Mycobacterium* sp. PH-06, which grows using 1,4-dioxane, deuterated 1,4-dioxane and GC-MS analysis detected only two metabolites, 1,4-dioxane-2-ol and ethylene glycol (Kim *et al*., 2009). Meanwhile, 2-hydroxyethoxyacetic acid (HEAA) was the sole metabolite detected with ^{14}C-labeled 1,4-dioxane in the co-metabolizing bacterium *Pseudonocardia* sp. strain ENV478 (Vainberg *et al.,* 2006).

A more comprehensive analysis of 1,4-dioxane metabolites was recently performed with the 1,4-dioxane-metabolizing *Pseudonocardia dioxanivorans* strain CB1190 (Mahendra *et al.*, 2007). By analyzing metabolites with Fourier transform ion cyclotron resonance-mass spectrometery, MS-MS, and by tracking distribution of ^{14}C-labeled 1,4-dioxane metabolites, the previously identified metabolites were confirmed, and 2-hydroxyethoxy-acetaldehyde, 1,2-dihydroxyethoxyacetic acid, 2-hydroxyethoxy-2-hydroxyacetic acid, glycoaldehyde, glyoxylic acid and CO_2 were also identified as 1,4-dioxane metabolites

(Mahendra *et al*., 2007). Based on these findings, a 1,4-dioxane biodegradation pathway was proposed, whereby all metabolites feed through oxalic acid into central metabolism (Mahendra *et al*., 2007).

These studies have improved our understanding of the fate of 1,4-dioxane during microbial biodegradation, but there is a knowledge gap in terms of the biochemical and genetic basis for this pathway. While monooxygenases or cytochrome P450 enzymes have been implicated in the initial activation of the 1,4-dioxane ring, the further processing of 1,4-dioxane metabolites has not been explored. In particular, the mechanism for incorporation of 1,4-dioxane metabolites into biomass in microorganisms that can grow with 1,4-dioxane as the sole carbon and energy source is unknown. In this work we used the recently-determined genome of CB1190 strain CB1190 (Sales *et al*., 2011) to investigate how 1,4-dioxane is assimilated into biomass to support growth. We present evidence highlighting the unique role of glyoxylate in bacterial 1,4-dioxane metabolism.

4.2. Materials and Methods.

4.2.1. Culture growth.

P. dioxanivorans strain CB1190 (designated as CB1190) was cultivated in ammonium mineral salts (AMS) medium (Parales *et al*., 1994). Replicate 250 mL screw-cap bottles were prepared with 50 mL AMS medium and were amended with the appropriate carbon source. These were then inoculated with 0.1 mL (1:500 dilution) 1,4-dioxane-grown CB1190 culture and were incubated at 30°C with shaking at 150 rpm. Sterile (un-inoculated) controls were prepared in parallel. Bottles were amended with 1,4-dioxane (4.7 mM final aqueous concentration), glycolate(2 mM), or pyruvate (45 mM). 1,4-Dioxane and glycolate were used at low concentrations to avoid toxicity. Of the known 1,4-dioxane degradation intermediates, glycolate was chosen since it best supported strain CB1190 growth. Glycolate bottles were neutralized with NaOH and were re-amended with a second dose of glycolate after the first dose was consumed.

4.2.2. Cell harvesting and RNA extraction for transcription studies.

Strain CB1190 grows as clumps on the liquid surface, so standard OD readings cannot be used to determine growth phase. Therefore, in order to generate highly active cultures, cells were harvested when approximately half of the added substrate had been consumed. For glycolate-grown cells, cell harvesting occurred one day after the second substrate amendment. Cells from replicate bottles were collected by filtration onto triplicate 0.45 µm cellulose ester filters (Gelman Sciences, Ann Arbor, MI), were scraped from the filters with a sterile scalpel, and were transferred to 2 mL screw-top microcentrifuge tubes containing 1 g 100-µm-diameter zirconia-silica beads (Biospec Products, Bartlesville, OK). Cells on each filter were collected from one, five, or six bottles, respectively, for pyruvate, 1,4-dioxane and glycolate treatments. Harvested cells were stored at -80°C until RNA extraction.

Nucleic acids were extracted using a modified version of the phenol method described previously (Johnson *et al.*, 2008). Briefly, each 2 mL microcentrifuge tube containing cells and zirconia-silica beads was filled with 250 µL lysis buffer (50 mM sodium acetate, 10 mM EDTA [pH 5.1]), 100 µL 10% sodium dodecyl sulfate, and 1.0 mL phenol (pH 8; Sigma-Aldrich, St. Louis, MO). Cells were lysed by heating to 65°C for 2 min, bead beating with a Mini Bead Beater (Biospec Products) for 2 min, incubating at 65°C for 8 min, and bead beating for an additional 2 min. Cellular debris was collected by centrifugation (5 min at 14,000 x *g*), and the aqueous lysate was transferred to a new microcentrifuge tube. The lysate was extracted twice with one volume of phenol-chloroform-isoamyl alcohol (pH 8) (24:24:1, vol/vol) and once with 1 volume of chloroform-isoamyl alcohol

(24:1, vol/vol) (Sigma-Aldrich). Nucleic acids were precipitated by adding 0.1 volume of 3 M sodium acetate and one volume of ice-cold isopropanol and storing the tube at -20°C overnight. The precipitate was collected by centrifugation (30 min at 21,000 x *g* at 4°C), washed once with 70% ethanol, and re-suspended in 100 µL nuclease-free water.

To obtain RNA, re-suspended nucleic acids were initially separated with the Allprep kit (Qiagen), and then the RNA was purified using the RNeasy kit (Qiagen). Following elution with 100 µL RNase-free water, any contaminating DNA was removed by two successive DNase I treatments using the DNA-free kit (Ambion, Austin, TX) according to the manufacturer's instructions. Pure RNA was obtained with a final cleanup with the RNeasy kit. RNA yields ranged from 6.3 - 24.8 µg per microcentrifuge tube of harvested cells.

4.2.3. Microarray design and analyses.

A draft version of the CB1190 strain CB1190 genome was obtained from the Joint Genome Institute and consisted of 6,896 protein-encoding sequences in 226 contigs. These were submitted to Affymetrix (Santa Clara, CA) for custom chip design. The final design consisted of a set of perfect match-only 25-mer probes, with each sequence targeted by 11-13 probes, and also included a standard set of Affymetrix controls for prokaryotic gene expression microarrays. Subsequent to microarray production, a finished genome sequence for strain CB1190 was made available (Sales *et al.*, 2011). The microarray probes were re-annotated to reflect the finished genome sequence. Re-annotated microarray probe sets targeted 6,391 (approximately 94%) of the coding sequences in the finished genome.

A total of five µg of total RNA was used as starting material for each microarray analysis. Triplicate microarrays were processed for each substrate treatment. cDNA was synthesized, fragmented, labeled, and hybridized to arrays according to the protocols outlined in section 3 of the Affymetrix GeneChip Expression Analysis technical manual, with the following changes: cDNA fragmentation was achieved by the addition of 0.07 U DNase I/µg cDNA, and the hybridization temperature was 52°C. Hybridized arrays were stained and washed according to Affymetrix protocol "Modified FlexMidi_euk2v3 for *P. aeruginosa* Array" and were scanned using an Affymetrix GeneChip Scanner 3000.

Microarray data was first processed with the RMAExpress software program (Bolstad, 2003) to compute gene expression summary values. Background adjustment and quantile normalization were performed across all microarrays. The average normalized expression ratio (1,4-dioxane or glycolate treatment/pyruvate control) was calculated for each gene. Ratios were considered significant if they were >2.0 or <0.5 and Student's t-test indicated a p-value (two-tailed) of <0.05.

4.2.4. Quantitative RT-PCR.

To verify microarray data, the relative transcription of 17 genes in the 1,4-dioxane and glycolate treatments were compared with the pyruvate treatment. cDNA was synthesized from 500 ng of RNA using the TaqMan reverse transcription kit (Applied Biosystems, Foster City, CA) with random hexamers in 15 µL reactions according the manufacturer's protocol. cDNA was diluted 100-fold and 2 µL were used in each subsequent qPCR reaction. All qPCR reactions were performed in triplicate with an Applied Biosystems StepOne Plus real-time PCR system. For most genes, SYBR Green chemistry was used, and each 20 µL reaction consisted of 1X Fast SYBR Green master mix, 2 µL of diluted cDNA and each primer at 0.5 mM. The PCR cycling conditions were: 95°C for 20 s, then 40 cycles of (95°C for 3 s, annealing for 30 s), followed by melting curve analysis. The genes *tpi*, *thiC*, and *rpoD* were determined by the geNorm method (Vandesompele *et al.*, 2002) to be stably transcribed across all treatments, and were thus used as internal references. TaqMan chemistry was used for these genes, and each qPCR reaction consisted of 1X Fast Universal Mix (Applied Biosystems), 2 µL of diluted cDNA, each primer at 0.5 mM, and the probe at 145 nM. The cycling conditions were: 95°C for 20 s, then 40 cycles of (95°C for 1 s, annealing for 20 s). The primer sequences, dye and quencher chemistry for each probe are listed in Table 4.1. The efficiency of each qPCR assay was determined with a serial dilution of cDNA derived from a 1,4-dioxane treatment replicate. Gene expression was normalized using the method of Vandesompele *et al*. (2002).

4.2.5. Cloning and heterologous expression of strain CB1190 genes.

Strain CB1190 genes Psed_3889 and Psed_3890 were PCR amplified and ligated into linearized *Rhodococcus* expression plasmid pTip-QC2 (Nakashima and Tamura, 2004) using the hetero-stagger cloning method (Liu, 1996). *Rhodococcus jostii* strain RHA1 was transformed with pTip-Psed_3889, pTip-Psed_3890, or pTip-QC2, and then expression of the inserted gene was induced by the addition of thiostrepton to the culture medium. Specifically, strain CB1190 gene Psed_3889 was amplified in two parallel PCR reactions using the primer pairs Psed_3889F1/R1 (F1: TATGAGCACCACCACC-GTCGC; R1: TAGATCTGGAACCGGAGCGGCCG) and Psed_3889F2/R2 (F2: TGA-GCACCAC-CACCGTCGC; R2: AGCTTAGATCTGGAACCGGAGCGGCCG). The gene Psed_3890 was amplified in two parallel PCR reactions using the primer pairs Psed_3890F1/R1 (F1: TATGCCTCGTATGAGGACGGTCG; R1: TAGATCTGACCG-CCCGGGCGA) and Psed_3890F2/R2 (F2: TGCCTCGTATGAGGACGGTCG; R2: AGCTTAGATCTGAC-CGCCCGGGCGA). Each 100 µL PCR reaction consisted of 1X HF Buffer, 200 nM dNTPs, 500 nM each primer, 10% DMSO, 2 units Phusion Hot Start II DNA polymerase (Finnzymes, Lafayette, CO) and 50 ng of strain CB1190 genomic DNA. Thermocycling conditions were as follows: 98°C for 3 min, then 30 cycles of (98°C for 20 s, 72°C for 3 min), then 72°C for 10 min. The appropriate amplicon was gel-purified, and then amplicons from the parallel PCR reactions were mixed in a 1:1 ratio, melted and re-annealed using the following conditions: 2 cycles of (95°C for 4 min, then ramp to 15°C at 0.1°C/

sec).

Plasmid pNit-QC2 was linearized with NdeI and HindIII (New England Biolabs, Ipswich, MA), gel purified, and then the plasmid and PCR insert were ligated at a 1:3 (plasmid:insert) ratio at 16°C overnight with T4 DNA ligase (New England Biolabs). Electrocompetent *E. coli* DH5α was transformed with 1 µL of ligation mix. Ampicillin-resistant colonies were screened for the appropriate construct, which were named pTip-Psed_3889 and pTip-Psed_3890.

A total of 50 ng of purified plasmid was used to transform electrocompetent *R. jostii* RHA1 according to the method of Kalscheuer *et al.* (1999), except that cells were not pre-incubated prior to electroporation, and the electroporation parameters were: 12 kV/cm, 800 Ω; and 25 µF. Cell-free extracts from *R. jostii*/pTip cells were prepared as described below for strain CB1190 cells.

Single colonies of *R. jostii* RHA1 containing plasmid pTip constructs were used to inoculate 3 mL of nutrient broth amended with chloramphenicol (34 µg/mL) and grown at 30°C with shaking at 150 rpm for 24 h. Baffled flasks (300 mL) containing 50 mL of nutrient broth amended with chloramphenicol and 1% each of sucrose and glycine were inoculated with 0.5 mL of 24 h culture and incubated at 30°C with shaking at 150 rpm. When the cultures had reached OD600 of 0.6-0.9 (~24 h), thiostrepton suspended in DMSO was added to a final concentration of 1 µg/mL and the cultures were incubated for a further 24 h. Cells were transferred to 50 mL centrifuge tubes, cooled on ice, centrifuged for 10 min at 8,000 x *g* at 4°C, and the cell pellets were stored at -80°C. Prior to preparation of cell-free extracts, the cell pellets were thawed on ice, washed with 5 mL of 10 mM sodium phosphate buffer (pH 7.0), centrifuged for 10 min, and then the pellet was suspended in 3 mL phosphate buffer.

4.2.6. Glyoxylate carboligase assay.

Strain CB1190 cells grown with either pyruvate or 1,4-dioxane were harvested by filtration and scraped cells were stored at -80°C. Cell-free extracts were prepared by re-suspending the cells in 1 mL of 10 mM sodium phosphate buffer (pH 7.0), adding 1 g of 100 µm-diameter zirconia-silica beads, and bead beating for four cycles of 1 min, with cooling on ice for 1 min between cycles. Cell debris was collected by centrifugation for 10 min at 16, 000 x *g* at 4°C, and the supernatant (cell-free extract) was aliquoted and stored at -80°C until use. Protein was determined by the method of Bradford (1976).

Glyoxylate carboligase activity was tested using the linked glyoxylate carboligase/tartronate semialdehyde reductase assay described by Cusa *et al*. (1999). Briefly, 2 mL reactions, containing 100 mM sodium phosphate (pH 8.0), 5 mM $MgCl_2$, 0.28 mM NADH, 0.5 mM thiamine pyrophosphate, and 200 µg of protein from cell-free extracts, were prepared in triplicate in 33 mL screw-top glass vials with Mininert caps. The headspace

was purged for 5 min with N_2, glyoxylate was added to 10 mM, and vials were incubated with shaking at 30°C for 15.5 h. Reactions were stopped and CO_2 liberated by the addition of 0.2 mL of 10 N sulfuric acid and shaking for 15 min. CO_2 was analyzed by gas chromatography-pulsed discharge detection (GC-PDD) and glyoxylate was analyzed by HPLC.

For assays with cell-free extracts from *R. jostii* RHA1 carrying pTip plasmid constructs, the assay vials were prepared as above for the following treatments: 1) pTip-Psed_3889 (8 µg of protein); 2) pTip-Psed_3890 (7 µg of protein); 3) pTip-Psed_3889-Psed_3890 (8 µg and 7 µg of protein of each, respectively); 4) pTip-QC2 (10 µg of protein); 5) Buffer control. CO_2 production was determined by GC-PDD, and glyoxylate consumption and glycerate production were determined by HPLC.

4.2.7. Growth with 1,4-[U-^{13}C]dioxane.

A series of strain CB1190 cultures was prepared with 1,4-[U-^{13}C]dioxane (99% pure; Sigma-Aldrich). Single serum bottles (160 mL; Wheaton Science Products, Millville, NJ) with black butyl rubber stoppers and containing 30 mL AMS medium were amended with 1.6 mM 1,4-[U-^{13}C]dioxane or 1.6 mM 1,4-[U-^{13}C]dioxane plus 3% CO_2. These bottles were inoculated with 0.1 mL (1:300 dilution) $^{12}C_4$-dioxane-grown strain CB1190 culture and were incubated at 30°C with shaking at 150 rpm. Following complete removal of the 1,4-dioxane, cells (0.1 mL) from each treatment were transferred to fresh triplicate bottles with the same amendments and were incubated with shaking until 1,4-dioxane removal was complete. Cells were then harvested by filtration, scraped from the filter, and stored at -80°C in 2 mL screw-top vials until isotopomer amino acid analysis.

4.2.8. Analytical methods.

Pyruvate, glycolate and glyoxylate consumption were monitored with a Waters (Milford, MA) model 2695 HPLC equipped with a model 2996 photodiode array detector and an Amidex HPX-87H column (Bio-Rad, Hercules, CA). Samples (50 µL) were analyzed isocratically with 5 mM H_2SO_4 at a flow rate of 0.6 mL/min, and the column was maintained at 30°C. 1,4-dioxane consumption was monitored by direct injection of 5 µL samples onto a Varian 3400 GC equipped with a FID detector and a 1% AT-1000 on carbograph1 packed column (Grace, Columbia, MD). Samples were analyzed isothermally at 170°C, and the injector and detector temperatures were 230°C and 250°C, respectively. CO_2 production in cell-free extracts was determined by injecting headspace samples (300 µL) onto a HP 5890 series II GC equipped with a TCD detector and a CTR1 column (Alltech Co.). The temperatures of oven, injector, and detector were maintained at 25, 25, and 30°C, respectively. The flow rate of helium carrier gas was set at 130 mL/min.

The preparation and isotopomeric analysis of proteinogenic amino acids were performed as previously described (Pingitore *et al.*, 2007; Tang *et al.*, 2007). In brief, biomass was hydrolyzed in 6 M HCl at 100°C for 24 h. During the hydrolysis, tryptophan and cysteine were degraded, while asparagine and glutamine were converted into aspartate and glutamate respectively. The amino acid solution was dried under air flush overnight. Amino acid samples were derivatized in tetrahydrofuran (THF) and N-(tert-butyl dimethylsilyl)-N-methyl-trifluoroacetamide (Sigma-Aldrich, St. Louis, MO) at 70°C for 1 h. A GC (Hewlett-Packard, model 6890, Agilent technologies, Palo Alto, CA) equipped with a DB5-MS column (J&W scientific, Folsom, CA) and a mass spectrometer (MS) (5975, Agilent technologies, Palo Alto, CA) was used for isotopomer analysis. Two types of charged fragments were clearly detected by GC-MS for the derivatized amino acids: the [M-57]+ which contains the entire amino acid; and the [M-159]+, which contains the amino acid without the 1st carbon (α carboxyl group). The [M-57]+ peaks in leucine, isoleucine and proline overlap with other peaks, so the [M-159]+ group were used to obtain the isotopomer labeling information of those amino acids. The final isotopomer labeling fractions were indicated as: M0 (unlabeled fraction), M1 (single labeled carbon fraction), M2 (fraction with two labeled carbons), M3 (fraction with three labeled carbons), and so forth.

4.2.9. Accession numbers.

Details of the microarray design, transcriptomic experimental design, and transcriptomic data have been deposited in the NCBI Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO Series accession number GSE33197.

Table 4.1. SYBR Green and TaqMan primers used in qRT-PCR analyses [a,b]

Target Gene ID	Gene product	Sense primer sequence	Antisense primer sequence	Probe sequence
Psed_0629	Methane monooxygenase	GAACCCGGAGCTGCACAA	TTCAGGTTCTGCTGGATCGT	-
Psed_0768	Phenol 2-monooxygenase	GCTCTTCGTGCCCTTCATGA	GAGAACCCGAACGACATCGT	-
Psed_0815	Methane/phenol/toluene hydroxylase	GGTGGAAGGACCGTGACAAC	GGCCAGGAAGCGATCGAT	-
Psed_3051	RNA polymerase sigma factor RpoD	TGATCCAGGAGGGCAACCT	TAGCCCTTGGTGTAGTCGAACTT	(TET)CTGATCCGTGCGGTC(TAMRA)
Psed_3417	Triosephosphate isomerase	CCGGCAACTGGAAGATGAAC	GGCGAGCTTCTGCAGGAA	(VIC)CACCTCGAGGCCATC(TAMRA)
Psed_3888	Hydropyruvate isomerase	CTTCCTGTTCGACCTGTTCCA	TGCACGTGGGCGATCAC	-
Psed_3889	Tartronate semialdehyde reductase	ACATGCTGAACCGGGACTTC	CTTCATGTCCTTGTGGTGCAA	-
Psed_3890	Glyoxylate carboligase	GCGCACCACGCACTACGACC	CGGCCGAAGACCCGGTTCATC	-
Psed_3891	Glycerate kinase	GATCGAGCAGGCCTACGC	TGCGAGCTCCTCCAGCAG	-
Psed_4782	Malate synthase	CGAGCAGACGGTCCGGGAGA	GTCGCTCATCGGCCGGTAGC	-
Psed_4783	Glycolate oxidase	GGCCGCATCTCCCCCGACTA	CTCGCCCATGTCCGCGATCC	-
Psed_4788	Glycolate oxidase	CCGACCTCGCGACGATGACG	GAGGGGTGGGCAGCAGCTTG	-
Psed_6168	Thiamine biosynthesis protein C	TGAAGGTCAACGCCAACATC	TGGCCCACACCAGTTTCTC	(FAM)CGTCGATCGAGGACG(TAMRA)
Psed_6782	Alcohol dehydrogenase	GCAGGTCGAGTCAAGGACTTTC	GCCGTGCGGCACCAT	-
Psed_6783	Fatty-acid-coA ligase	CTCTAGTGGACGAGGAGGAACTG	TCGCTTAGGCACCTTGTAACC	-
Psed_6970	FAD/FMN-dependent dehydrogenase	GAGTTGACTTCGATCTGCTTACGA	GGCCGCATCAACCATGAA	-
Psed_6971	Alcohol dehydrogenase	TTGCTGGGATGGTGAAGGA	CCATGAGGCACCATTGCTT	-
Psed_6975	Aldehyde dehydrogenase	TGACATCGAGCAGGCTGTTG	CAGGTCTGCCCGTTGTTGT	-
Psed_6976	Monooxygenase oxidoreductase domain	GGCTGGGACCGGACGTA	TTGTTCGGCAGCTTGTATCG	(TET)TACCCCCATGAGCGCGGGAA(TAMRA)
Psed_6981	Aldehyde dehydrogenase	GGAGCGCCGGTTATTATCAA	TGACCCAAGAACAGCGATGA	-

[a] All primers sets were annealed at 60°C, except for Psed_4783, which was annealed at 65°C

[b] Except for where probe sequences are listed, all PCR reactions were performed with SYBR-Green chemistry

Table 4.2 Homologous genes potentially encoding steps in the 1,4- dioxane metabolic pathway.

Enzymatic Activity	Substrate	Product	Homologues[a]
Hydroxylase	1,4-Dioxane	2-Hydroxy-1,4,-dioxane	Psed_0629-0632
	2-Hydroxyethoxy-acetic acid	1,2-Dihydroethoxy-acetic acid	Psed_0766-0770
			Psed_0812-0815
			Psed_1155-1159
			Psed_1436-1439
			Psed_6058-6062
			Psed-6066-6067
			Psed_6977-6980
Secondary alcohol dehydrogenase	2-Hydroxy-1,4,-dioxane	1,4-Dioxane-2-one	Psed_0131
			Psed_2070
			Psed_4156
Aldehyde dehydrogenase	2-Hydroxy-ethoxyacetaldehyde	2-Hydroxyethoxy-acetic acid	Psed_1151
			Psed_1385
	Glycoaldehyde	Glycolate	Psed_1709
			Psed_1800
			Psed_1899
			Psed_2161
			Psed_2162
			Psed_2510
			Psed_2977
			Psed_3350
			Psed_6458
			Psed_6975
			Psed_6981
Aldehyde reductase	Glyoxal	Glycoaldehyde	Psed_2147
			Psed_5591
			Psed_6971
Alcohol oxidoreductase	Ethylene glycol	Glycoaldehyde	Psed_0301
			Psed_6782
			Psed_6971
Glycolate oxidase	Glycolate	Glyoxylate	Psed_4783-4785
			Psed_4788-4790
Malate synthase	Glyoxylate + AcetylCoA	Malate	**Psed_4782**
Glyoxylate carboligase	Glyoxylate	Tatronate semialdehyde	**Psed_3890**

[a] Genes in bold were upregulated during growth with dioxane relative to growth with pyruvate

Table 4.3. CB1190 genes up-regulated only on 1,4-dioxane, relative to pyruvate.

Gene	Protein	log_2FC Dioxane vs. Pyruvate	log_2FC Glycolate vs. Pyruvate
Psed_0038	regulatory protein ArsR	1.22	-1.31
Psed_1076	short-chain dehydrogenase/reductase SDR	1.35	0.79
Psed_1594	protein of unknown function UPF0016	1.19	0.24
Psed_1658	Trimethylamine-N-oxide reductase (cytochrome c)	2.43	-0.74
Psed_2752	hypothetical protein	1.12	0.77
Psed_3041	NADH dehydrogenase (ubiquinone) 24 kDa subunit	1.07	0.08
Psed_3564	Aromatic-amino-acid transaminase	1.55	0.32
Psed_3653	hypothetical protein	1.51	-0.02
Psed_3934	major facilitator superfamily MFS_1	1.22	0.55
Psed_3935	GntR domain protein	1.46	0.22
Psed_4146	Potassium-transporting ATPase B chain	1.58	0.10
Psed_4147	Potassium-transporting ATPase A chain	1.27	0.10
Psed_4148	K+-transporting ATPase, F subunit	1.81	0.35
Psed_4149	hypothetical protein	1.14	0.36
Psed_4306	hypothetical protein	1.05	1.00
Psed_4424	hypothetical protein	1.42	0.44
Psed_5135	malic protein NAD-binding	1.40	0.47
Psed_5524	hydrolase	1.08	0.35
Psed_5938	glycoside hydrolase family 13 domain-containing protein	1.21	0.54
Psed_6241	hypothetical protein	1.05	0.07
Psed_6259	FMN-dependent oxidoreductase, nitrilotriacetate monooxygenase family	1.51	0.21
Psed_6261	ABC-type transporter, integral membrane subunit	1.31	-0.01
Psed_6262	ABC-type transporter, integral membrane subunit	1.20	0.00
Psed_6263	Extracellular ligand-binding receptor	1.21	-0.17
Psed_6728	Amidase	1.37	0.07
Psed_6729	hypothetical protein	1.91	0.03
Psed_6730	Luciferase-like, subgroup	1.63	-0.39
Psed_6732	MaoC domain protein dehydratase	1.19	0.00
Psed_6740	hypothetical protein	1.12	0.68
Psed_6742	NLP/P60 protein	1.50	0.08
Psed_6743	hypothetical protein	2.52	-0.67
Psed_6744	hypothetical protein	1.97	-0.24
Psed_6745	ATP-binding protein	1.91	-0.15

Psed_6746	hypothetical protein	2.60	-0.78
Psed_6747	hypothetical protein	1.92	-0.54
Psed_6748	hypothetical protein	2.28	-0.71
Psed_6749	hypothetical protein	3.05	-0.73
Psed_6750	hypothetical protein	2.09	-0.25
Psed_6751	Transglycosylase-like domain protein	2.33	-0.66
Psed_6752	hypothetical protein	2.15	-0.29
Psed_6753	hypothetical protein	1.77	-0.06
Psed_6754	hypothetical protein	1.86	-0.38
Psed_6755	hypothetical protein	1.83	-0.13
Psed_6757	hypothetical protein	1.03	-0.64
Psed_6758	hypothetical protein	1.17	0.00
Psed_6888	C-5 cytosine-specific DNA methylase	1.17	-0.22
Psed_6913	hypothetical protein	1.27	-0.01
Psed_6970	D-lactate dehydrogenase (cytochrome)	2.80	-0.60
Psed_6971	Hydroxyacid-oxoacid transhydrogenase	2.47	-0.55
Psed_6972	GntR domain protein	1.93	-0.70
Psed_6973	hypothetical protein	1.53	-0.56
Psed_6974	Ethyl tert-butyl ether degradation EthD	1.85	-0.47
Psed_6975	Betaine-aldehyde dehydrogenase	1.47	-0.41
Psed_6977	Ferredoxin--NAD(+) reductase	2.26	-0.44
Psed_6978	methane/phenol/toluene hydroxylase	1.60	-0.29
Psed_6979	monooxygenase component MmoB/DmpM	1.98	-0.38
Psed_6980	hypothetical protein	2.60	-0.61
Psed_6981	Aldehyde Dehydrogenase	3.38	-0.33
Psed_6982	Mn2+/Fe2+ transporter, NRAMP family	3.87	-0.08
Psed_7002	transcription factor WhiB	1.51	-0.58
Psed_7003	hypothetical protein	1.48	-0.10
Psed_7007	hypothetical protein	1.40	0.18
Psed_7008	hypothetical protein	1.20	-0.09

Table 4.4. Strain CB1190 genes up-regulated on both 1,4-dioxane and glycolate, relative to pyruvate.

Gene	Protein	log_2FC Dioxane vs. Pyruvate	log_2FC Glycolate vs. Pyruvate
Psed_3889	2-hydroxy-3-oxopropionate reductase	7.06	7.02
Psed_3888	Hydroxypyruvate isomerase	6.99	6.89
Psed_3890	glyoxylate carboligase	6.75	6.83
Psed_3891	glycerate kinase	5.64	5.81
Psed_4788	D-lactate dehydrogenase (cytochrome)	5.50	5.46
Psed_4790	protein of unknown function DUF224 cysteine-rich region domain protein	4.84	4.88
Psed_4789	FAD linked oxidase domain protein	4.74	4.61
Psed_0350	Bile acid:sodium symporter	1.60	3.74
Psed_6787	Formyl-CoA transferase	3.89	3.68
Psed_5371	hypothetical protein	3.34	3.66
Psed_1302	protein of unknown function DUF156	1.85	3.53
Psed_4782	Malate synthase	3.38	3.51
Psed_4755	ammonium transporter	1.14	3.29
Psed_1304	heavy metal translocating P-type ATPase	1.38	3.23
Psed_6780	hypothetical protein	3.18	3.12
Psed_5736	Transglycosylase-like domain protein	1.41	2.98
Psed_1303	Heavy metal transport/detoxification protein	1.43	2.95
Psed_6779	Integrase catalytic region	2.90	2.44
Psed_6782	Hydroxyacid-oxoacid transhydrogenase	2.33	2.24
Psed_6175	regulatory protein TetR	1.73	1.98
Psed_6784	Alkylglycerone-phosphate synthase	1.98	1.96
Psed_3522	response regulator receiver	1.74	1.93
Psed_4577	Cold-shock protein DNA-binding	1.20	1.93
Psed_1584	6-phosphofructokinase	1.76	1.85
Psed_5025	4-hydroxyacetophenone monooxygenase	2.42	1.82
Psed_6600	acyl-CoA dehydrogenase domain-containing protein	2.00	1.70
Psed_3918	FAD linked oxidase domain protein	1.37	1.55
Psed_4512	ABC-type transporter, integral membrane subunit	1.25	1.47
Psed_2371	ABC-type transporter, periplasmic subunit	1.05	1.47
Psed_5026	regulatory protein TetR	1.19	1.41
Psed_2030	Linalool 8-monooxygenase	1.09	1.29
Psed_1555	peptidase S1 and S6 chymotrypsin/Hap	1.51	1.27
Psed_4513	cobalamin (vitamin B12) biosynthesis CbiX protein	1.24	1.25

Psed_6791	IstB domain protein ATP-binding protein	1.79	1.24
Psed_5404	NAD(P)(+) transhydrogenase (AB-specific)	1.02	1.18
Psed_5405	nicotinamide nucleotide transhydrogenase alpha subunit 2 PntAB	1.04	1.10
Psed_5406	NAD(P)(+) transhydrogenase (AB-specific)	1.06	1.09

Table 4.5. Normalized relative expression levels for selected strain CB1190 genes analyzed by qRT-PCR

	Gene name	Gene product	Gene ID	Replicon[a]	Expression ratio[b] Dioxane/ Pyruvate	Expression ratio[b] Glycolate/ Pyruvate
Monooxygenase genes lacking probes on microarray	***thmA***	**THF monooxygenase a-subunit**	**Psed_6976**	**p2**	**15 ± 0.2**	**1.0 ± 0.5**
	prmA	Methane monooxygenase	Psed_0629	c	0.4 ± 0.1	0.8 ± 0.5
		Phenol 2-monooxygenase	Psed_0768	c	2 ± 2	0.9 ± 0.4
		Methane/phenol/toluene hydroxylase	Psed_0815	c	1.1 ± 0.5	0.3 + 0.4
Dioxane metabolism intermediates-related genes		Alcohol dehydrogenase	Psed_6782	p1	7.3 ± 0.8	3.3 ± 0.4
		Fatty-acid-CoA ligase	Psed_6783	p1	24 ± 2	7 ± 5
		FAD/FMN-dependent dehydrogenase	**Psed_6970**	**p2**	**6.1 ± 0.8**	**1.5 ± 0.9**
		Alcohol dehydrogenase	**Psed_6971**	**p2**	**8.7 ± 0.8**	**1.3 ± 0.4**
		Aldehyde dehydrogenase	**Psed_6975**	**p2**	**23 ± 2**	**0.9 ± 0.3**
		Aldehyde dehydrogenase	**Psed_6981**	**p2**	**11 ± 1**	**1.5 ± 0.9**
Glycolate and glyoxylate metabolism-related genes	*glcD1*	Glycolate oxidase	Psed_4783	c	0.3 ± 0.1	0.7 ± 0.5
	glcD2	Glycolate oxidase	Psed_4788	c	3.6 ± 0.3	2.8 ± 0.5
	glcB	Malate synthase G	Psed_4782	c	5.4 ± 0.2	2 ± 1
	hyi	Hydroxypyruvate isomerase	Psed_3888	c	5 ± 1	3.1 ± 0.3
	glxR	Tartronate semialdehyde reductase	Psed_3889	c	13 ± 1	6 ± 2
	gcl	Glyoxylate carboligase	Psed_3890	c	18 ± 2	10 ± 4
	glxK	Glycerate kinase	Psed_3891	c	1.9 ± 0.6	1.9 ± 0.7

[a] c = chromosome; p1 = plasmid pPSED01; p2 = plasmid pPSED02

[b] Genes up-regulated with dioxane but not with glycolate are indicated in **bold**

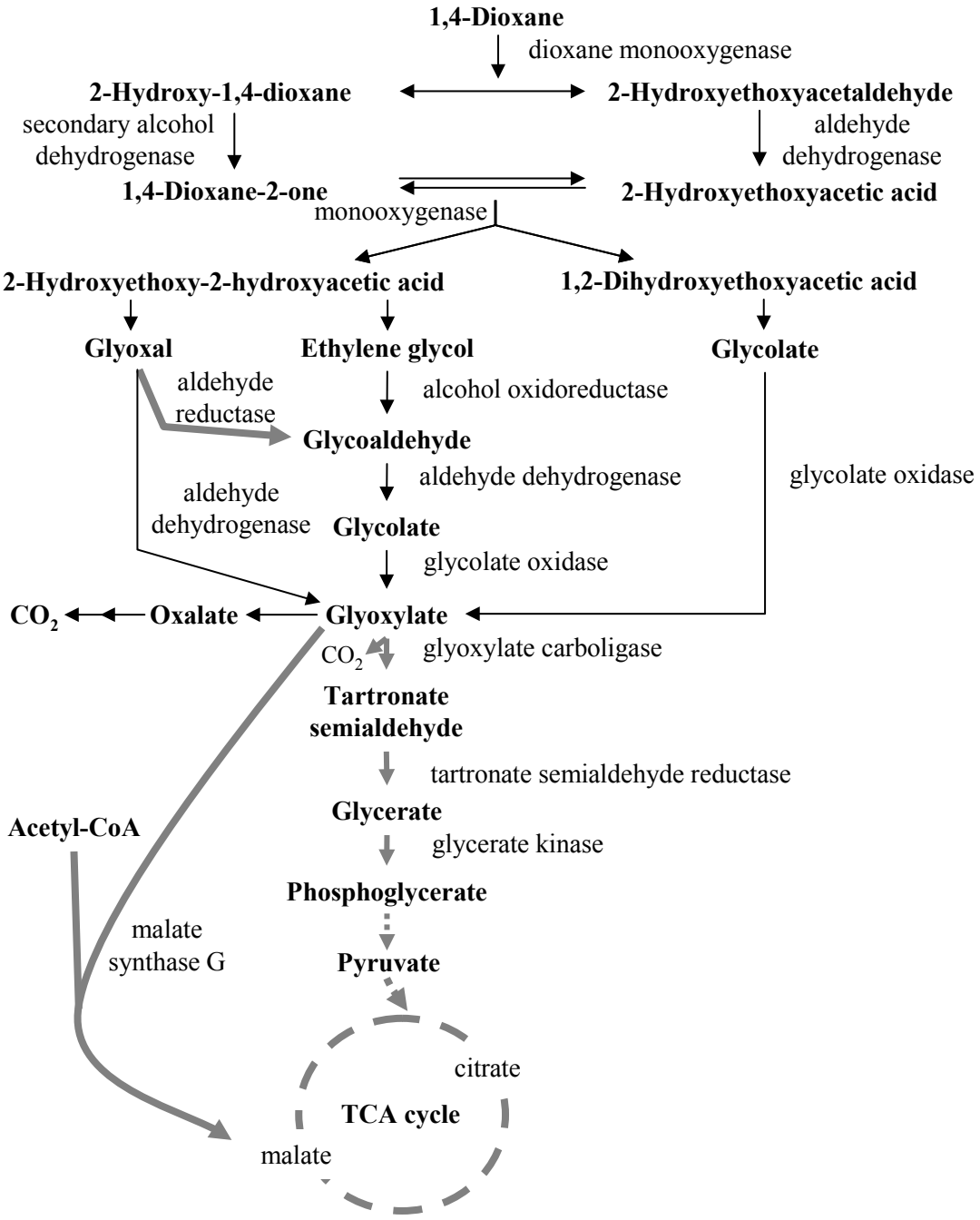


Figure 4.1. Proposed pathways and genes involved in 1,4-dioxane metabolism by strain CB1190. Black arrows indicate the transformations previously proposed by Mahendra *et al.* (2007). Grey arrows indicate transformations supported by *in silico* or experimental results in the current work. Dashed arrows indicate multi-step transformations.

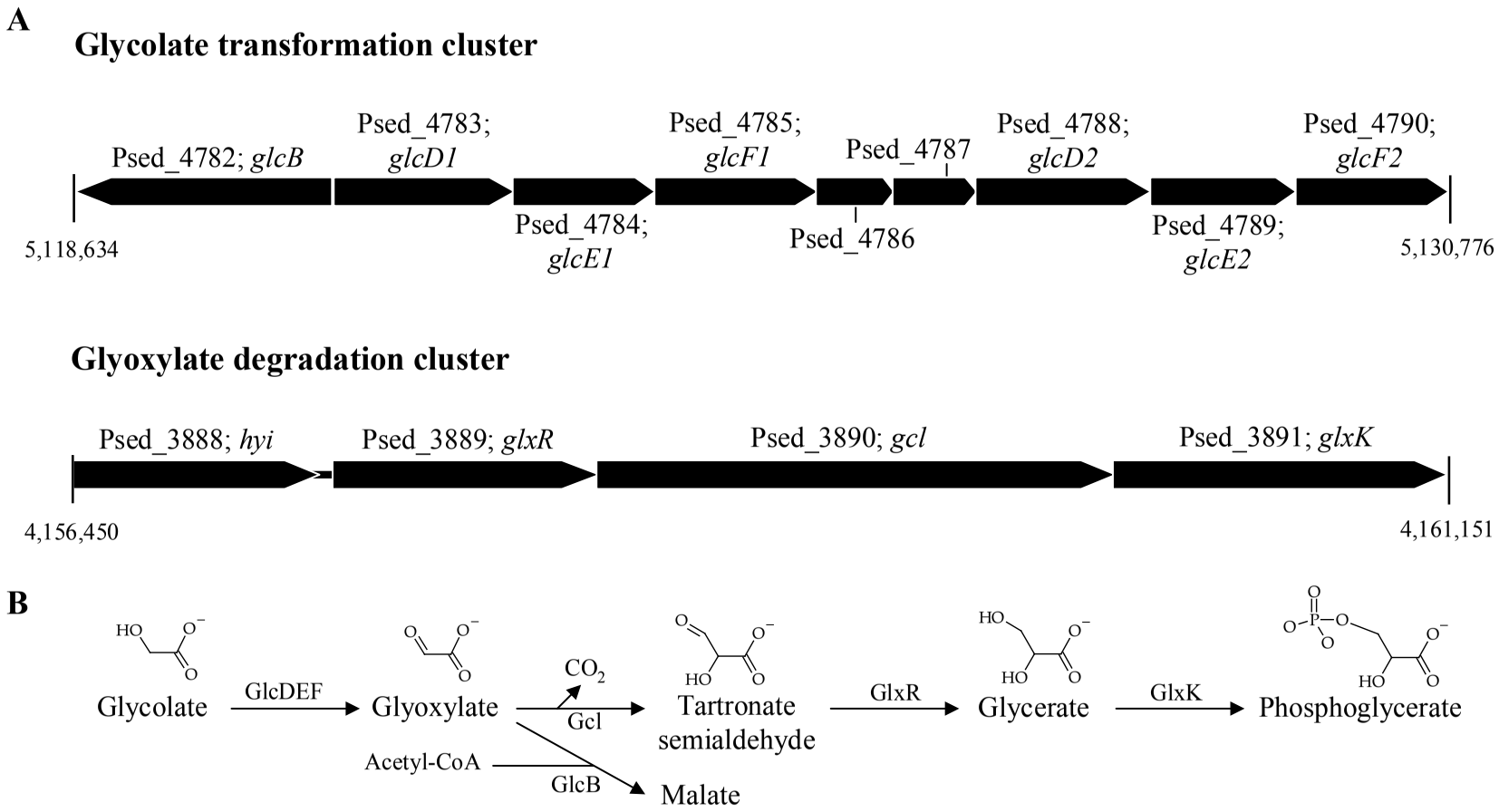


Figure 4.2. Strain CB1190 chromosomal regions of interest implicated in glycolate and glyoxylate transformations during dioxane metabolism. A) Gene clusters implicated in glycolate and glyoxylate metabolism. B) Proposed transformations of glycolate and glyoxylate catalyzed by enzymes putatively encoded by *P. dioxanivorans* gene clusters.

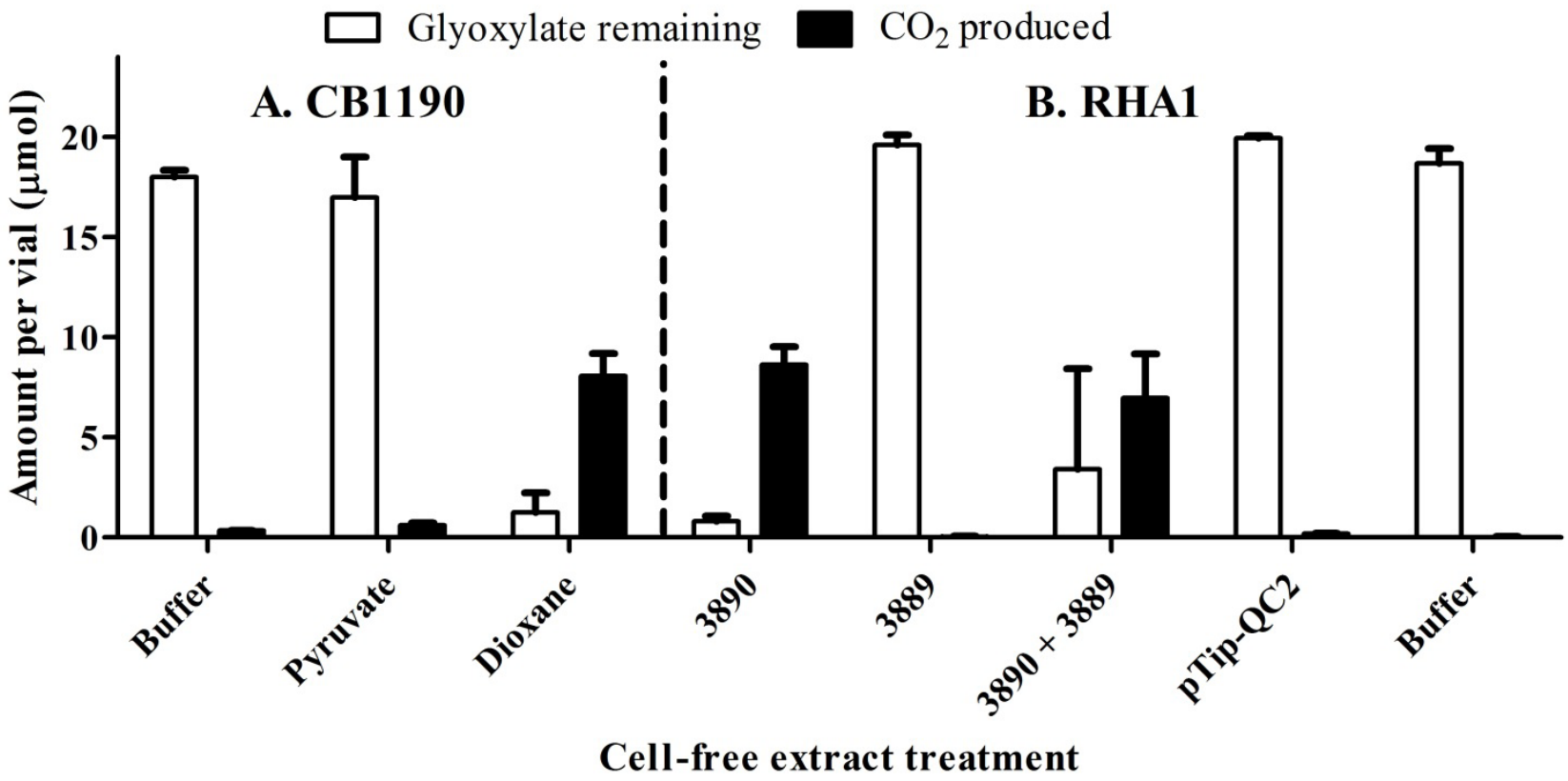


Figure 4.3. Glyoxylate carboligase activity in strain CB1190 and strain RHA 1 pTip cell-free extracts. A) Buffer or cell-free extracts from pyruvate- or dioxane-grown strain CB1190 cells were amended with 10 mM glyoxylate and then glyoxylate remaining and CO_2 production were determined after 15.5 h incubation. **B)** The same assay was performed with cell-free extracts prepared from strain RHA1 cells with pTip-Psed_3890 (3890; putative glyoxylate carboligase gene), pTip-3889 (3889; putative tartronate semialdehyde reductase gene), a mix of the two, pTip-QC2 (empty vector), or buffer.

Table 4.6. Stable isotopic lebeling profile of proteogenic amino acids from strain CB1190 cells grown with 1,4-[U-^{13}C]dioxane and atmospheric CO_2.

Amino acids	Precursors	Ions	$[M-57]^+$	$[M-159]^+$
Alanine	Pyruvate	M0	0.00	0.00
		M1	0.00	0.03
		M2	0.03	0.97
		M3	0.96	
Valine	Pyruvate	M0	0.00	0.00
		M1	0.00	0.00
		M2	0.00	0.00
		M3	0.00	0.04
		M4	0.04	0.94
		M5	0.94	
Leucine	Pyruvate	M0	0.00	0.00
	Acetyl-CoA	M1	0.00	0.00
		M2	0.02	0.00
		M3	0.06	0.01
		M4	0.03	0.06
		M5	0.13	0.93
		M6	0.77	
Isoleucine	Pyruvate	M0	0.00	0.00
	Threonine	M1	0.00	0.00
		M2	0.00	0.00
		M3	0.04	0.02
		M4	0.04	0.09
		M5	0.11	0.88
		M6	0.80	
Aspartate	Oxaloacetate	M0	0.00	0.00
(Asparagine)		M1	0.00	0.01
		M2	0.02	0.06
		M3	0.08	0.92
		M4	0.90	
Threonine	Aspartate	M0	0.00	0.00
		M1	0.00	0.03
		M2	0.02	0.08
		M3	0.09	0.89
		M4	0.89	
Serine	Glycerate-3P	M0	0.00	0.01
		M1	0.00	0.03
		M2	0.04	0.96

		M3	0.95	
Glycine	Glycerate-3P	M0	0.01	0.02
		M1	0.03	0.98
		M2	0.95	
Phenylalanine	Phosphoenol-pyruvate	M0	0.00	0.00
		M1	0.00	0.00
	Erythrose 4-phosphate	M2	0.00	0.00
		M3	0.00	0.00
		M4	0.00	0.00
		M5	0.00	0.00
		M6	0.00	0.00
		M7	0.00	0.06
		M8	0.06	0.92
		M9	0.92	
Tyrosine	Phosphoenol-pyruvate	M0	0.00	0.00
		M1	0.00	0.00
	Erythrose 4-phosphate	M2	0.00	0.00
		M3	0.00	0.00
		M4	0.00	0.00
		M5	0.00	0.00
		M6	0.00	0.02
		M7	0.00	0.07
		M8	0.06	0.90
		M9	0.93	
Glutamate	2-Ketoglutarate	M0	0.00	0.00
(Glutamine)		M1	0.00	0.00
		M2	0.00	0.02
		M3	0.02	0.05
		M4	0.08	0.91
		M5	0.88	

Table 4.7 . Stable isotopic labeling profiles of proteogenic amino acids from strain CB1190 cells grown with 1,4-[U-13C]dioxane and elevated CO_2

Amino acids	Precursors	Ions	$[M-57]^+$	$[M-159]^+$
Alanine	Pyruvate	M0	0.00	0.00
		M1	0.00	0.03
		M2	0.06	0.97
		M3	0.93	
Valine	Pyruvate	M0	0.00	0.00
		M1	0.00	0.00
		M2	0.00	0.00
		M3	0.00	0.05
		M4	0.06	0.94
		M5	0.93	
Leucine	Pyruvate	M0	0.00	0.00
	Acetyl-CoA	M1	0.00	0.00
		M2	0.00	0.00
		M3	0.04	0.00
		M4	0.02	0.06
		M5	0.12	0.93
		M6	0.81	
Isoleucine	Pyruvate	M0	0.00	0.00
	Threonine	M1	0.00	0.00
		M2	0.00	0.00
		M3	0.02	0.02
		M4	0.03	0.22
		M5	0.27	0.75
		M6	0.67	
Lysine	Pyruvate	M0	0.00	0.00
	Aspartate	M1	0.00	0.00
		M2	0.00	0.02
		M3	0.00	0.02
		M4	0.04	0.22
		M5	0.28	0.72
		M6	0.66	
Aspartate	Oxaloacetate	M0	0.00	0.00
(Asparagine)		M1	0.00	0.01
		M2	0.04	0.22
		M3	0.28	0.76
		M4	0.67	
Methionine	Aspartate	M0	0.00	0.02

(weak peak)	Methyl-THF	M1	0.00	0.02
		M2	0.00	0.04
		M3	0.07	0.23
		M4	0.29	0.70
		M5	0.64	
Threonine	Aspartate	M0	0.00	0.00
		M1	0.00	0.02
		M2	0.03	0.21
		M3	0.28	0.77
		M4	0.68	
Serine	Glycerate-3P	M0	0.00	0.00
		M1	0.00	0.02
		M2	0.16	0.97
		M3	0.82	
Glycine	Glycerate-3P	M0	0.01	0.02
		M1	0.23	0.98
		M2	0.76	
Phenylalanine	Phosphoenol-pyruvate	M0	0.00	0.00
		M1	0.00	0.00
	Erythrose 4-phosphate	M2	0.00	0.00
		M3	0.00	0.00
		M4	0.00	0.00
		M5	0.00	0.00
		M6	0.00	0.00
		M7	0.00	0.07
		M8	0.08	0.91
		M9	0.90	
Tyrosine	Phosphoenol-pyruvate	M0	0.00	0.00
		M1	0.00	0.00
	Erythrose 4-phosphate	M2	0.00	0.00
		M3	0.00	0.00
		M4	0.00	0.00
		M5	0.00	0.00
		M6	0.00	0.02
		M7	0.00	0.08
		M8	0.08	0.89
		M9	0.89	
Histidine	Phosphoribosylpyrophosphate	M0	0.00	0.00
		M1	0.00	0.00
		M2	0.15	0.32

		M3	0.17	0.78
		M4	0.72	0.00
		M5	0.00	0.00
		M6	0.01	
Glutamate (Glutamine)	2-Ketoglutarate	M0	0.00	0.00
		M1	0.00	0.00
		M2	0.00	0.03
		M3	0.04	0.07
		M4	0.25	0.90
		M5	0.70	

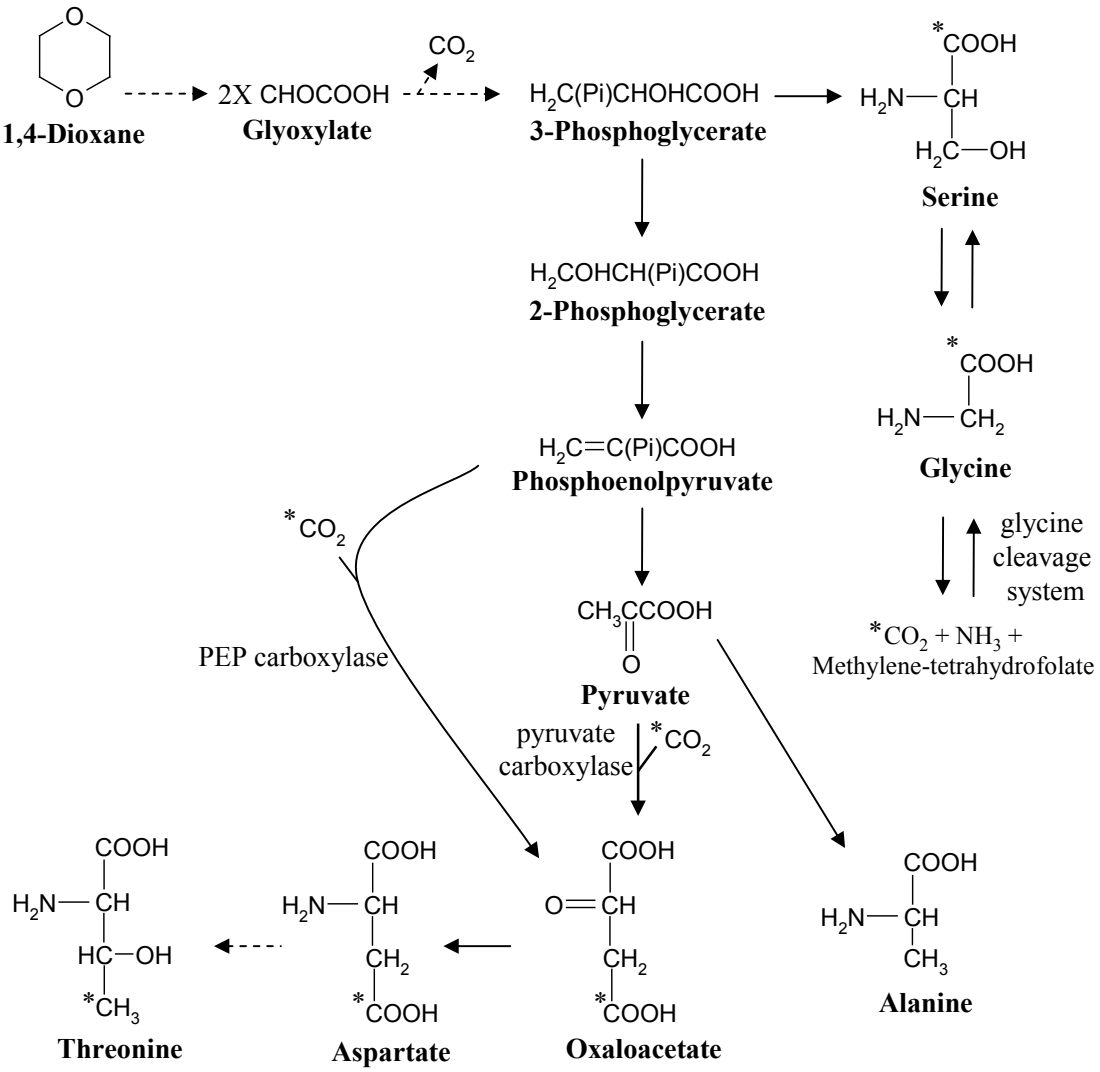


Figure 4.4. Proposed assimilation of unlabeled carbon during growth of strain CB1190 with uniformly ^{13}C-labeled dioxane. Unlabeled carbons are indicated with an asterisk (*); all other carbons are considered ^{13}C labeled. The dashed arrow indicates a multi-step reaction. The amino acids valine, phenylalanine and tyrosine were omitted for simplicity.

Table 4.8. Analysis of energy generation from 1,4-dioxane metabolite transformations in strain CB1190.

Substrate	Product	Net NAD(P)H	Net ATP	Net GTP
1,4-dioxane	hydroxyethoxyacetaldehyde	-1		
hydroxyethoxyacetaldehyde	hydroxyethoxyacetic acid	1		
hydroxyethoxyacetic acid	dihydroxyethoxyacetic acid	-1		
glyoxal	glycoaldehyde	-1		
ethylene glycol	glycoaldehyde	1		
2 glycoaldehyde[a]	2 glycolate	2		
2 glycolate	2 glyoxylate	2		
2 glyoxylate	tartronate semialdehyde			
tartronate semialdehyde	glycerate	-1		
glycerate	phosphoglycerate		-1	
phosphoglycerate	PEP			
PEP	pyruvate		1	
pyruvate	TCA cycle	4		1
Net		6	0	1
Total ATP (mol/mol dioxane)[b]		19		

[a] Assuming that glyoxal is transformed to glycoaldehyde

[b] Assuming that GTP is equivalent to ATP

4.3. Results and Discussion.

4.3.1. *In silico* analysis of 1,4-dioxane degradation pathway genes.

The pathway for 1,4-dioxane degradation that was proposed by Mahendra *et al*. (2007) (Figure 4.1, black arrows) was the starting point for this study. A literature search for descriptions of enzymatic activities catalyzing the proposed 1,4-dioxane metabolic transformations was performed, and the amino acid sequence of those enzymes were used to identify homologues in the strain CB1190 genome sequence (Table 4.2) using blastp (Altschul *et al*., 1994).

1,4-Dioxane degradation is initiated by the activity of a monooxygenase (Mahendra and Alvarez-Cohen, 2006; Mahendra *et al*., 2007; Vainberg *et al*., 2006). The CB1190 genome carries eight bacterial multi-component monooxygenase gene clusters, seven of which are encoded on the chromosome and one that is on plasmid pPSED02 (Chapter 3). The transformation of the hydroxylated 1,4-dioxane metabolite is proposed to involve spontaneous ether cleavage and/or aldehyde/carboxylic acid and/or secondary alcohol/ketone conversions (Mahendra *et al*., 2007). Strain CB1190 has 13 genes encoding proteins with at least 30% amino acid identity with characterized bacterial aldehyde dehydrogenases (*E. coli* AldA [accession NP_415933], *E. coli* AldB [accession AAC76612], *Rhodococcus erythropolis* AldH [accession AAZ14956]), which catalyze the NAD(P)+-dependent oxidation of aldehydes to carboxylic acids. Eleven of these genes are located on the chromosome, while the remaining two are on plasmid pPSED02 (Table 4.2). Three genes encoding proteins with 29-36% amino acid identity to a secondary alcohol dehydrogenase (accession CAD36475) of the actinomycete *Rhodococcus ruber* are on the chromosome; this enzyme catalyzes the NAD+-dependent transformation of secondary alcohols to ketones. The second proposed hydroxylation reaction generating HEAA or 1,2-dihydroxyethoxyacetic acid has also been postulated to be catalyzed by a monooxygenase (Vainberg *et al*., 2006; Mahendra *et al*., 2007).

The subsequent cleavage of the second ether bond leads to the production of two-carbon intermediates, of which glyoxal, ethylene glycol, glycoaldehyde, glycolate, glyoxylate and oxalate have been identified (Mahendra *et al.*, 2007). The strain CB1190 genome has three genes encoding proteins with 27-34% amino acid identity to *E. coli* 1,2-propanediol oxidoreductase (accession AP_003365), which transforms ethylene glycol to glycoaldehyde (Conway and Ingram., 1989). Glycoaldehyde is transformed to glycolate by aldehyde dehydrogenases (see above), while glycolate is converted to glyoxylate by glycolate oxidase (Kornberg and Elsden, 1961). In *E. coli*, glycolate oxidase is encoded by *glcDEF* (Pellicer *et al*., 1996); strain CB1190 has two adjacent homologues, *glcD1E1F1* and *glcD2E2F2* (Figure 4.2). These homologues share 72%, 47%, and 64% amino acid identity for *GlcD*, *GlcE* and *GlcF*, respectively.

Mahendra *et al*. (2007) proposed that glyoxal is transformed to glyoxylate, and although

this enzymatic activity has not to date been explicitly described in the literature, the numerous aldehyde dehydrogenases encoded in the CB1190 genome could potentially catalyze this reaction. Alternatively, aldehyde reductase homologues (*E. coli* YqhD [accession Q46856], *Bacillus subtilis* YvgN [accession O32210]) that catalyze the transformation of glyoxal to glycoaldehyde were identified. Furthermore, in *E. coli,* the glyoxalase I/II system, encoded by the genes *gloA* and *gloB*, uses (*S)*-glutathione to transform glyoxal to glycolate (Thornalley, 1998); however, no homologous genes were identified in the CB1190 genome.

Since glyoxylate has been identified as a metabolite of 1,4-dioxane (Mahendra *et al.*, 2007; Nakamiya *et al.*, 2005}, the CB1190 genome was examined for genes involved in known glyoxylate assimilation pathways. Strain CB1190 has a cluster of genes (Figure 4.2) homologous to those encoding the glyoxylate carboligase pathway that converts glyoxylate through tartronate semialdehyde and glycerate to phosphoglycerate, in order to provide both carbon and energy for growth in *E. coli* (Krakow *et al.*, 1961). Strain CB1190 has the key glyoxylate shunt pathway gene *glcB* encoding malate synthase, which assimilates glyoxylate directly into the TCA cycle via malate (Kornberg and Elsden, 1961), and an isocitrate lyase gene (Psed_4635), which encodes for the enzyme that transforms carbon from isocitrate into the glyoxylate shunt pathway.

While the transformation of glyoxylate to oxalate has been reported in *Pseudomonas fluorescens* (Singh *et al.*, 2009), the gene sequence for the enzyme catalyzing this reaction has not yet been determined in bacteria. Finally, aerobic bacterial growth on oxalate was shown to be dependent on the oxalate decarboxylase *OxdC*, which converts oxalate to formate (Tanner and Bornemann, 2000). However, the strain CB1190 genome lacks a homologue for this enzyme.

4.3.2. Gene expression during growth on 1,4-dioxane, glycolate and pyruvate

Gene expression microarrays targeting the strain CB1190 genome were used to determine the genes that are differentially regulated during growth on 1,4-dioxane or glycolate relative to growth on pyruvate. The expression of 383 genes differed significantly; 97 genes were up-regulated with 1,4-dioxane relative to the pyruvate control, whereas 286 genes were down-regulated (Appendix 5). When strain CB1190 was grown with glycolate, the expression of 506 genes differed significantly relative to pyruvate-grown cells, with 203 genes up-regulated and 303 down-regulated (Appendix 6). A comparison of genes differentially regulated with either 1,4-dioxane or glycolate relative to pyruvate identified 38 genes that were up-regulated with both substrates (Table 4.4), whereas 121 genes were down-regulated with both substrates.

The replicon location of the 1,4-dioxane- and glycolate-induced genes was examined. For 1,4-dioxane-grown cells, 27 and 17 genes were induced from plasmids pPSED01 and pPSED02, respectively, with the remaining induced genes on the chromosome. For

glycolate-grown cells, 15 induced genes were from plasmid pPSED01, while the rest were on the chromosome; no genes were induced from plasmid pPSED02. Sixty-four genes were up-regulated with 1,4-dioxane but not with glycolate, relative to pyruvate (Table 4.3). Of these genes, 21 were from plasmid pPSED01, while 17 genes were from plasmid pPSED02. Of the 1,4-dioxane-induced plasmid pPSED01 genes, 14 of these encode hypothetical proteins.

4.3.3. Up-regulation of a multi-component gene cluster during 1,4-dioxane metabolism.

The initial step in bacterial 1,4-dioxane biotransformation is known to involve a monooxygenase, leading to a hydroxylated product that is further transformed through undefined reactions to C2 compounds (Mahendra *et al*., 2007). While the strain CB1190 genome encodes eight monooxygenase gene clusters (Chapter 3), microarray analysis of 1,4-dioxane-induced transcription revealed that only the plasmid pPSED02-encoded monooxygenase cluster was up-regulated when compared with growth on pyruvate (Table 4.3). Up-regulation of this cluster was verified with qRT-PCR using primers targeting Psed_6976, encoding a homologue of the THF monooxygenase a-subunit (Table 4.4). Three chromosomally-encoded monooxygenase gene clusters were missing from the microarray, so their potential involvement in 1,4-dioxane metabolism was tested using qRT-PCR with primers targeting the genes encoding the a-subunits. As Table 4.4 shows, no significant differential transcription was observed for these three monooxygenase genes (Psed_0629, Psed_0768, and Psed_0815) in cDNA from 1,4-dioxane-, glycolate- or pyruvate-grown cells.

The 1,4-dioxane-induced, plasmid pPSED02-endoded monooxygenase gene cluster is homologous to the *thm* gene cluster involved in THF utilization in *Pseudonocardia tetrahydrofuranoxydans* strain K1 (Thiemer *et al*., 2004). This gene cluster has also been identified in the DNA of *Pseudonocardia* strain ENV478 (Masuda, 2009), and in both strains, *P. tetrahydrofuranoxydans* and *Pseudonocardia* sp. ENV478, growth with THF induces transcription of genes in the cluster (Masuda, 2009; Thiemer *et al*., 2003). *P. tetrahydrofuranoxydans* and *Pseudonocardia* sp. ENV478 degrade 1,4-dioxane co-metabolically when induced with THF (Mahendra and Alvarez-Cohen., 2006; Thiemer *et al*., 2003; Vainberg *et al*., 2006) . These results for *P. tetrahydrofuranoxydans* and *Pseudonocardia* sp. ENV478 did not directly link the THF-induced monooxygenase gene cluster to biochemical 1,4-dioxane transformation activity. However, the up-regulation of the homologous *thm* monooxygenase gene cluster in strain CB1190 during 1,4-dioxane metabolism, reported here, does support the involvement of the *thm* monooxygenase gene clusters in 1,4-dioxane degradation in *P. tetrahydrofuranoxydans* and *Pseudonocardia* sp. ENV478.

4.3.4. Genes potentially contributing to transformation of C_4 1,4-dioxane metabolites

After the initial monooxygenase-catalyzed hydroxylation, the C_4 1,4-dioxane metabolites undergo a series of transformations that ultimately yield two C_2 compounds (Figure 4.1). The set of genes on plasmid pPSED02 that are induced by 1,4-dioxane but not by glycolate could potentially be involved in these transformations (Table 4.3). The operon containing the *thm* monooxygenase-encoding gene cluster also includes genes encoding two aldehyde dehydrogenases (Psed_6975 and Psed_6981), while upstream of the monooxygenase cluster and in the reverse orientation is an up-regulated gene cluster encoding an alcohol dehydrogenase and a FAD/FMN-dependent dehydrogenase, as well as a transcriptional regulator (Psed_6970-6972). 1,4-Dioxane-dependent up-regulation of these genes was verified by qRT-PCR (Table 4.5). The aldehyde-carboxylic acid conversion of C_4 1,4-dioxane metabolites could be catalyzed by these 1,4-dioxane-induced aldehyde dehydrogenases. In contrast, a candidate enzyme for the secondary alcohol-ketone conversion of C_4 metabolites (*i.e.*, 2-hydroxy-1,4-dioxane to 1,4-dioxane-2-one) is not immediately clear, since none of the secondary alcohol dehydrogenase genes identified by *in silico* analysis (Table 4.1) were specifically up-regulated by 1,4-dioxane. The alcohol dehydrogenase-encoding Psed_6971 or constitutively expressed secondary alcohol dehydrogenases (not identified with the differential-transcription techniques used here) could be involved in this reaction. Future studies are being planned to clone the up-regulated dehydrogenases and reductases to functionally test their role in the transformation of C_4 1,4-dioxane metabolites.

The 1,4-dioxane metabolic pathway also requires a second hydroxylation and two ether cleavage steps. Ether cleavage may occur spontaneously following hydroxylation, or it may occur enzymatically. A heterogeneous group of ether cleaving enzymes have been described in a review (White *et al*., 1997), and an ether-bond cleaving diglycolate dehydrogenase has been purified from *P. tetrahydrofuranoxydans* (Yamashita *et al.*, 2004). However, genomic and transcriptomic analysis of 1,4-dioxane metabolism did not identify such enzymes in strain CB1190.

4.3.5. Genes potentially contributing to transformation of C_2 1,4-dioxane metabolites to glyoxylate.

The plasmid pPSED02-encoded putative aldehyde and alcohol dehydrogenases that were up-regulated during growth on 1,4-dioxane but not on glycolate could also contribute to the transformation of the 1,4-dioxane metabolites glyoxal, glycoaldehyde and ethylene glycol. In *E. coli* the aldehyde reductase *YqhD* catalyzes the NADPH-dependent reduction of glyoxal to glycoaldehyde (Lee *et al*., 2010), while in *Bacillus subtilis* the non-homologous *YvgN* catalyzes the same reaction (Sakai *et al*., 2001). The up-regulated aldehyde dehydrogenase gene products share only low amino acid sequence identity with these proteins, but Psed_6975 has 35% identity to *E. coli AldA*, which catalyzes the NAD^+-dependent oxidation of glycoaldehyde to glycolate (Hidalgo *et al*., 1991). Boronat *et al*. (1983) identified an *E. coli* mutant that employed the activity of propanediol oxidoreductase *fucO* (Conway and Ingram, 1989) to transform ethylene glycol to glycoaldehyde, which was further transformed to glycolate. The putative alcohol dehydrogenase gene

Psed_6971 that is up-regulated by 1,4-dioxane shares 42% amino acid similarity to *E. coli* 1,2-propanediol oxidoreductase, so its product may catalyze the conversion of ethylene glycol to glycoaldehyde during 1,4-dioxane metabolism. It should be noted that the glycolate- and 1,4-dioxane-induced alcohol dehydrogenase gene Psed_6782 has similar amino acid identity to *E. coli* propanediol oxidoreductase, although its role in glycolate metabolism is not clear.

The set of genes up-regulated with both 1,4-dioxane and glycolate relative to pyruvate include the putative glycolate oxidase-encoding chromosomal gene cluster *glcD2E2F*2 (Psed_4788-4790) (Table 4.4). In contrast, the adjacent homologous *glcD1E1F*1 gene cluster was not up-regulated with either 1,4-dioxane or glycolate. This lack of up-regulation was confirmed with qRT-PCR (Table 4.5). The enzyme encoded by *glcD2E2F*2 likely catalyzes the transformation of glycolate to glyoxylate (Pellicer *et al.*, 1996). In *E. coli*, genes for malate synthase G (*glcB* – see discussion below) and *glcDEF* are proximal, oriented in the same direction, and are co-transcribed during growth with glycolate (Pellicer *et al.*, 1999). While *glcB* (Psed_4782) and *glcD2E2F2* are in close proximity in strain CB1190, they have opposing orientation and are separated by the homologous but non-identical *glcD1E1F1* (Psed_4783-4785) cluster that was not up-regulated during growth with either 1,4-dioxane or glycolate (Fig.2). Between *glcD2E2F2* and *glcD1E1F1* is a gene (Psed_4787) encoding a putative GntR family transcriptional regulator, which may be involved in regulation of this glycolate oxidase homologue.

4.3.6. Glyoxylate metabolism during 1,4-dioxane degradation in strain CB1190.

Genes encoding two divergent routes for glyoxylate assimilation were up-regulated with both 1,4-dioxane and glycolate, as determined by microarray analysis. The first route was through the *glcB*-encoded malate synthase G (Psed_4782) (Table 4.4), although qRT-PCR did not confirm this up-regulation (Table 4.5). The up-regulated gene cluster encoding glyoxylate carboligase, tartronate semialdehyde reductase, hydroxypyruvate isomerase and glycerate kinase (Psed_3888-3891; Figure 4.2) represents the second route for potential glyoxylate assimilation. The up-regulation of three genes from this cluster was verified by qRT-PCR (Table 4.5).

These transcriptional results point to glyoxylate, rather than oxalate (Figure 4.1), as the key intermediate in the assimilation of carbon into central metabolism during 1,4-dioxane degradation by strain CB1190. In the glyoxylate carboligase pathway, two glyoxylate molecules are combined by glyoxylate carboligase, producing a C_3-compound that is eventually incorporated into glycolysis at phosphoglycerate (Krakow *et al*., 1961) (Figures 4.1 and 4.2). In the malate synthase G pathway, glyoxylate and acetyl-CoA are condensed to form malate, which is incorporated into the TCA cycle (Kornberg and Elsden, 1961) (Figures 4.1 and 4.2). As noted above, the glyoxylate carboligase pathway supports growth through both carbon and energy conservation, whereas malate synthase G is involved in anaplerotic reactions but is not known to generate energy to support growth in the absence of glyoxylate carboligase with glyoxylate as a sole substrate (Orn-

ston and Ornston, 1969). Therefore, the growth of strain CB1190 on 1,4-dioxane is likely dependent on the glyoxylate carboligase pathway, and is likely only enhanced by malate synthase G.

Given that strain CB1190 is differentiated from several other 1,4-dioxane-degrading bacteria by its ability to derive both carbon and energy from 1,4-dioxane degradation, and that the glyoxylate carboligase pathway represents a potential route to both carbon and energy generation during 1,4-dioxane metabolism, confirmation of the activity of this pathway in 1,4-dioxane-grown cells was tested. Glyxoylate carboligase (Gcl) activity (2 glyoxylate $\rightarrow$ CO_2 + tartronate semialdehyde, Figure 4.2) was tested in cell-free extracts prepared from strain CB1190 cells. As Figure 4.3 shows, pre-growth on 1,4-dioxane resulted in significantly greater consumption of glyoxylate than pre-growth on pyruvate. Glyoxylate disappearance was accompanied by a stoichiometric production of CO_2, a specific product of the glyoxylate carboligase reaction. Heating of the 1,4-dioxane cell-free extracts for 10 min at 80°C reduced CO_2 production to that observed with the buffer treatment (data not shown). No CO_2 was detected in 1,4-dioxane cell-free extracts when glyoxylate was omitted.

To verify the putative roles of strain CB1190 genes in the 1,4-dioxane-induced glyoxylate carboligase pathway, the genes Psed_3890 (putative glyoxylate carboligase) and Psed_3889 (putative tartronate semialdehyde reductase, Figure 4.2) were cloned and over-expressed in *R. jostii* RHA1 using the thiostrepton-inducible plasmid pTip-QC2 (Nakashima and Tamura, 2004). As expected, glyoxylate was consumed and CO_2 produced only in cell-free extracts from Psed_3890-expressing RHA1, or when these extracts were mixed with extracts from Psed-3889-expressing RHA1 (Figure 4.3B). When cell-free extracts from RHA1/pTip-Psed_3890 and RHA1/pTip-Psed_3889 were mixed and amended with glyoxylate, glyoxylate was consumed, CO_2 was generated, and a small amount of glycerate was produced (0.71±0.23 μmol per vial). No glycerate was produced with cell-free extracts from either construct alone, or with cell-free extracts from RHA1 with empty pTip-QC2. The amount of glycerate detected with the mix of RHA1/pTip-Psed_3890 and *R. jostii*/pTip-Psed_3889 cell-free extracts represented ~7% of that expected if tartronate semialdehyde was stoichiometrically transformed to glycerate (Figure 4.2). Tartronate semialdehyde was not detectable with the HPLC method used, so it is unclear whether the small amount of glycerate detected was due to only a minor transformation of tartronate semialdehyde or due to the further transformation of the generated glycerate to unspecified products. Regardless, these results support the annotation of Psed_3890 as a glyoxylate carboligase gene and Psed_3889 as a tartronate semialdehyde reductase gene.

The glyoxylate carboligase pathway ultimately leads to the assimilation of carbon as three-carbon metabolites in glycolysis as 3-phosphoglycerate and later pyruvate (Hansen and Hayashi, 1962). Isotopomer amino acid tracer analysis was employed using ^{13}C-uniformly-labeled 1,4-dioxane to verify that carbon from 1,4-dioxane enters central metabolism via the glyoxylate carboligase pathway as three-carbon compounds. Strain CB1190 was grown either with 1,4-[U-^{13}C]dioxane as the sole carbon source or with 1,4-[U-^{13}C] dioxane and elevated (3%) CO_2. With 1,4-[U-^{13}C]dioxane as the sole carbon source, all of the detected amino acids were heavily labeled (Table 4.6), which confirmed, as expected,

that strain CB1190 can directly use 1,4-dioxane as a sole carbon source.

Pyruvate-derived alanine, valine, phenylalanine and tyrosine were predominantly ^{13}C-labeled when strain CB1190 was grown with 1,4-[U-^{13}C]dioxane as the sole carbon source (Table 4.6), and the addition of elevated unlabeled CO_2 did not affect these labeling patterns (Table 4.7). These results are in contrast to aspartate, threonine, serine and glycine; for these, a small proportion of unlabeled carbon was integrated when the cells were grown with ^{13}C-dioxane as the sole carbon source, and this proportion increased greatly when strain CB1190 was grown with 13C-dioxane and elevated unlabeled CO_2. These results indicate that carbon from CO_2 was assimilated during the synthesis of these amino acids (Figure 4.4).

For the oxaloacetate-derived aspartate and threonine, the unlabeled carbon was present at a non-first carbon position (M-159 results, Tables 4.6 and 4.7) under both experimental conditions. These results indicate that the anaplerotic pathway transforming either phosphoenolpyruvate (PEP) or pyruvate to oxaloacetate through the addition of CO_2 (Kornberg, 1966; Wood *et al.*, 1966) was active during 1,4-dioxane metabolism (Figure 4.4). Strain CB1190 has homologous genes for both PEP carboxylase (Psed_6164) and pyruvate carboxylase (Psed_3032), which catalyze these anaplerotic reactions. The enzyme that catalyzes the reverse of the PEP-utilizing process, PEP carboxykinase, is putatively encoded by Psed_6619, and this gene was down-regulated during growth with both 1,4-dioxane and glycolate, relative to pyruvate (Appendices 5 and 6, respectively). Taken together, these results indicate that with 1,4-dioxane, carbon flows predominantly from the direction of PEP towards the TCA cycle, and support the proposed role of the three-carbon compound-generating glyoxylate carboligase pathway in 1,4-dioxane metabolism. In contrast, when grown with pyruvate, strain CB1190 needs to generate PEP and further gluconeogenic compounds necessary for amino acid synthesis, which explains the relative up-regulation of the gene (Psed_6619) encoding the PEP-generating PEP carboxykinase.

The inconsistency between the labeling patterns of 3-phosphoglycerate-derived serine and glycine and pyruvate-derived alanine, valine, phenylalanine and tyrosine is noteworthy, since 3-phosphoglycerate is a precursor of pyruvate in glycolysis. An explanation for this inconsistency is an alternative pathway of serine and glycine biosynthesis, specifically, by the reverse activity of the glycine cleavage system (GCS). The GCS converts glycine and tetrahydrofolate to CO_2, NH_3 and methylene-tetrahydrofolate (Sagers and Gunsalus, 1961), and it is putatively encoded by the strain CB1190 genome (W. Zhuang *et al*., manuscript in preparation). The GCS is reversible (Kochi and Kikuchi, 1969), and reverse GCS activity in the presence of elevated unlabeled CO_2 would result in increased unlabeled carbon in the glycine pool, and consequently, in the serine pool as well (Figure 4.4). Thus, during 1,4-dioxane metabolism, it is possible that serine and glycine are synthesized from both uniformLy-labeled, glyoxylate carboligase pathway-generated 3-phosphoglycerate and from the unlabeled-carbon-contributing reverse GCS.

Glyoxylate could contribute to the serine pool in yet another way. The strain CB1190 glyoxylate carboligase cluster has a gene (*hyi*) for hydroxypyruvate isomerase, which

catalyzes the isomerization of hydroxypyruvate to tartronate semialdehyde (Ashiuchi and Misono, 1999) and which is up-regulated in strain CB1190 during growth with 1,4-dioxane (Tables 4.3). Hydroxypyruvate can be used as a precursor for serine biosynthesis by the activity of transaminases (Sallach, 1956). However, no genes with significant homology to known amino acid-glyoxylate aminotransferase or amino acid-hydroxypyruvate transminase genes were up-regulated in strain CB1190 during growth with 1,4-dioxane or glycolate, so the role of hydroxypyruvate isomerase in 1,4-dioxane metabolism remains unclear.

4.3.7. Energy generation in strain CB1190 dioxane metabolism.

Based on the current work, a revised 1,4-dioxane degradation pathway for strain CB1190 is proposed, linking 1,4-dioxane metabolites to central metabolism (Figure 4.1). The employment by strain CB1190 of the glyoxylate carboligase pathway during 1,4-dioxane degradation provides the basis for energy production, since this pathway results in the pyruvate precursors that can then be metabolized to yield NADH and ATP (Krakow *et al*., 1961). An analysis (Table 4.8) of the reducing equivalents consumed or produced in the individual steps proposed for 1,4-dioxane metabolism in Fig. 1 reveals a maximum net yield of 6 NADH and one GTP per 1,4-dioxane molecule. Assuming an equivalence of 3 NADH per ATP, this means the theoretical maximum yield of ATP generated per dioxane is 19. Mahendra and Alvarez-Cohen (Mahendra *et al.*, 2007) previously reported a yield of 0.09 g protein per g dioxane for strain CB1190, so assuming that protein constitutes 50% of dry cell weight and integrating this cell yield with the theoretical maximum yield of ATP from dioxane, this results in a YATP (Bauchop and Elsden, 1960) of 0.83 g dry cell weight per mol ATP.

This work represents the first insights into the genetic basis for microbial 1,4-dioxane metabolism and provides a more complete picture of the pathway leading to carbon assimilation and energy generation. It will be useful to determine if 1,4-dioxane supports growth through similar pathways in the other reported 1,4-dioxane-metabolizing microorganisms, including *P. benzenivorans* B5 (Mahendra *et al*., 2007), *Mycobacterium* sp. PH-06 (Kim *et al.*, 2009), *C. sinensis* (Nakamiya *et al.*, 2005) and *R. ruber* 219 (Bernhardt and Diekmann, 1991).

Chapter 5

Examining the role of a tetrahydrofuran monooxygenase in the degradation of 1,4-dioxane and THF by *Pseudonocardia dioxanivorans* strain CB1190

5.1. Introduction.

Increasing evidence and awareness of the prevalence of 1,4-dioxane and tetrahydrofuran (THF) contamination in groundwater plumes, due primarily to their use as chlorinated solvents stabilizers, has prompted investigations of treatment strategies to remove them from the environment. One strategy of interest is biological degradation of these cyclic ethers. A number of studies have reported the ability of pure and mixed cultures of bacteria and fungi to degrade 1,4-dioxane aerobically (Bernhardt and Diekmann, 1991; Burback and Perry, 1993; Parales *et al*., 1994; Roy *et al*., 1994; Patt and Abebe, 1995; Raj *et al*., 1997; Skinner *et al*., 2009; Zenker *et al*., 2000; Nakimaya *et al*., 2005; Vainberg *et al*., 2006; Mahendra, 2006; Kim *et al*., 2009) and one study reported anaerobic degradation (Shen *et al*., 2008). The primary mechanism for aerobic biodegradation of 1,4-dioxane is co-metabolism, by which another substrate is used to provide carbon and energy to the microorganism. However, a handful of bacteria, including *Rhodococcus ruber* 219 (Bernhardt and Diekmann, 1991), *Mycobacterium* sp. PH-06 (Kim *et al*., 2009), *Pseudonocardia dioxanivorans* CB1190 (Parales *et al*., 1994), *Pseudonocardia benzenivorans* B5 and the fungus *Cordyceps sinensis* (Nakimaya *et al*., 2005) are capable of growth on 1,4-dioxane as their sole carbon and energy source. In contrast, metabolism of THF as a sole carbon and energy source among bacteria is more common. A number of actinomycetes can utilize THF for growth, including *Rhodococcus ruber* strains 219 (Bernhardt and Diekmann, 1991; Bock *et al*., 2006), M2 (Daye *et al*., 2004) and ENV425 (Vainberg *et al*., 2006; Fournier *et al*., 2009), a number of *Pseudonocardia* strains including *Pseudonocardia dioxanivorans* CB1190 (Parales et al., 1994), *Pseudonocardia benzenivorans* B5 (Mahendra and Alvarez-Cohen, 2006), *Pseudonocardia sulfidooxydans* (Kampfer *et al*., 2006; Mahendra and Alvarez-Cohen, 2006), *Pseudonocardia hydrocarbooxydans* (Kampfer *et al*., 2006), *Pseudonocardia tetrahydrofuranoxydans* K1 (Kohlweyer *et al*., 2000; Thiemer *et al*., 2003) and *Pseudonocardia sp.* ENV478 (Vainberg *et al*., 2006). Fungal growth on THF has also been observed in *Cordyceps sinensis* (Nakimaya *et al*., 2005), *Aureobasidium pullulans* (Patt and Abebe, 1995) and *Graphium sp.* ATCC 58400 (Skinner *et al*., 2009).

The initial step in the aerobic biodegradation of both 1,4-dioxane and THF is catalyzed by a monooxygenase reaction. Metabolic pathways for THF degradation have been proposed for *R. ruber* 219 (Bernhardt and Diekmann, 1991), *P. tetratrhydrofuranoxydans* (Thiemer *et al*., 2003), and *Graphium* sp. (Skinner *et al*., 2009). Common among these metabolic pathways is the initial oxidation of THF to 2-hydroxytetrahydrofuran and the formation of succinate as the downstream intermediate. No intermediates of THF degradation by *R. ruber* 219 have been detected, but the ability of *R. ruber* 219 to grow on the hypothetical intermediates γ-butyrolactone and succinate have been exhibited (Bernhardt and Diekmann, 1991). In *P. tetrahydrofuranoxydans*, a gene cluster involved in the utilization of THF was cloned and sequenced. This gene cluster contained a four-subunit multicomponent monooxygenase, *thmADBC*, involved in the oxidation of THF to 2-hydroxytetrahydrofuran; a succinate semialdehyde dehydrogenase, *sad*, responsible for the conversion of succinic semialdehyde to succinate; and an aldehyde dehydrogenase, *aldH*. Northern blot analysis of *P. tetrahydrofuranoxydans* indicated that *thmADBC* and *sad* genes were transcribed during growth on THF but *aldH* was only transcribed during growth on the linear ether 1,4-butanediol (Thiemer *et al*., 2003). No intermediates of THF

degradation by *P. tetrahydrofuranoxydans* were detected, but the enzyme activity of *sad* for the NAD-dependent aldehyde dehydrogenase conversion of succinic semialdehyde to succinate was demonstrated in THF-grown cells and not succinate-grown cells. In the fungus *Graphium* sp., a cytochrome P450 monooxygenase enzyme was suggested to catalyze the initial oxidation of THF. The only intermediate of THF degradation detected in *Graphium* sp., or any other strain, has been γ-butyrolactone (Skinner *et al*., 2009). In both *P. tetrahdyrofuranoxydans* and *Graphium* sp., the conversion of 2-hydroxytetrahydrofuran to γ-butyrolactone is proposed to be catalyzed by an alcohol dehydrogenase. Furthermore, the conversion of γ-butyrolactone to 4-hydroxybutyrate is proposed to be an abiotic process and the subsequent reaction of 4-hydroxybutyrate to succinic semialdehyde is catalyzed by an alcohol dehydrogenase. Homologues of the *thm* cluster from *P. tetrahydrofuranoxydans* have been found in *Pseudonocardia* sp. ENV478 (Vainberg *et al*., 2006), *Rhodococcus* sp. YYL (Yao *et al*., 2009), and Strain CB1190 CB1190 (Sales *et al*., 2011).

The biochemical and genetic basis for the degradation pathway for 1,4-dioxane is less understood. A number of studies have demonstrated that co-metabolic degradation of 1,4-dioxane can be initiated by a number of multicomponent monooxygenases, including methane monooxygenases, propane monooxygenases, toluene monooxygenases, and tetrahydrofuran monooxygenases (Burback and Perry, 1993; Mahendra and Alvarez-Cohen, 2006; Vainberg *et al*., 2006). The production of CO_2 due to the mineralization of 1,4-dioxane was first observed in Strain CB1190 CB1190 (Parales *et al*., 1994). The intermediates ethylene glycol, glycolic acid, and oxalic acid were detected as products of metabolic degradation of 1,4-dioxane by a fungal isolate, *Cordyceps sinensis* (Nakamiya *et al*., 2005). Accumulation of the intermediate β-hydroxyethoxyacetic acid (HEAA) was observed during co-metabolic degradation of 1,4-dioxane by *Pseudonocardia sp.* ENV478 grown on THF (Vainberg et al., 2006). Using electron spray triple quadrupole ion trap-mass spectrometry and Fourier transform ion cyclotron resonance-mass spectrometry, HEAA and twelve additional intermediates of 1,4-dioxane degradation by co-metabolism and metabolism in monooxygenase-expressing bacteria were detected, including 2-hydroxy-1,4-dioxane, 2-hydroxyethoxyacetaldehyde, 1,4-dioxane-2-one, 1,2-dihydroxyethoxyacetic acid, 2-hydroxyethoxy-2-hydroxyacetic acid, ethylene glycol, glycolate, glycoaldehyde, glyoxal, glyoxylic acid, oxalic acid, and formic acid (Mahendra *et al*., 2007). Even though a 1,4-dioxane degradation pathway was proposed based on these detected intermediates, other than biochemical evidence that the initial oxidation is catalyzed by a monooxygenase, the enzymes and genes associated with the other steps were not suggested.

In Chapter 3, genes encoding for multicomponent monooxygenase and cytochrome P450 enzymes in the genome sequence of strain CB1190 were elucidated. In Chapter 4, analyses of differentially expressed genes during growth on 1,4-dioxane and glycolate, relative to pyruvate, were used to identify genes likely involved in the degradation pathway of 1,4-dioxane. While the transcriptional data in Chapter 4 showed that the THF monooxygenase gene cluster *thmADBC* was the only multicomponent monooxygenase up-regulated on 1,4-dioxane and not glycolate, it is unclear if the THF monooxygenase is only involved in the oxidation of 1,4-dioxane or if the THF monooxygenase is also involved in the oxidation of HEAA, as proposed by Mahendra *et al*. (2007).

In this chapter, the function of the THF monooxygenase in the degradation of 1,4-dioxane and THF is characterized. Transcriptomic microarray data from strain CB1190 grown on 1,4-dioxane and THF and their respective intermediates, glycolate and succinate, are analyzed. Metabolic degradation of the intermediate HEAA by strain CB1190 is demonstrated. Finally, biochemical analysis of HEAA degradation and functional examinations of heterologous clones expressing the genes *thmABCD* demonstrate that degradation of HEAA is not catalyzed by the THF monooxygenase.

5.2. Materials and Methods.

5.2.1. Chemicals.

All chemicals used in medium preparation were of ACS reagent grade or better. 1,4-Dioxane (99.8%), glucose (99%), and sodium succinate (99%) were obtained from Sigma-Aldrich (Milwaukee, WI). THF (99.5%) was purchased from Acros Organics (Morris Plains, NJ). High-purity (99.5%) propane and acetylene were purchased from Matheson Gas Products (Twinsburg, OH). The potassium salt of β-hydroxyethoxyacetic acid (HEAA) was prepared by CanSyn Chemical Corporation (Toronto, ON). HPLC grade acetonitrile, ammonium formate, and formic acid were purchased from Fisher Scientific, Inc. (Fairlawn, New Jersey) to make mobile phases for liquid chromatography. Deionized (DI) water, produced from a Barnstead Nanopure II water purifying system, was used for preparation of medium, stock solutions, mobile phases, and buffers.

5.2.2. Laboratory strains.

Pseudonocardia dioxanivorans CB1190 (Parales *et al*., 1994; Mahendra and Alvarez-Cohen, 2005) (strain CB1190) was a gift from Dr. Rebecca Parales, University of California, Davis. *Rhodococcus jostii* RHA1 was a gift from Drs. William Mohn and Lindsay Eltis, University of British Columbia, Vancouver. Electrocompentent *Escherichia coli* DH5α was purchased from New Engliand Biolabs (Ipswich, MA).

5.2.3. Culture conditions.

Cells of strain CB1190 were grown in ammonium mineral salts (AMS) liquid medium (Parales *et al*., 1994). One liter of AMS contained 0.66 g of $(NH_4)_2SO_4$, 1.0 g of $MgSO_4 \cdot 7H_2O$, and 0.015 g of $CaCl_2 \cdot 2H_2O$, 1.0 mL of AMS trace elements, 1.0 mL of stock A, and 20 mL of 1.0 M phosphate buffer (added after autoclaved sterilization). The AMS trace elements contained (per liter) 0.5 g of $FeSO_4 \cdot 7H_2O$, 0.4 g of $ZnSO_4 \cdot 7H_2O$, 0.02 g of $MnSO_4 \cdot H_2O$, 0.015 g of H_3BO_3, 0.01 g of $NiCl_2 \cdot 6H_2O$, 0.25 g of EDTA, 0.05 g of $CoCl_2 \cdot 6H_2O$, and 0.005 g of $CuCl_2 \cdot 2H_2O$. The AMS stock A contained (per liter) 5.0 g of Fe-Na EDTA and 2.0 g of $NaMoO_4 \cdot 2H_2O$. The 1 M phosphate contained 113.0 g of K_2HPO_4 and 47.0 g of KH_2PO_4. The growth substrate 1,4-dioxane, THF, succinate, or glucose were added to the culture medium to achieve a final concentration of 5 mM. HEAA was added to cultures as a growth substrate with a final concentration of 1.5 mM. Cultures were incubated aerobically while shaking at 150 rpm at 30ºC. The liquid me-

dium was always less than 20% of the total volume of the culture bottles to prevent mass transfer limitation of oxygen. Strain CB1190 was also maintained on 1.5% Bacto agar plates containing R2A (Becton, Dickinson and Company, Franklin Lakes, NJ).

5.2.4. Cell harvesting and RNA isolation for transcriptional studies.

Strain CB1190 cells were harvested for transriptomic microarray analysis and quantitative reverse transcriptase PCR (qRT-PCR) experiments using the method described in Chapter 4. Cells from replicate bottles were collected by filtration onto several 0.22 µm PVDF Duarpore membrane filters (Millipore, Billerica, MA). The cells from each filter were scraped with a sterile scalpel, and were transferred to 2 mL screw-top microcentrifuge tubes containing 1 gram of 100-µm-diameter zirconia-silica beads (Biospec Products, Bartlesville, OK) in microcentrifuge tubes. Harvested cells were stored at -80ºC until total nucleic acids extraction.

Nucleic acids were extracted using a modified version of the phenol method described previously (Johnson, 2008). Briefly, each 2 mL microcentrifuge tube containing cells and zirconia-silica beads was filled with 250 µL lysis buffer (50 mM sodium acetate, 10 mM EDTA [pH 5.1]), 100 µL 10% sodium dodecyl sulfate, and 1.0 mL phenol (pH 8; Sigma-Aldrich, St. Louis, MO). Cells were lysed by heating to 65°C for 2 min, bead beating with a Mini Bead Beater (Biospec Products) for 2 min, incubating at 65°C for 8 min, and bead beating for an additional 2 min. Cellular debris was collected by centrifugation (5 min at 14,000 x *g*), and the aqueous lysate was transferred to a new microcentrifuge tube. The lysate was extracted twice with one volume of phenol-chloroform-isoamyl alcohol (pH 8) (24:24:1, vol/vol) and once with 1 volume of chloroform-isoamyl alcohol (24:1, vol/vol) (Sigma-Aldrich). Nucleic acids were precipitated by adding 0.1 volume of 3 M sodium acetate and one volume of ice-cold isopropanol and storing the tube at -20°C overnight. The precipitate was collected by centrifugation (30 min at 21,000 x *g* at 4°C), washed once with 70% ethanol, and re-suspended in 100 µL nuclease-free water.

To obtain RNA, re-suspended nucleic acids were initially separated with the Allprep kit (Qiagen), and then the RNA was purified using the RNeasy kit (Qiagen). Following elution with 100 µL RNase-free water, RNA was subjected to DNase I treatments using the DNA-free kit (Ambion, Austin, TX) according to the manufacturer's instructions until all contaminating DNA was removed (C_T value > 35). Pure RNA was obtained with a final cleanup using the RNeasy kit.

For RT-PCR analysis of the THF monooxygenase gene cluster thmADBC, strain CB1190 cells were grown on AMS medium with 1,4-dioxane, isopropanol, or THF.

5.2.5. Analytical methods.

1,4-Dioxane and THF removal was monitored by gas chromatography (GC) with a flame ionization detector (FID). Liquid samples containing cells were filtered with 0.2 µm syringe filters and 5 µL samples were injected directly into a Varian 3400 GC equipped with a FID detector and a 1% AT-1000 on a carbograph1 packed column (Grace, Columbia, MD). Samples were analyzed isothermally at 170°C, and the injector and detector temperatures were 230°C and 250°C, respectively.

The production and degradation of HEAA was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a hydrophilic interaction chromatography (HILIC) column. Samples were prepared by filtration with 0.2 µm syringe filters to remove cells. Standards and samples were measured on an Agilent Technologies 1200 Series LC system equipped with an Agilent Technologies Zorbax HILIC Plus column (4.6 mm x 100 mm, 3.5 µm) coupled to an Agilent Technologies 6410 tandem triple quadrupole (QQQ) mass spectrometer (Santa Clara, CA). The LC solvents used were Solvent A: aqueous buffer and Solvent B: acetonitrile. The aqueous buffer was 0.45 µm filtered, 10 mM ammonium formate in water with formic acid to adjust acidity (pH 5). The flow rate was 0.5 mL/min. The gradient was: t = 0 min., 95% B; t = 10 min., 40% B; t = 13 min., 95% B; t = 15 min., 95% B. The injection volume for samples and standards was 10 µL. Column effluent was introduced into the electrospray chamber, where the electrospray ionization was set to 3000 V in negative mode (ESI-). Nitrogen was used as the nebulizing gas at 30 psi, 325ºC, and a gas flow of 11 L/min. Multiple reaction monitoring (MRM) was used to monitor the transitions from the parent ion of HEAA, *m/z* 119 $[C_4H_7O_4]^-$ to the major product ions of *m/z* 119 $[C_4H_7O_4]^-$, *m/z* 101 $[C_4H_5O_2]^-$, *m/z* 75 $[C_2H_3O_3]^-$, and *m/z* 31 $[CH_3O]^-$ during a run. The collision gas used for MRM was argon, with the collision energy of 6 V and a fragmentor of 70 V for all MRM transitions. The summation of the peak areas for all MRM transitions was used for quantification of standards and samples.

5.2.6. Transcriptomics microarray analysis.

Five µg of total RNA was used as starting material for each microarray chip. Triplicate microarray chips were processed for each substrate treatment. cDNA was synthesized, fragmented, labeled, and hybridized to arrays according to the protocols outlined in section 3 of the Affymetrix GeneChip Expression Analysis technical manual, with the following changes: cDNA fragmentation was achieved by the addition of 0.07 U DNase I/µg cDNA, and the hybridization temperature was 52°C. Hybridized arrays were stained and washed according to Affymetrix protocol "Modified FlexMidi_euk2v3 for *P. aeruginosa* Array" and were scanned using an Affymetrix GeneChip Scanner 3000.

All microarray data analyses were performed in the R statistical programming environment (www.r-project.org) using packages available from Bioconductor version 2.9 (www.bioconductor.org) (Gentleman *et al*., 2004). Hybridization signal intensities for probe sets were calculated using the "rma" function from the "affy" package (Gautier *et al*., 2004). The "rma" function implements the computation of the RMA expression measure (Irizar-

ry *et al*., 2003), which summarizes the probe intensities for each probe set, by background adjustment, quantile normalization (Bolstad *et al*., 2003), and the median polish linear fitting procedure.

Identification of differentially expressed genes was accomplished using the "limma" package (Smyth, 2004), which implements a linear models approach to analyzing designed microarray experiments. This approach first required fitting the microarray data to a linear model, specified by a design matrix that indicates which RNA samples have been applied to each array, using the function "lmFit". Since the expression data was from Affymetrix GeneChips, the linear modeling was the same as one-way analysis of variance (ANOVA). The second step in this approach involved specifying the comparisons to be made between groups of arrays in a contrast matrix, such as contrasting all treatments (1,4-dioxane, glycolate, THF, and succinate) to the control (pyruvate) and direct comparison between treatments (*e.g*., 1,4-dioxane to glycolate or THF to succinate). This contrast matrix was applied to the linear fitted microarray data using the function "contrast.fit". Estimated log-fold changes ($\log_2$FC) and statistics used to assess differential expression between contrasts were determined using an empirical Bayes method, implemented in the function "eBayes". The *P* values returned from linear modeling were adjusted to correct for multiple hypothesis testing by applying the Benjamini and Hochberg procedure (Benjamini and Hochberg, 1995). This procedure allowed for the control of the false discovery rate (FDR) to 1% (*i.e.*, by only considering contrasts with an adjusted *p*-value less than 0.01). Genes that met the criteria of FDR < 1% and a $\log_2\text{FC} \geq |1|$ were considered differentially expressed.

5.2.7. Transcriptional analyses.

For induction analysis of *thmA* and *prmA* gene expression, a two-step qRT-PCR method was used. Total RNA for qRT-PCR was isolated, using the procedure described in 5.2.4, from strain CB1190*s* cells after 8 hours of exposure to either 5 mM of 1,4-dioxane, 5 mM of THF, 20% (vol./vol. of total headspace) of propane, or 5 mM of glucose (control). Cells for induction analysis were grown in AMS medium with 5 mM of glucose and harvested by filtration. The harvested cells were washed with and resuspended in AMS medium. The resuspended cells were aliquoted so each treatment and control condition was tested in triplicate. The isolated RNA was used to synthesize cDNA using the Taqman reverse transcription kit (Applied Biosystems). Taqman chemistry was used for qPCR reactions targeting *thmA* (Psed_6976), *prmA* (Psed_0639), *tpi* (Psed_3417), *thiC* (Psed_6168), and *rpoD* (Psed_0376). The genes *tpi*, *thiC*, and *rpoD* were determined by the geNorm method (Vandesompelee *et al*., 2002) to be stably transcribed across all treatments, and were thus used as internal references. Each qPCR reaction, which was run in triplicate for each biological replicate on an Applied Biosystems StepOne Plus real-time PCR system, consisted of 1X Fast Universal Mix (Applied Biosystems), 2 µL of diluted cDNA, each primer at 0.5 mM, and the probe at 145 nM. The cycling conditions were: 95°C for 20 s, then 40 cycles of 95°C for 1 s followed by annealing for 20 s. The efficiency of each qPCR assay was determined with a serial dilution of cDNA derived from a

dioxane treatment replicate. Gene expression was normalized using the method of Vandesompele *et al.* (2002).

Cells for RT-PCR analysis of the THF monooxygenase genes *thmADBC* were grown on 1,4-dioxane, isoproponal, or THF and harvested by centrifugation. RNA was isolated using the RNeasy kit. RT-PCR was performed using the Qiagen One Step RT-PCR kit. The primers used for amplification of each fragment were: fragment A, thmA_For1 5'-ATGACTGCCCCACCGATGAAG-3' and thmA_Rev 5'- AACGGTAGTAGTC-GTCATTCACC-3'; fragment B, thmA_For2 5'-CCTATAAGCGAGTGGAATAC-3' and thmD_Rev 5'- CGATGAGTAACGCCGAACGACTC-3'; fragment C, thmD_For 5'-GCTGCGCCAGATGTCAGAGG-3' and thmC_Rev1 5'-CCACATAATCATAAGC-GACGTCG-3'; and fragment D, thmB_For 5'-CTCATGAGCGCGAGATCGAG-3'; and thmC_Rev2 5'-CCACATAATCATAAGCGACGTCG -3'. RT-PCR products are visualized on an electrophoresis gel.

5.2.8. Cloning of strain CB1190 THF monooxygenase genes into *R. jostii* RHA1.

The development of vector construct containing the THF monooxygenase genes *thmADBC* was accomplished by the following. The 4.3 Kb fragment of strain CB1190 genes Psed_6976-6979 was amplified with forward primer 5'-ATGC**GGTACC**CAC-CATATAGAGGCGCCATC-3' and reverse primer 5'- ATGC**TCTAGA**GGCGAGAT-CACCTTGATGATCC -3', where the gene-binding sequence is underlined and the KpnI and XbaI restriction sites, respectively, are in bold. Each 100 µL PCR reaction consisted of 1X PCR buffer, 200 nM dNTPs, 500 nM each primer, 2 units Pfu polymerase and 50 ng of strain CB1190 genomic DNA. Thermocycling conditions were as follows: 95°C for 3 min, then 26 cycles of (95°C for 1 min, 55°C for 1 min, 72°C for 5 min), then 72°C for 10 min. The appropriate amplicon was then gel-purified.

The PCR amplicon and plasmid pK18 (Pridmore, 1987) were digested with KpnI and XbaI (New England Biolabs) and the digested plasmid was desphosphorylated with Antartic Phosphatase (New England Biolabs). The plasmid and PCR insert were ligated at a 1:3 (plasmid:insert) ratio at 16°C overnight with T4 DNA ligase (New England Biolabs) and used to transform electrocompetent *E. coli* DH5α.

The Psed_6976-6979 fragment encoding for the THF monooxygene genes *thmADBC* was then subcloned into plasmid pTip-QC2 (Nakashima and Tamura, 2004). The fragments was amplified with forward primer 5'-AAGGAGATATA**CATATG**ACTGCCCCACC-GAT-GAA-3' and reverse primer 5'-GTATGCGGCCGCCAT**GGAATT**CTACGACT-CAGA-GTTGATCAGCTCGAT-3', where the gene-binding sequence is underlined and the NdeI and XhoI restriction sites, respectively, are in bold. Each 100 mL PCR reaction consisted of 1X HF Buffer, 200 nM dNTPs, 500 nM each primer, 3% DMSO, 2 units Phusion Hot Start II DNA polymerase (Finnzymes) and 10 ng of plasmid pT7-7/thfmo DNA. Thermocycling conditions were as follows: 98°C for 3 min, then 30 cycles

of (98°C for 20 s, 72°C for 6 min), then 72°C for 10 min. The appropriate amplicon was then gel-purified.

Plasmid pTip-QC2 was linearized with NdeI and EcoRI (New England Biolabs), gel purified, and then the plasmid and PCR insert were ligated at a 1:3 (plasmid:insert) ratio at 16°C overnight with T4 DNA ligase (New England Biolabs). Electrocompetent *E. coli* DH5α was transformed via electroporation with 1 μL of ligation mix. Ampicillin-resistant colonies were screened for the appropriate construct, which was named pTip-thfmo.

Fifty ng of purified plasmid pTip-thfmo or empty vector pTip-QC2 were used to transform electrocompetent *Rhodococcus jostii* strain RHA1 according to the method of Kalscheuer *et al.* (1999), except that cells were not pre-incubated prior to electroporation, and the electroporation parameters were: 12 kV/cm, 800 Ω; and 25 μF.

5.2.9. Heterologous expression of THF monooxygenase in *R. jostii* RHA1.

Single colonies of *R. jostii* RHA1 (strain RHA1) containing the plasmid pTip constructs were used to inoculate 10 mL of nutrient broth amended with chloramphenicol (34 μg/mL) and grown at 30°C with shaking at 150 rpm for 48 h. Two liter flasks containing 0.5 L of nutrient broth amended with chloramphenicol and 1% each of sucrose and glycine were inoculated with 10 mL of 48 h culture and incubated at 30°C with shaking at 175 rpm. When the cultures had reached OD600 of 0.8 (~21 h), thiostrepton dissolved in DMSO was added to a final concentration of 1 μg/mL and the cultures were incubated for a further 26 h. Cells were transferred to 500 mL centrifuge bottles (Beckman Coulter, Brea, CA), cooled on ice, centrifuged for 10 min at 5,000 x g at 4°C, and then cell pellets were washed with 40 mL of ice-cold buffer (20 mM sodium phosphate, pH 7.0, 0.05% Tween-80). The cells were pelleted, washed again with 20 mL buffer, and resuspended in 10 mL buffer. This cell suspension was aliquoted (1 mL) to 1.5 mL microcentrifuge tubes, cells were pellets, the supernatant was removed, and the cell pellet aliquots were stored at -80°C until use in enzyme assays.

5.2.10. Inhibition and transformation assays.

The inhibitory effect of acetylene on HEAA degradation was examined. Axenic cultures of strain CB1190 grown on 1,4-dioxane (10 mM) in AMS medium were harvested by filtration. Cells were washed on the filter with 0.1 M phosphate buffer (pH 7) to remove residual 1,4-dioxane. The washed cells were resuspended in phosphate buffer and split in half. One half of the cell suspension was exposed briefly to 5% (vol./vol.) acetylene in the headspace for 10 minutes while shaking at 30ºC. The cell suspensions were then stripped with nitrogen gas for 5 minutes to remove residual acetylene. The non-acetylene-exposed

half of the cell suspension was treated identically, without the addition of acetylene. Finally, HEAA was amended into the cell suspensions and abiotic controls to achieve an initial concentration of 1.5 mM and the concentration of HEAA in each suspension was monitored over time. Acetylene-exposed, non-acetylene-exposed, and abiotic controls were all performed in triplicate.

Monitoring the transformation of THF and 1,4-dioxane and the production of HEAA during 1,4-dioxane degradation in heterologous expression clones was accomplished as follows. Aliquots of strain RHA1 carrying plasmid pTip constructs were thawed and resuspended in 1 mL of cold buffer. The transformation of 1,4-dioxane and THF and production of HEAA by cell suspensions of RHA1/pTip-thfmo or RHA1/pTip-QC2 was tested in 2 mL microcentrifuge tubes containing 1 mL of buffer and cell suspension. Each triplicate tube received 100 µL of cell suspension, and was amended with 1,4-dioxane (2.5 mM), THF (4.0 mM) or HEAA (10 mM) from aqueous stocks. Buffer controls (no cell suspension) vials were prepared in parallel. Vials were incubated at 30°C with horizontal shaking at 150 rpm. At several timepoints, 200 µL were removed and analyzed for the disappearance of the amended compound. GC-FID (for 1,4-dioxane and THF) and LC-MS/MS (HEAA) was used for analyzing samples.

5.3. Results.

5.3.1. Transcriptomics of strain CB1190 growth on cyclic ethers: 1,4-dioxane and THF.

The transcriptomes of 1,4-dioxane-, glycolate-, THF- and succinate-grown strain CB1190 were determined by microarray analysis. In comparison to the reference transcriptome of pyruvate-grown strain CB1190, a total of 1,332 genes were differentially expressed ($|\log_2 FC| \geq 1$, FDR < 0.01) in at least one of these transcriptomes (Appendix 11). When the 1,4-dioxane-, glycolate-, THF-, and succinate-grown transcriptomes were individually contrasted against the pyruvate-grown transcriptome, the number of differentially expressed genes were 362, 482, 717, and 580, respectively (Appendix 5- 8). Growth on 1,4-dioxane versus pyruvate led to the up-regulation of 97 genes and the down-regulation of 265 genes. Growth on glycolate versus pyruvate led to the up-regulation of 190 genes and the down-regulation of 293 genes. Growth on THF versus pyruvate led to the up-regulation of 224 genes and the down-regulation of 493 genes. Growth on succinate versus pyruvate led to the up-regulation of 377 genes and the down-regulation of 203 genes.

Hierarchical clustering of the top 1,332 differentially expressed genes from each sample is shown as a heat plot in Figure 5.1. Despite inherent variability among biological replicates, the dendrogram (at the top of the heat plot) groups samples according to their growth condition. The same dendrogram also groups 1,4-dioxane and glycolate samples together and THF and succinate samples together. This grouping supports the hypothesis that the pathways used for glycolate metabolism should be a subset of the pathways for 1,4-dioxane metabolism and the pathways used for succinate metabolism should be a subset of the pathways for THF metabolism, since glycolate and succinate are an intermediate of each metabolic pathway, respectively. The degree of variation in genome-wide regulation of gene expression for each growth condition compared against the reference condition is visible in the volcano plots (Figure 5.2), which graph the log odds ratio (the $\log_e$ of the probability that a gene is differentially expressed over the probability it is not) versus the $\log_2 FC$ of each gene.

5.3.2. Direct comparison of 1,4-dioxane and glycolate transcriptomes.

In order to pinpoint the genes involved in the initial steps of 1,4-dioxane degradation, the sets of genes differentially expressed during growth on 1,4-dioxane and glycolate, relative to pyruvate, were compared (Chapter 4). Specifically, a transcriptomic microarray analysis was performed based on the assumption that genes involved in the conversion of 1,4-dioxane to glycolate would not be up-regulated on 1,4-dioxane relative to pyruvate but would not up-regulated on glycolate relative to pyruvate. The results of this transcrip-

tomic comparison identified 65 genes that were up-regulated only on 1,4-dioxane (relative to pyruvate), see Table 4.3.

A direct comparison of genes differentially expressed between 1,4-dioxane- and glycolate-grown strain CB1190 rather than relative to pyruvate is reported here. The results of this direct comparison (Appendix 9) showed that a total of 370 genes are differentially expressed ($|log2FC| \geq 1$ and FDR<0.01), of which, 165 are up-regulated and 205 are down-regulated [the list of up-regulated genes in the direct comparison of 1,4-dioxane and glycolate is larger since the analysis in Chapter 4 eliminated genes that were not up-regulated on 1,4-dioxane relative to pyruvate ($log_2FC_{dioxane/pyruvate} < 1$) but were more highly expressed than on glycolate [$log_2FC_{dioxane/pyruvate} > log_2FC_{glycolate/pyruvate)}$]. Evaluation of the 165 genes up-regulated in the direct comparison between 1,4-dioxane and glycolate and the 63 genes up-regulated on 1,4-dioxane relative to pyruvate identified a total of 49 common genes (Appendix 12) resulting in 14 genes (Psed_1076, Psed_1594, Psed_2752, Psed_3041, Psed_3934, Psed_4148, Psed_4149, Psed_4306, Psed_4424, Psed_5135, Psed_5524, Psed_5938, Psed_6241, Psed_6740) that were not differentially expressed ($|log2FC| \geq 1$ and FDR < 0.01) in the direct 1,4-dioxane and glycolate comparison.

5.3.3. Contrasting the THF and succinate transcriptomes.

Succinate has been shown to be an intermediate of aerobic degradation in the bacterium *P. tetrahdyrofuranoxydans* (Thiemer *et al*., 2004) and the fungus Graphium *sp.* (ATCC 58400) (Skinner *et al*., 2009). Therefore, in order to determine the genes involved in the degradation of THF in strain CB1190, the transcriptomes during THF and succinate growth were examined. A total of 830 genes were differentially expressed on THF versus succinate, of which, 220 were up-regulated and 610 were down-regulated (Appendix 10). Similar to the analysis method used to produce the results in section 4.2.3, a total of 92 genes are up-regulated on THF and not on succinate, both relative to pyruvate (Table 5.1). Of the 220 genes up-regulated on THF versus succinate, only 65 are found in Table 5.1. The remaining 155 genes that are up-regulated on THF versus succinate are either not up-regulated on THF relative to pyruvate (i.e.,$log_2FC_{THF/pyruvate} < 1$) or up-regulated on succinate relative to pyruvate ($log_2FC_{succinate/pyruvate} > 1$) but the difference between $log_2FC_{THF/pyruvate}$ - $log_2FC_{succinate/pyruvate}$ is greater than 1. The 27 genes in red text in Table 5.1 (up-regulated on THF and not succinate, relative to pyruvate) are not found in Appendix 10 because these genes were not found to be differentially expressed between THF- and succinate-grown strain CB1190 cells.

The *thm* gene cluster (Psed_6974 to Psed_6982) located on plasmid pPSED02 is found in the set of genes up-regulated on THF but not succinate, relative to pyruvate (Table 5.1). The most highly up-regulated gene in the *thm* cluster is the Mn2+/Fe2+ transporter ($log_2FC_{THF/pyruvate} = 3.95$). Another gene (Psed_6971) located just upstream of the *thm* cluster on plasmid pPSED02 is also up-regulated. The gene Psed_6971 is annotated to encode for a hydroxyacid-oxoacid transhydrogenase, which is known to convert γ-hydroxybutyrate to succinic semialdehyde, using α-ketogluturate as a hydrogen accep-

tor (Kaufmann *et al*, 1988). Other oxoacids, including oxaloacetate and α-ketoapidate can also be used as a hydrogen acceptor but with lower activity. Although it is not listed in Table 5.1, the gene Psed_6970 is up-regulated on THF versus succinate ($log_2FC_{THF/succinate}$ = 2.91, adjusted-*p*-value = 2.06e-5) and encodes for a lactate dehydrogenase, a homologue of γ-hydroxybutyrate dehydrogenase, which converts γ-hydroxybutyrate to succinic semialdehyde using NADH.

5.3.4. Induction of *thmA* gene expression by 1,4-dioxane and THF.

As mentioned in Chapter 4, the microarray used in this study is missing a number of genes from the finished strain CB1190 genome and does not include sequences determined to be pseudogenes. The sequence Psed_6976 was designated as a pseudogene and was subsequently left off the microarray. However, sequence analysis described in Chapter 3 determined that the Psed_6976 shares 98.9% amino acid identity, 94% nucleotide identity, and the same gene order with the tetrahydrofuran monooxygenase α-subunit sequence, *thmA,* characterized in *P. tetrahydrofuranoxydans*. RT-PCR analysis of the THF monooxygenase genes *thmADBC* (Figure 5.3) indicated that all of the genes in this cluster were transcribed during growth on isopropanol, 1,4-dioxane, or THF. Amplification of fragment A demonstrated that Psed_6976 is not a pseudogene but an expressible *thmA* gene. The weak amplification of fragments B and D that span *thmAD* and *thmDBC*, respectively, suggested that the reductase component (*thmD*) is weakly co-transcribed with its neighboring open-reading frames. The presence of fragment D shows that two genes *thmB* and *thmC* were co-transcribed.

In Chapter 4, qRT-PCR analysis had been used to quantify the up-regulation of *thmA* (Psed_6976) during growth on 1,4-dioxane. Here, qRT-PCR was used to quantify the induction of *thmA* gene expression after 8 hours of exposure to 1,4-dioxane, THF, or propane. Using cells exposed to 5 mM of glucose as a control, 1,4-dioxane, THF, and propane were determined to induce 24.4 ± 7.7, 10.2 ± 8.5, and 6.2 ± 0.5 fold up-regulation of *thmA*, respectively, as shown in Figure 5.4. A similar analysis was performed for the gene propane monooxygenase α-subunit *prmA* (Psed_0629). Exposure to 1,4-dioxane and THF did not lead to significant differential expression of *prmA*, with a differential-fold expression of 2.0 ± 0.3 and 1.0 ± 0.3, respectively. However, propane significantly up-regulated the *prmA* gene with a differential-fold expression of 64.0 ± 14.4.

5.3.5. Growth of *P. dioxanivorans* strain CB1190 on HEAA.

Growth of strain CB1190 on identified intermediates of 1,4-dioxane, such as ethylene glycol, glycolate, glycoaldehyde, glyoxal, glyoxylic acid, oxalic acid, and formic acid have been confirmed (Mahendra *et al*, 2007). However, growth on 2-hydroxy-1,4-dioxane, 1,4-dioxane-2-one (dioxanone), β-hydroxyethoxyacetic acid (HEAA), and 1,2-di-

hydroxyethoxy-2-hydroxyethoxy acetic acid have not been determined. Many of these compounds are notorious for polymerizing in their pure form. However, we were able to successfully obtain synthesized HEAA in its salt form in order to demonstrate growth of strain CB1190 on HEAA as a sole carbon and energy source for the first time. During the growth of strain CB1190 on 1.5 mM HEAA in AMS, removal of HEAA was recorded and plotted in Figure 5.5. Starting with a 1:500 inoculation of strain CB1190 cells previously growing on 1,4-dioxane, complete removal was accomplished within 11 days. The lag in the degradation of HEAA agrees with a visual observation of a corresponding lag in biomass accumulation for the first few days following addition of HEAA.

5.3.6. Effect of brief acetylene-exposure on HEAA degradation.

Brief exposure to acetylene gas is known to cause irreversible inhibition of monooxygenase enzyme activity (Colby *et al*., 1975; Stirling and Dalton, 1979; Prior and Dalton, 1985; Zahn and DiSpirito, 1996; Hamamura *et al*., 1999; Sharp *et al*., 2006) and has been shown to specifically inhibit co-metabolic and metabolic degradation of 1,4-dioxane (Mahendra and Alvarez-Cohen, 2006; Mahendra *et al*., 2007). Results reported here and in prior studies have corroborated the fact that the first step in the degradation pathway of 1,4-dioxane is its oxidation by a monooxygenase enzyme. Mahendra *et al*. (2007) proposed that a monooxygenase is also involved in the hydroxylation of HEAA and that this monooxygenase is likely the same enzyme that catalyzes the hydroxylation of 1,4-dioxane. In order to test this hypothesis, strain CB1190 cells were briefly exposed to acetylene gas. Results in Figure 5.6 indicate that strain CB1190 cells grown on 1,4-dioxane and briefly exposed to acetylene gas are able to completely degrade 1.25 mM of HEAA within 90 hours. The degree of inhibition caused by brief exposure is minimal, as shown by the similar degradation curves for the exposed and non-exposed cells (Figure 5.6).

5.3.7. Functional activity of *thmADBC*-expressing *R. jostii* RHA1 clones.

The gene cluster *thmADBC* encoding for the 4-subunit THF multicomponent monooxygenase in strain CB1190 was successfully cloned into competent *Rhodococcus jostii* RHA1 on a pTip vector (RHA1/pTip-thfmo). The RHA1/pTip-thfmo clones were grown on a nutrient broth and expression was induced by thiostreptin. Harvested clones were resuspended in phosphate buffer (pH = 7) to test the function of the THF monooxygenase on 1,4-dioxane and THF (Figure 5.7 and 5.8, respectively). The RHA1/pTip-thfmo expression clone suspensions demonstrated the ability to almost completely transform 2.5 mM of 1,4-dioxane into HEAA within 3 days, which corresponds to a degradation rate of 0.94 mM/d. In Figure 5.7, the generation rate of HEAA was stoichiometric with the 1,4-dioxane transformation rate. As a control, RHA1 clones containing an empty pTip vector (RHA1/pTip-QC2) were not capable of transforming 1,4-dioxane. The compound HEAA was detectable at extremely low concentrations in the abiotic and pTip vector

controls, which means that HEAA was a minor impurity in the 1,4-dioxane stock from the manufacturer.

The RHA1/pTip-thfmo expression clones also demonstrated the ability to degrade the compound THF. The initial 4 mM of THF amended into each RHA1/pTip-thfmo expression clone sample was almost completely transformed within 3 days. The rate of THF transformation by the clones is 1.48 mM/d. The amount of THF loss in the abiotic and pTip-QC2 controls was minimal and was attributable to the volatility of THF [Henry's constant $H_c = 7.05 \times 10^{-5}$ atm-m^3/mol] (Mohr *et al.*, 2010).

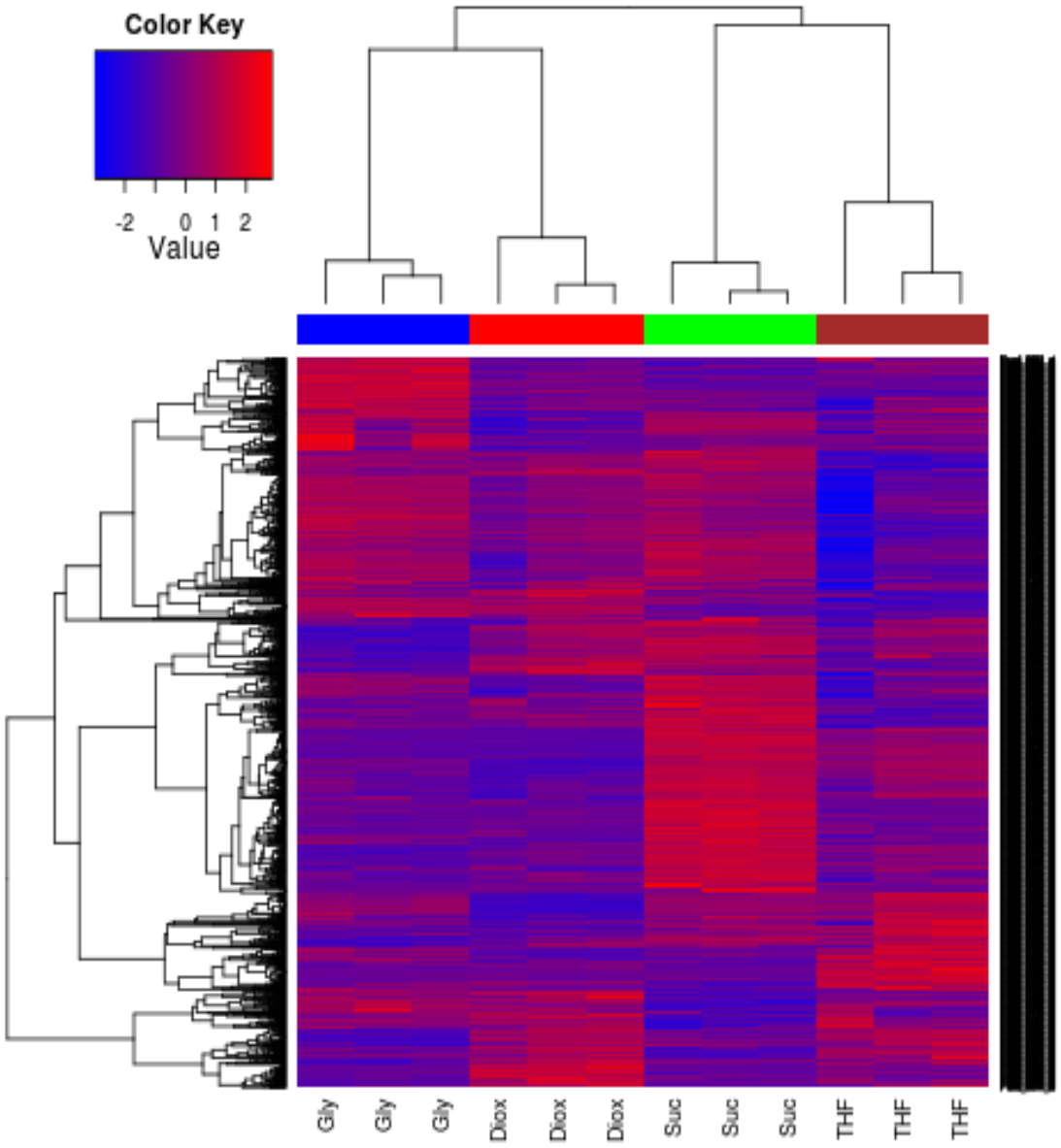


Figure 5.1. Hierarchical clustering and dendrogram of differentially expressed genes. Differentially expressed genes ($\log_2$FC > |1|, FDR < 0.01) from triplicate microarrays for each condition, 1,4-dioxane, glycolate, THF, and succinate, are grouped by hierarchical clustering (dendrogram on left side of heat map). The microarrays are clustered according to eigenvalues determined by multivariate analysis performed by implementing the "heatplot" function in the "made4" package in the R statistical programming environment. The color gradient from blue to red represent increasing microarray RMA expression values (scaled according to "heatplot").

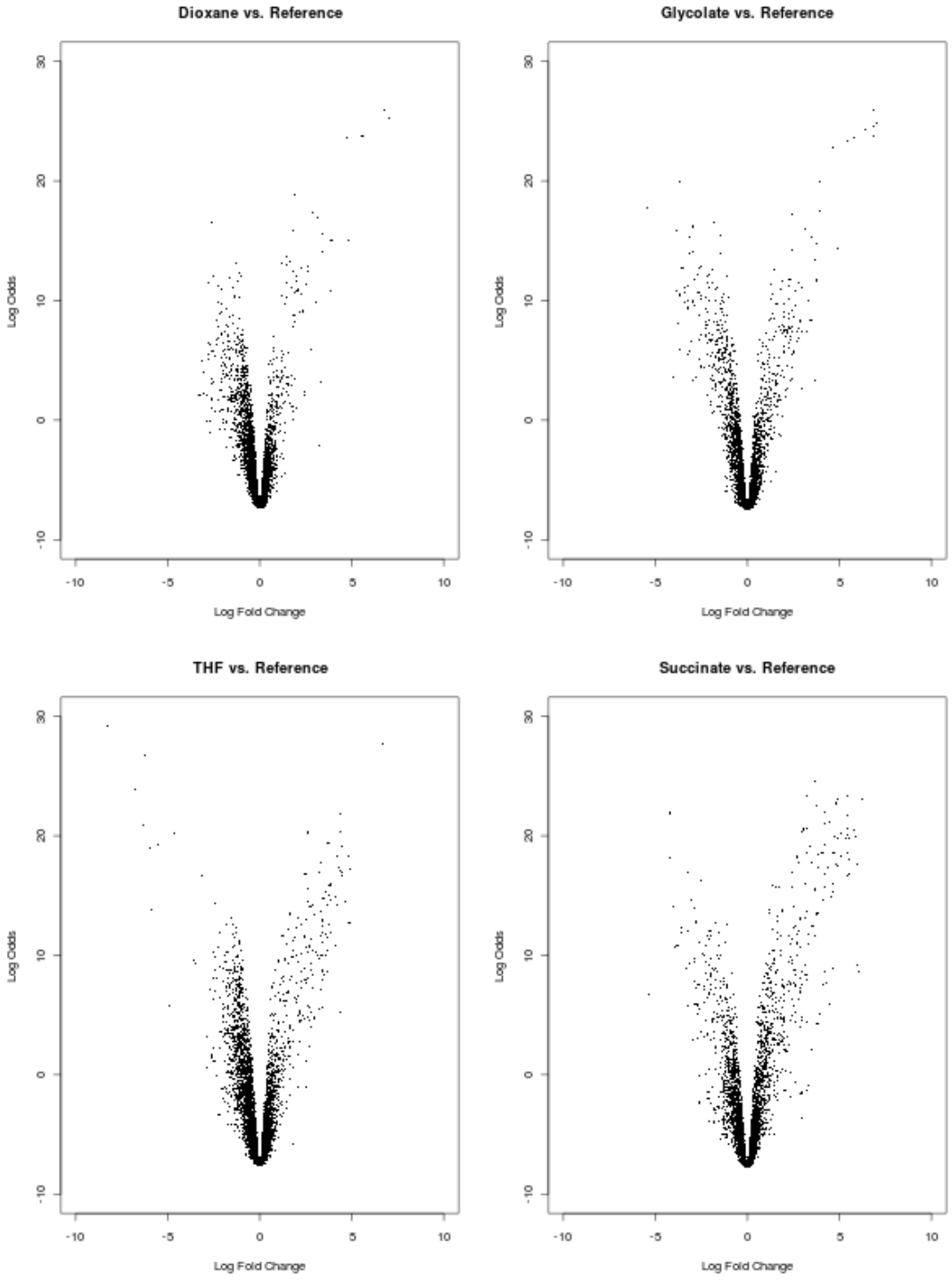


Figure 5.2. Volcano plots. Each plot graphs the "Log Fold-Change" (log_2FC) and the "Log Odds" for every gene on the microarray between a test condition (either 1,4-dioxane-, glycolate-, succinate- or THF-grown strain CB1190) and the reference condition (pyruvate-grown strain CB1190). The "Log Odds" is the log_e of the probability that a gene is differentially expressed over the probability it is not.

Table 5.1 Genes up-regulated on THF but not succinate, relative to pyruvate. Up-regulated genes have a $log_2FC \geq 1$, FDR (adjusted *p*-value) < 0.01. [Genes highlighted in Red are not differentially expressed log_2FC and FDR <0.01 when comparing the transcriptomes of THF- and succinate-grown strain CB1190 directly.]

Gene	Protein	THF vs. Pyruvate		Succinate vs. Pyruvate	
		log_2FC	**adjusted p-value**	**log_2FC**	**adjusted p-value**
Psed_0094	YCII-related	2.46	1.82E-06	0.77	1.98E-02
Psed_0095	RNA polymerase sigma factor, sigma-70 family	1.52	1.28E-06	0.21	3.01E-01
Psed_0129	protein of unknown function DUF59	1.62	7.26E-05	0.67	3.67E-02
Psed_0130	amidohydrolase 2	2.17	2.87E-05	0.83	3.29E-02
Psed_0131	Alcohol dehydrogenase GroES domain protein	1.99	1.44E-05	0.60	6.51E-02
Psed_0288	S-(hydroxymethyl)glutathione dehydrogenase	1.76	8.80E-07	0.18	4.31E-01
Psed_0355	hypothetical protein	1.26	8.93E-06	-0.41	3.86E-02
Psed_0357	helix-turn-helix domain protein	1.08	4.94E-06	0.06	7.65E-01
Psed_0382	hypothetical protein	1.16	7.36E-03	0.84	5.05E-02
Psed_0554	hypothetical protein	2.06	2.85E-03	0.59	3.96E-01
Psed_0934	YCII-related	2.58	6.30E-07	-0.16	6.72E-01
Psed_1301	Redoxin domain protein	1.03	5.57E-03	-0.05	9.25E-01
Psed_1303	Heavy metal transport/detoxification protein	1.05	2.65E-03	-0.72	3.18E-02
Psed_1584	6-phosphofructokinase	1.05	8.35E-05	-0.49	2.22E-02
Psed_1967	major facilitator superfamily MFS_1	1.85	7.63E-08	0.09	6.76E-01
Psed_1968	hypothetical protein	2.11	7.38E-08	0.27	1.86E-01
Psed_2321	ABC-type sugar transport system periplasmic component-like	1.22	6.89E-04	-0.29	3.91E-01
Psed_3256	hypothetical protein	3.34	6.17E-06	0.99	4.96E-02
Psed_3442	protein of unknown function DUF939	1.05	8.63E-05	0.74	1.70E-03

Psed_3564	Aromatic-amino-acid transaminase	1.66	1.63E-04	0.47	1.97E-01
Psed_3652	hypothetical protein	1.52	1.32E-04	-0.25	4.80E-01
Psed_3669	hypothetical protein	2.28	3.92E-06	0.66	4.66E-02
Psed_3986	Peptidase M23	1.05	3.08E-03	0.54	1.07E-01
Psed_4083	hypothetical protein	1.23	8.85E-04	0.80	1.84E-02
Psed_4236	hypothetical protein	1.03	3.68E-04	-0.01	9.83E-01
Psed_4268	membrane protein of uknown function UCP014873	2.06	3.52E-04	0.12	8.60E-01
Psed_4321	hypothetical protein	1.55	1.68E-04	-0.63	6.24E-02
Psed_4322	Dihydrolipoyl dehydrogenase	2.21	5.18E-08	-0.42	4.42E-02
Psed_4699	protein of unknown function DUF1365	1.62	4.24E-04	0.83	3.79E-02
Psed_4702	protein of unknown function DUF1295	3.20	2.18E-05	0.52	3.76E-01
Psed_4753	nitrogen regulatory protein P-II	1.15	2.38E-04	0.06	8.59E-01
Psed_4754	nitrogen regulatory protein P-II	1.34	3.94E-04	0.41	2.14E-01
Psed_4937	hypothetical protein	1.06	5.06E-04	-0.61	2.31E-02
Psed_4938	regulatory protein MarR	2.83	1.92E-06	-0.16	7.30E-01
Psed_4939	Lipase	6.70	2.21E-13	-0.18	4.01E-01
Psed_4940	fumarylacetoacetate (FAA) hydrolase	5.21	6.82E-10	-0.01	9.79E-01
Psed_4941	Formyl-CoA transferase	2.78	1.92E-06	0.02	9.71E-01
Psed_5082	YVTN beta-propeller repeat-containing protein	1.20	2.34E-07	0.23	7.05E-02
Psed_5176	hypothetical protein	1.61	2.21E-04	0.66	6.88E-02
Psed_5224	hypothetical protein	1.19	9.71E-03	-0.55	2.42E-01
Psed_5245	regulatory protein TetR	1.13	2.38E-04	-0.15	6.27E-01
Psed_5246	uncharacterized peroxidase-related enzyme	1.64	6.01E-05	0.01	9.81E-01
Psed_5247	hypothetical protein	2.10	9.49E-06	-0.13	7.52E-01
Psed_5248	Nitroreductase	4.37	9.86E-11	-0.04	9.00E-01
Psed_5249	NADPH:quinone reductase	3.45	2.19E-08	-0.25	4.25E-01
Psed_5250	major facilitator superfamily MFS_1	1.04	1.13E-06	0.10	4.88E-01

Psed_5736	Transglycosylase-like domain protein	2.30	6.47E-05	0.94	3.63E-02
Psed_5747	hypothetical protein	1.83	5.87E-07	0.53	1.60E-02
Psed_5748	hypothetical protein	1.10	1.23E-05	0.58	2.97E-03
Psed_5749	hypothetical protein	1.31	7.51E-04	0.70	4.43E-02
Psed_5752	hypothetical protein	1.04	5.54E-05	0.32	1.05E-01
Psed_5753	hypothetical protein	1.73	2.97E-06	0.29	2.45E-01
Psed_5755	hypothetical protein	1.21	3.14E-06	0.66	7.48E-04
Psed_5756	hypothetical protein	1.16	1.31E-03	0.61	6.52E-02
Psed_5757	hypothetical protein	1.79	8.17E-05	0.18	6.69E-01
Psed_5758	hypothetical protein	2.27	5.81E-06	0.60	7.81E-02
Psed_5759	hypothetical protein	2.39	3.16E-05	0.45	3.14E-01
Psed_5760	hypothetical protein	2.22	4.68E-05	0.26	5.85E-01
Psed_5761	hypothetical protein	2.02	4.35E-06	0.61	3.74E-02
Psed_5762	hypothetical protein	1.63	3.56E-06	0.33	1.52E-01
Psed_5763	hypothetical protein	1.35	2.24E-04	0.35	2.65E-01
Psed_5764	hypothetical protein	1.31	5.77E-04	0.22	5.69E-01
Psed_5765	hypothetical protein	1.01	7.80E-06	0.21	1.80E-01
Psed_5766	hypothetical protein	1.43	7.73E-06	0.53	1.95E-02
Psed_5767	hypothetical protein	1.50	6.11E-06	0.14	5.78E-01
Psed_5768	hypothetical protein	1.43	3.67E-07	0.56	1.86E-03
Psed_5769	hypothetical protein	1.55	7.37E-06	0.19	4.71E-01
Psed_5770	hypothetical protein	1.29	4.99E-04	0.29	3.92E-01
Psed_5778	cell divisionFtsK/SpoIIIE	1.33	1.30E-05	0.12	6.49E-01
Psed_5779	hypothetical protein	2.01	1.40E-07	0.08	7.57E-01
Psed_5780	hypothetical protein	1.37	2.32E-04	0.78	1.48E-02
Psed_5781	hypothetical protein	1.29	3.07E-03	0.81	4.93E-02
Psed_6160	heavy metal translocating P-type ATPase	1.24	2.99E-04	0.92	3.60E-03

Psed_6249	Ribulose bisphosphate carboxylase large chain	1.15	5.81E-04	-0.20	5.57E-01
Psed_6665	NLP/P60 protein	2.19	1.63E-04	0.27	6.25E-01
Psed_6780	hypothetical protein	1.57	7.91E-06	-0.10	7.57E-01
Psed_6806	hypothetical protein	1.31	3.62E-04	-0.12	7.63E-01
Psed_6807	hypothetical protein	2.79	2.82E-05	-0.11	8.67E-01
Psed_6812	hypothetical protein	1.37	1.85E-05	0.92	6.79E-04
Psed_6847	hypothetical protein	1.11	4.62E-04	0.08	8.20E-01
Psed_6857	protein of unknown function DUF156	1.03	4.44E-04	0.72	7.36E-03
Psed_6879	S-adenosylmethionine synthase	1.82	8.09E-04	-0.23	7.01E-01
Psed_6970	D-lactate dehydrogenase (cytochrome)	2.71	4.71E-05	-0.20	7.56E-01
Psed_6971	Hydroxyacid-oxoacid transhydrogenase	2.17	3.00E-05	0.44	2.60E-01
Psed_6972	GntR domain protein	1.66	6.94E-04	0.31	5.24E-01
Psed_6974	Ethyl tert-butyl ether degradation EthD	1.82	3.25E-07	0.92	1.96E-04
Psed_6975	Betaine-aldehyde dehydrogenase	1.63	5.64E-08	0.73	1.26E-04
Psed_6977	Ferredoxin--NAD(+) reductase	2.48	8.02E-08	0.70	6.09E-03
Psed_6978	methane/phenol/toluene hydroxylase	1.59	1.55E-07	0.54	2.75E-03
Psed_6979	monooxygenase component MmoB/DmpM	2.23	1.07E-07	0.68	4.47E-03
Psed_6981	Aldehyde Dehydrogenase	3.35	7.63E-08	0.88	9.56E-03
Psed_6982	Mn2+/Fe2+ transporter, NRAMP family	3.46	2.56E-06	0.25	6.61E-01

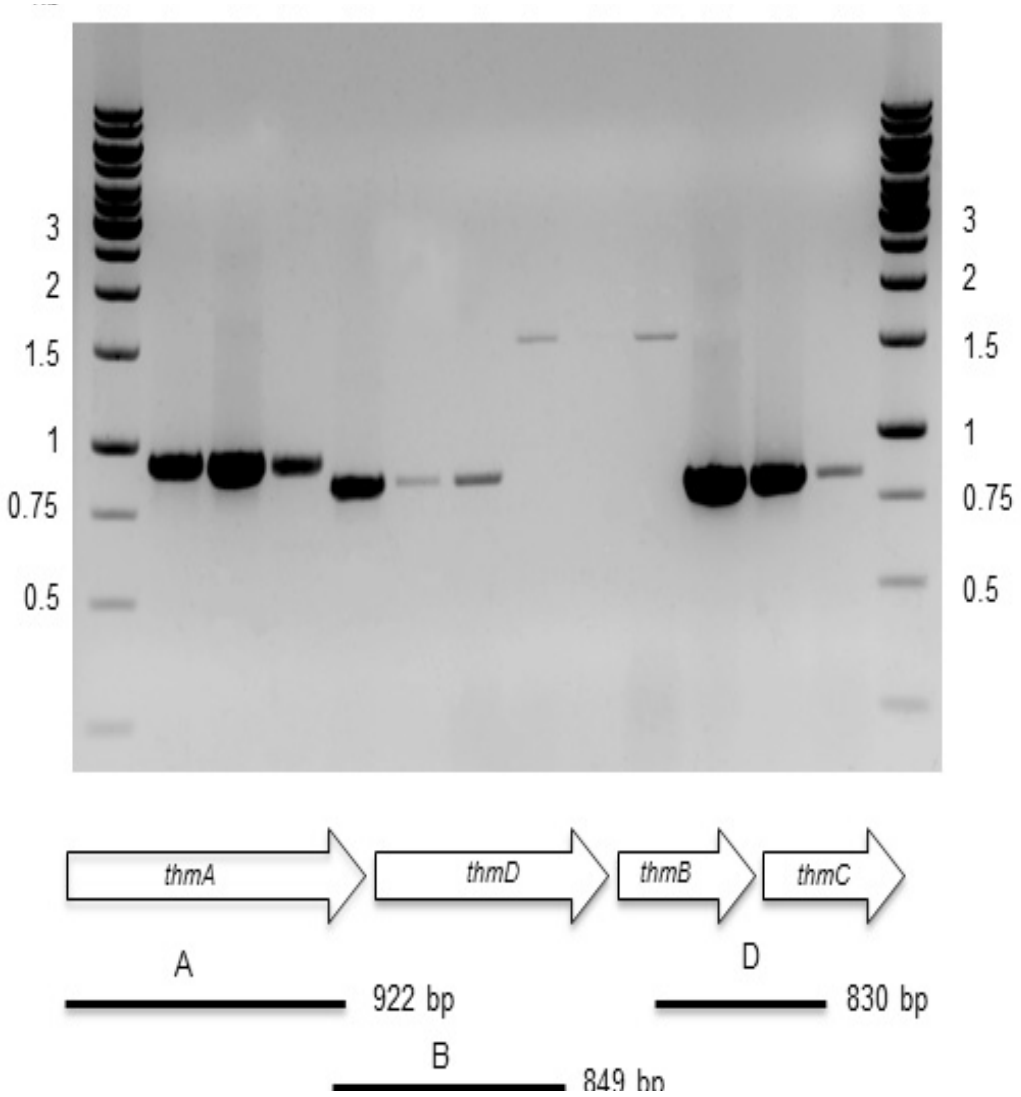


Figure 5.3. RT-PCR analysis of the THF monooxygenase genes. Lanes 1-3, primers thmA_For1 and thmA_Rev were used to amplify fragment A. Lanes 4-6, primers thmA_ For2 and thmD_Rev were used to amplify fragment B. Lanes 7-9, primers thmD_For and thmC_Rev1 were used to amplify fragment C. Lanes 10-12, primers thmB_For and thmC_Rev2 were used to amplify fragment D. RNA used in reactions shown in lanes 1, 4, 7, and 10 was purified from isopropanol-grown cells, RNA used in reactions shown in lanes 2, 5, 8, and 11 was purified from 1,4-dioxane-grown cells, and RNA used in reactions shown in lanes 3, 6, 9, and 12 was purified from THF-grown cells. Lanes M, molecular weight markers. No products were detected in reactions lacking reverse transcriptase.

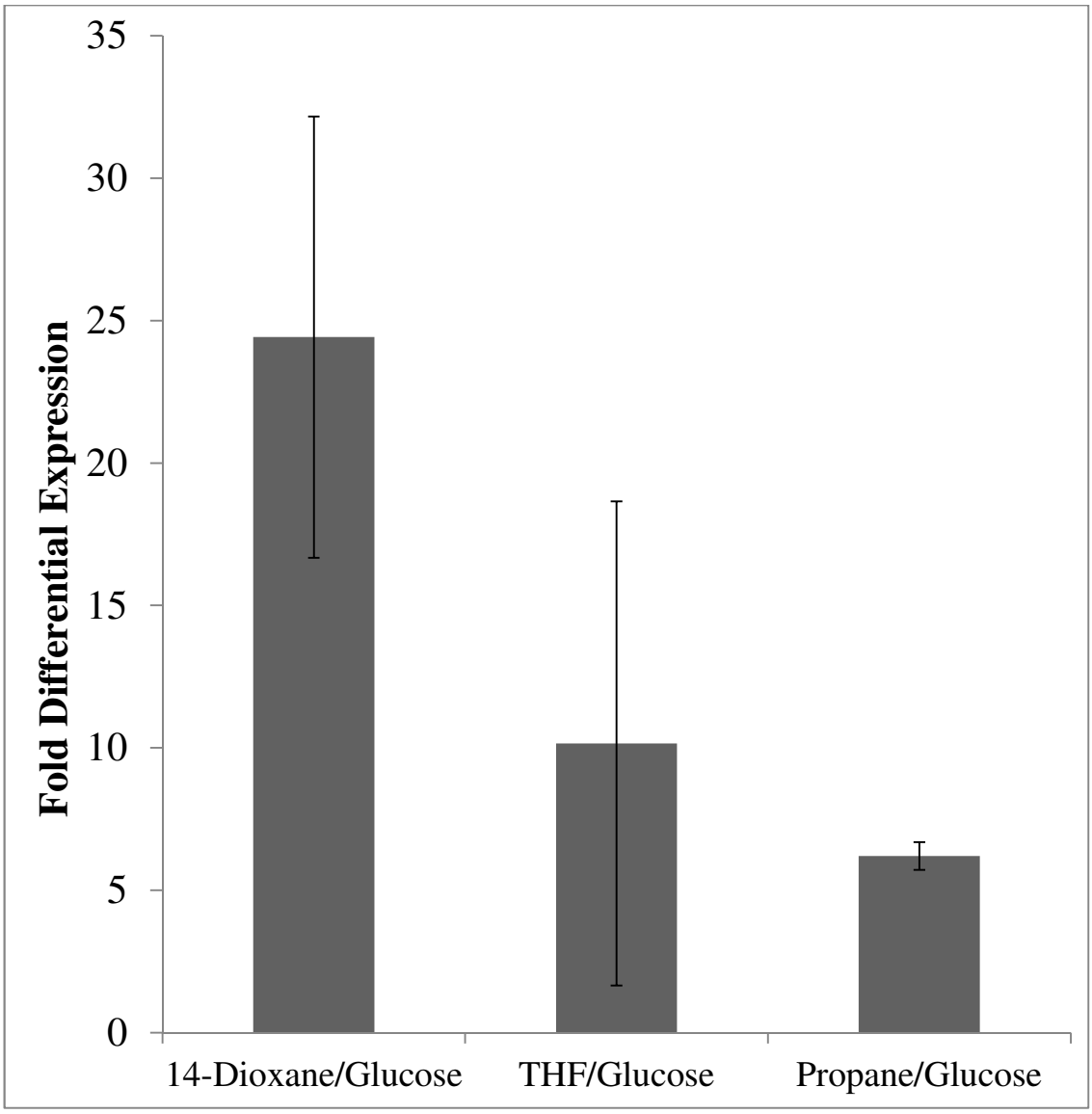


Figure 5.4. Induction of *thmA* gene expression. Glucose-grown strain CB1190 were exposed to 1,4-dioxane (5 mM), THF (5 mM), or propane (20% vol./vol. total headspace). After 8 hours of exposure, RNA was extracted and transcripts were quantified using RT-qPCR. Fold-differential expression values were calculated relative to the expression of cells exposed to 5mM glucose (control) and were normalized to multiple housekeeping genes, RNA polymerase sigma factor *RpoD* (Psed_0376) , triphosphate isomerase *tpi* (Psed_3417), and thiamine biosynthesis protein *thiC* (Psed_6168).

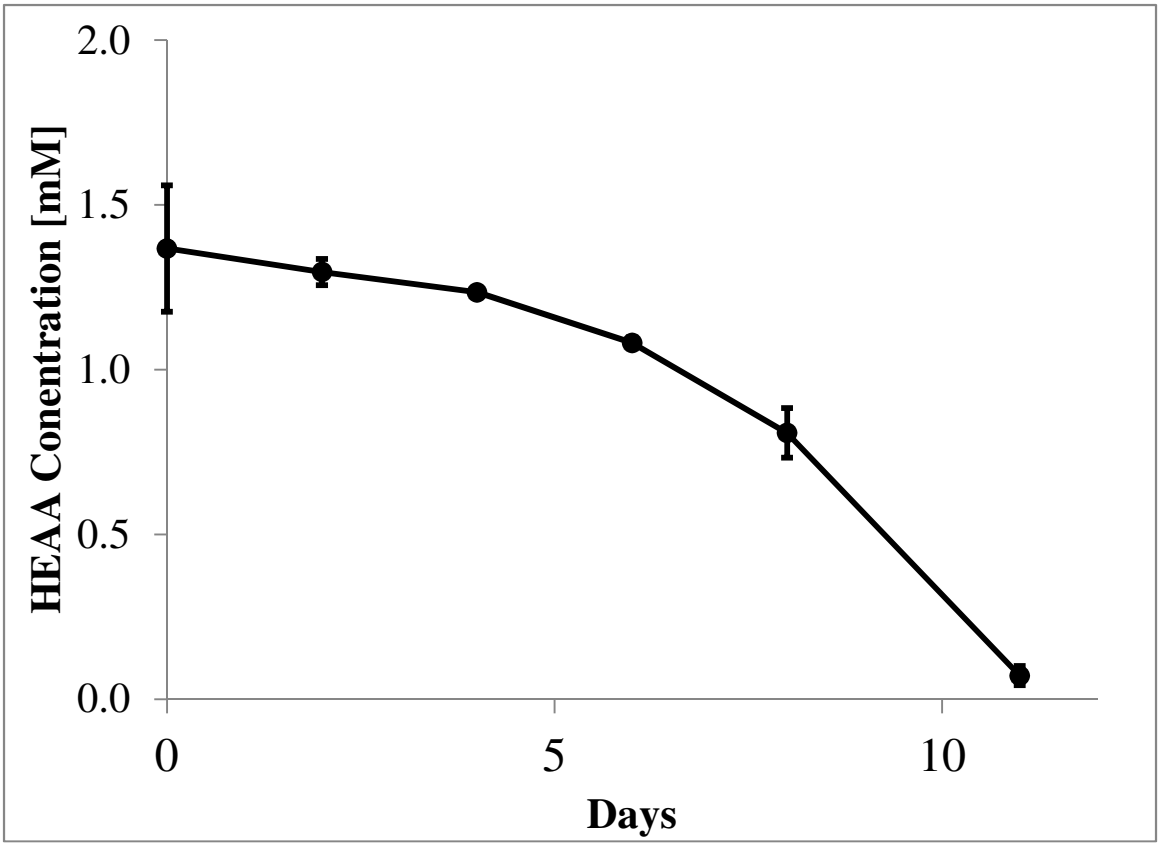


Figure 5.5. Metabolic degradation of HEAA. Cells strain CB1190 (n=5) were grown in AMS medium were ameneded with 1.5 mM of HEAA.

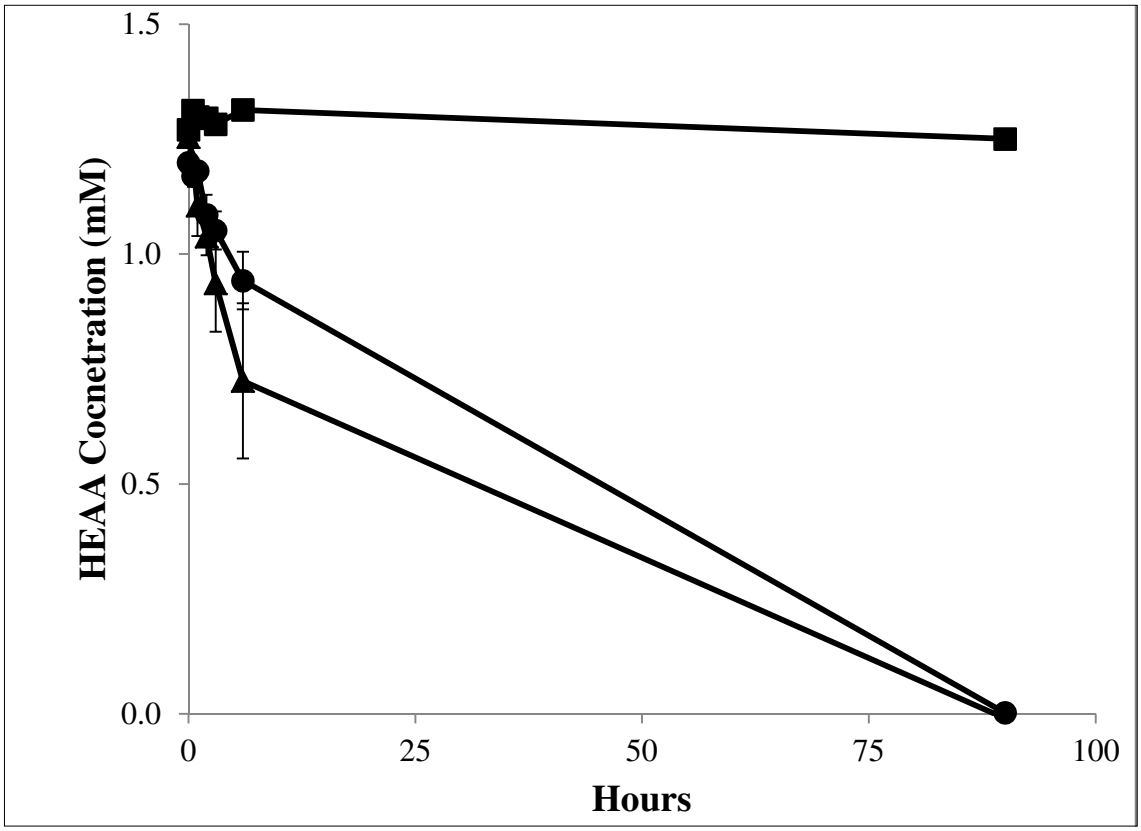


Figure 5.6. Effect of acetylene on HEAA degradation. Removal of HEAA was monitored for three conditions: ■ (square), abiotic samples; ▲ (triangle), acetylene-exposed cells; and ● (circle), non-acetylene exposed cells. Each condition was tested in triplicate (n=3). Cells of strain CB1190 were grown on 1,4-dioxane, washed, and resuspended in phosphate buffer (pH = 7).

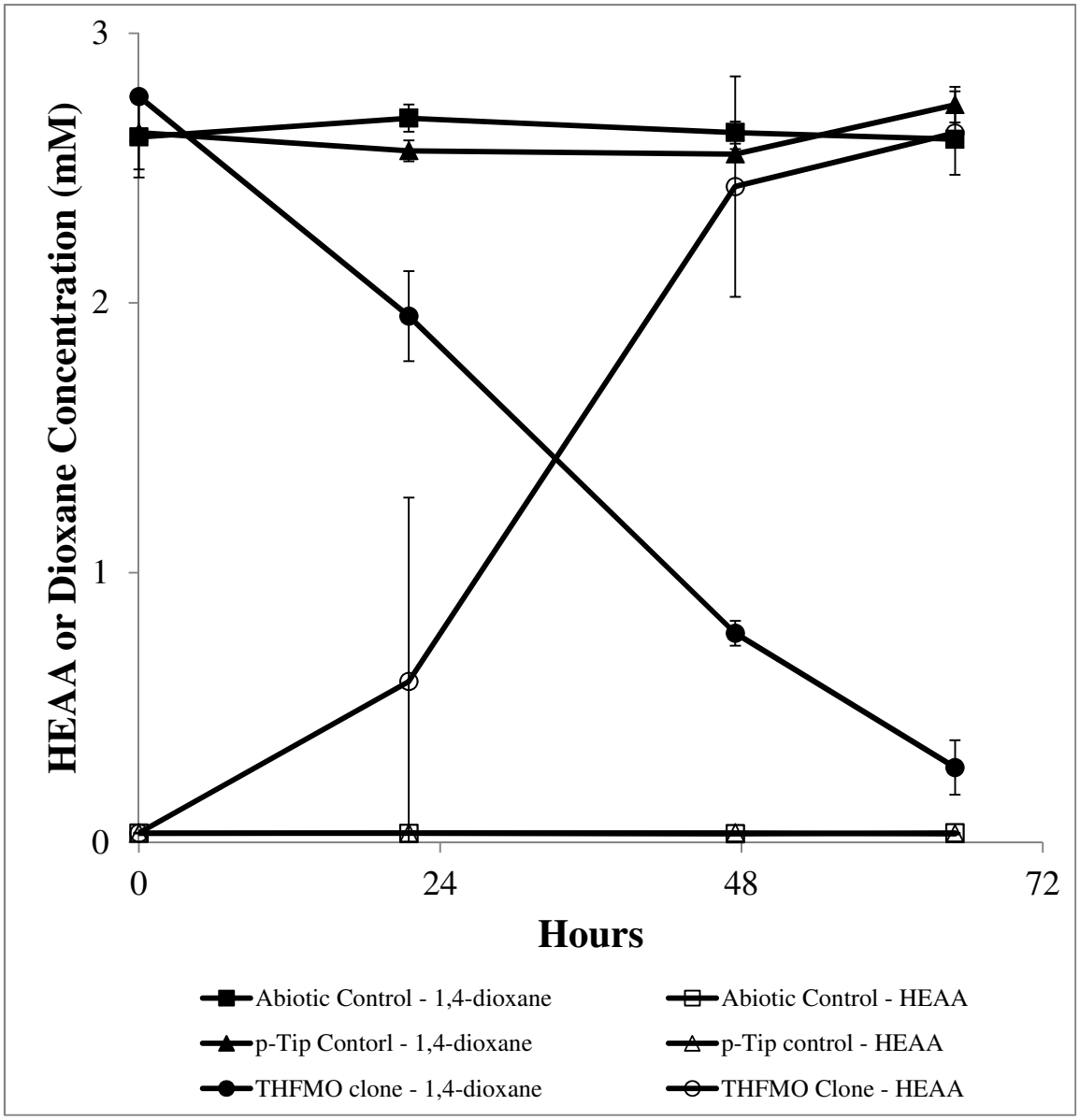


Figure 5.7. Biotransformation of 1,4-dioxane to HEAA by THF monooxygenase expression clones. RHA1/pTip-thfmo were amended with 2.5 mM of 1,4-dioxane. Abiotic controls and clones containing an empty pTip vector (RHA1/pTip-QC2) were also amended with 2.mM of 1,4-dioxane. 1,4-Dioxane concentrations were determined by GC-FID and HEAA concentrations were determined by LC-MS/MS. Points and respective error bars represent the average concentration and standard error for each condition run in duplicate (n = 2).

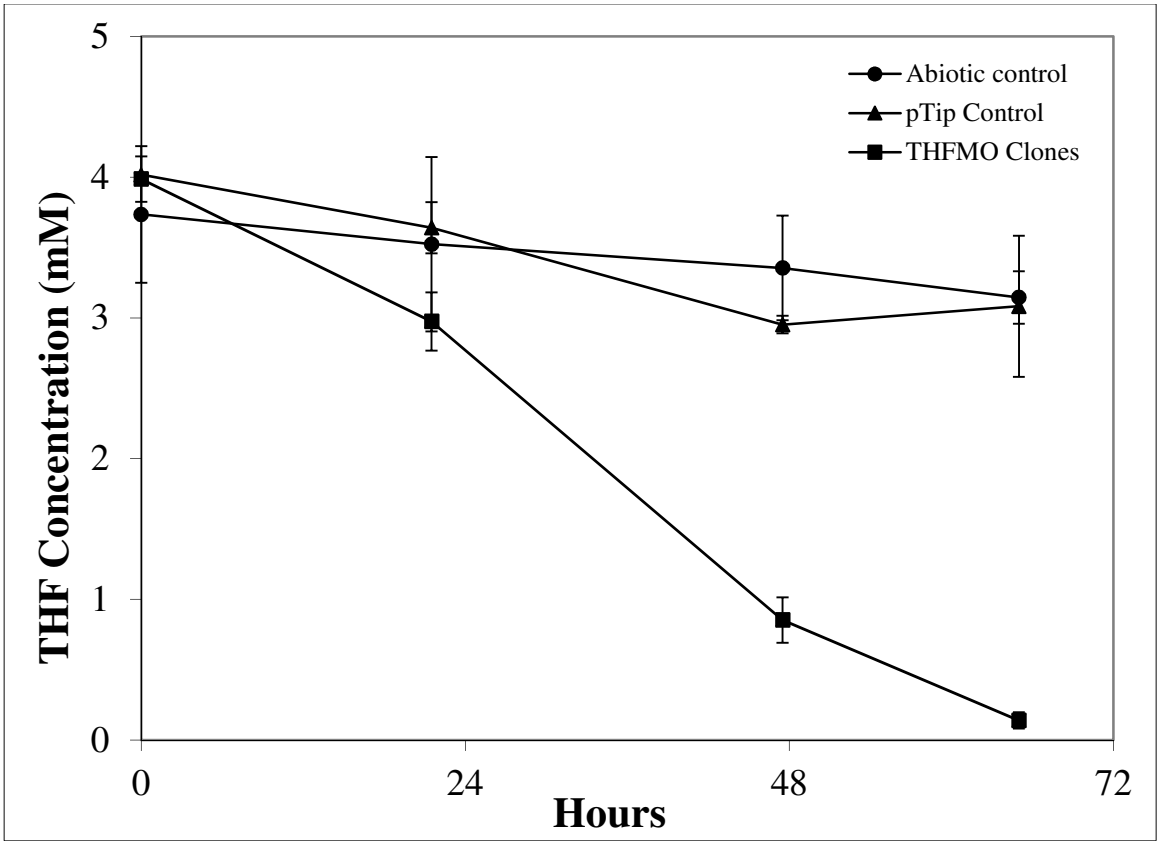


Figure 5.8. Biotransformation of THF by THF monooxygenase expression clones. RHA1/pTip-thfmo expression clones were amended with 4 mM of THF. Abiotic controls and clones containing an empty pTip vector (RHA1*i*/pTip-QC2) were also amended with 4 mM of THF. The THF concentrations were determined by GC-FID. Points and respective error bars represent the average concentration and standard error for each condition run in duplicate (n = 2).

5.4. Discussion.

In this chapter, the involvement of the THF monooxygenase gene cluster, *thmADBC*, in the initial hydroxylation of 1,4-dioxane and THF in strain CB1190 was demonstrated. Transcriptional analyses based on microarray and RT-qPCR results reported in Chapter 4 had identified THF monooxygenase genes *thmADBC* as the only bacterial multicomponent monooxygenase in the strain CB1190 to be up-regulated only during growth on 1,4-dioxane and not glycolate, relative to pyruvate. Direct comparison of the 1,4-dioxane and glycolate transcriptomes also shows *thmADBC* to be significantly up-regulated during growth on 1,4-dioxane. The transcriptional evidence of the involvement of the THF monooxygenase in 1,4-dioxane biotransformation was corroborated by phenotypic evidence using clones of *R. jostii* RHA1 containing an expression vector with the *thmADBC* sequence (RHA1/pTip-thfmo). These expression clones demonstrated the ability to transform 1,4-dioxane rapidly to HEAA (Figure 5.7). In addition to the THF monooxygenase genes, microarray results reported here and in Chapter 4 are in agreement that the entire *thm* cluster (Psed_6974 to Psed_6982) and genes immediately upstream (Psed_6971 to Psed_6973) are up-regulated. The involvement in 1,4-dioxane degradation of the non-monooxygenase genes in the *thm* cluster proposed in Chapter 4 was verified in this chapter using molecular and biochemical studies.

The RHA1/pTip-thfmo expression clones also demonstrated that the enzyme catalyzing the transformation of HEAA, a 1,4-dioxane degradation intermediate, is not the THF monooxygenase. Stoichiometric accumulation of HEAA as a dead-end product was observed as the RHA1/pTip-thfmo expression clones transformed 1,4-dioxane. This result is in disagreement with the hypothesis that the same monooxygenase was involved in the hydroxylation of both 1,4-dioxane and HEAA (Mahendra *et al*., 2007). The accumulation of HEAA as a dead-end product, however, has been reported in another isolate, *Pseudonocardia* sp. ENV478, which possesses a *thm* gene cluster (Vainberg *et al*., 2006; Masuda, 2009). Both *Pseudonocardia* sp. ENV478 and *P. tetrahydrofuranoxydans* are only capable of co-metabolic degradation of 1,4-dioxane during growth on THF. Although *P. tetrahdyrofuranoxydans* cannot grow on 1,4-dioxane, it was not found to accumulate HEAA (Mahendra et al., 2007). Therefore, strain CB1190 and *P. tetrahydrofuranoxydans* both possess genes encoding for an enzyme that catalyzes the transformation of HEAA that *Pseudonocardia* sp. ENV478 does not. Prior studies showed that acetylene inhibits 1,4-dioxane degradation in strain CB1190 grown on either THF or 1,4-dioxane (Mahendra et al., 2007). However, results reported here indicate that acetylene does not inhibit HEAA degradation in strain CB1190 cells grown on 1,4-dioxane. These biochemical responses to acetylene and the fact that HEAA accumulates during 1,4-dioxane degradation by the RHA1 expression clones reveal that a monooxygenase is not responsible for HEAA biotransformation. An ether-cleaving enzyme, a diglycolic acid (DGA) dehydrogenase (DGADH), was identified in *P. tetrahydrofuranoxydans*, whose N-terminal amino acid sequence is homologous to superoxide dismutases (Yamashita *et al*., 2005) and to the manganese iron superoxide dismutase (Psed_5669) in strain CB1190 (blastp indicates 67% identity and 73% similarity). However, this superoxide dismutase is not differentially expressed during growth on 1,4-dioxane versus glycolate or 1,4-dioxane versus pyruvate. The N-terminal amino acid sequence from a DGADH purified form *Sphingomonas terrae* is homologous to a lactate dehydrogenase in *Sinorhizobium meliloti* and to a

glycolate oxidase in *Mesorhizobium loti* (Enokibara and Kawai, 1997; Yamashita et al., 2005). In strain CB1190, the *S. terrae* DGADH sequence shares 61% identity and sim-Larity to (*S*)-2-hydroxy-acid oxidase (Psed_5322), but this gene is also not differentially expressed during growth on 1,4-dioxane relative to growth on glycolate or pyruvate. A variety of enzymes have been reported for ether bond cleavage: monooxygenases, dioxygenases, ether hydrolases, carbon-oxygen lyases, peroxidases, laccases, and β-etherases (White *et al.*, 1997). The lack of acetylene inhibition suggests that monooxygenases are not involved. Although any gene up-regulated on 1,4-dioxane that could potentially be involved in ether bond splitting of HEAA, these differentially expressed genes are likely candidates: one encoding a peroxidase (Psed_2329) which has a $\log_2 FC_{1,4\text{-dioxane/glycolate}}$ of 1.82; one encoding a hydroxyacid-oxoacid transhydrogenase (Psed_6971) which has a $\log_2 FC_{1,4\text{-dioxane/glycolate}}$ of 3.02; and one encoding a lactate dehydrogenase (Psed_6972) which has a $\log_2 FC_{1,4\text{-dioxane/glycolate}}$ of 3.39. It is also a possibility that the enzyme that catalyzes HEAA biotransformation is not differentially expressed on 1,4-dioxane relative to either pyruvate or glycolate. Regardless, further studies are needed to identify the HEAA-degrading enzyme.

The THF metabolic pathway in strain CB1190*s* is examined for the first time in this chapter. Transcriptomic microarray analysis of THF- and succinate-grown strain CB1190 cells agreed with the Northen blot analysis performed with *P. tetrahydrofuranoxydans* that showed genes in the *thm* cluster to be expressed during THF utilization (Thiemer et al., 2003). Although a probe set for the THF monooxygenase oxygenase component α-subunit *thmA* is not on the microarray, RT-qPCR shows that THF induces gene expression of *thmA* by 10.2-fold ($\log_2 FC = 3.35$), relative to glucose. The only disagreement between the Northern blot analysis performed with *P. tetrahydrofuranoxydans* and the transcriptional analyses performed with strain CB1190 is that the aldehyde dehydrogenase gene (Psed_6981), which has been proposed to be involved in the conversion of 4-hydroxybutyraldehyde to 4-hydroxybutyrate in *P. tetrahydrofuranoxydans* and *Graphium* sp. ATCC 58400 (Skinner *et al*., 2009), is up-regulated during growth on THF in strain CB1190 and not in *P. tetrahydrofuranoxydans*. An alcohol dehydrogenase had been previously proposed to catalyze the conversion of 2-hydroxytetrahydrofuran to γ-butyrolactone (Skinner et al., 2009). Among the genes only up-regulated on THF relative to pyruvate, is one annotated as the alcohol dehydrogenase GroES domain protein (Psed_0131). Although the activity of this alcohol dehydrogenase needs to be confirmed, this is the first evidence of induced alcohol dehydrogenase gene expression during THF degradation. The conversion of 4-hydroxybutyrate to succinate semialdehyde is proposed to be catalyzed by the product of at least one of two differentially expressed genes on the plasmid pPSED02, a lactate dehydrogenase (Psed_6970) or hydroxyacid-oxoacid transhydrogenase (Psed_6971). Therefore, based on transcriptomic microarray analysis, the proposed THF degradation pathway starts with (i) the hydroxylation of THF to 2-hydroxytetrahydrofuran by the THF monooxygenase (*thmADBC*), (ii) conversion of 2-hydroxydtetrahydforuan to γ-butyrolactone by the alcohol dehydrogenase (Psed_0131), (iii) abiotic hydrolysis of γ-butyrolactone produces 4-hydroxybutyrate, (iv) conversion of 4-hydroxybutyrate to succinate semialedhyde via either transhydrogenase (Psed_6971) or dehydrogenase (Psed_6970), (v) the oxidation of succinate semialdehyde to succinate by succinate semiladelhyde dehydrogenase (*sad*), (vi) the succinate finally enters the tricarboxylate cycle. The 4-hydroxybutyrate could potentially be polymerized to poly-beta-hydroxybutyrate (PHB) since two poly-beta-hydroxybutyrate polymerase genes

(Psed_2979 and Psed_2990) exist on the strain CB1190 chromosome. The function of the THF monooxygenase was confirmed by the ability of the *R. jostii*/pTip-thfmo expression clones to degrade THF. Further studies that identify the intermediates of THF degradation by strain CB1190 and that confirm the function of the genes proposed to be involved in the metabolism of THF should be tested.

Chapter 6

An inducible propane monooxygenase is responsible for *N*-nitrosodimethylamine degradation by *Rhodococcus jostii* RHA1

6.1. Introduction.

Recently recognized as a drinking water contaminant (Mitch *et al.*, 2003), N-nitrosodimethylamine (NDMA) is now closely monitored by municipal water providers to minimize human exposure (OEHHA, 2006; EBMUD, 2006; MOE, 2006). Concern has developed due to NDMA's potent mutagenicity and carcinogenicity (IARC, 1987) coupled with increasing awareness of its presence as a groundwater contaminant associated with liquid rocket propellants, certain industrial processes, and chlorine-based water reuse projects (Mitch *et al.*, 2003; MOE, 2006; Sedlak *et al.*, 2005). The combination of high subsurface mobility coupled with poor attenuation by volatilization, sorption, and abiotic and biological processes (Mitch *et al.*, 2003) has resulted in groundwater plumes that contain measurable quantities of NDMA following decades and miles of subsurface propagation (WateReuse, 2006). Despite its recalcitrance in groundwater, it has recently been shown that NDMA can be attenuated in wastewater treatment systems (Sedlak *et al.*, 2005) and soils (Bradley *et al.*, 2005; Drewes *et al.*, 2006; Yang *et al.*, 2005), presumably through the involvement of microorganisms. This dichotomy between persistence and potential biodegradability necessitates a more detailed understanding of the biochemical mechanisms that contribute to NDMA degradation.

Microorganisms grown on substrates such as propane, methane, and toluene have been shown to rapidly oxidize NDMA in the laboratory (Fournier *et al.*, 2006; Sharp *et al.*, 2005). In these cases, evidence from inhibition and induction experiments along with observations of requisite oxygen consumption suggests that propane monooxygenases (PrMO), soluble methane monooxygenases (sMMO), and toluene monooxygenases (TMO) are most likely involved in these transformations. In addition, experiments with *Escherichia coli* clones expressing TMO inserts confirmed the role of toluene 4-monooxygenase (T4MO) in NDMA oxidation, while cupric selection for soluble rather than particulate MMO confirmed the role of sMMO (Sharp *et al.*, 2005). The involvement of PrMO is less understood, as the traditional boundary can blur between enzymes oxidizing gaseous and liquid *n*-alkanes. Liquid alkanes are typically oxidized by alkane monooxygenases (AlkMO), but AlkMO can be induced by propane in some bacteria but not in others (Hamamura *et al.*, 2001; Lopes Ferreira *et al.*, 2007). Regardless of the class of monooxygenase involved, NDMA is transformed with little observed benefit to the cells and no evidence of cellular growth, despite the production of oxidized products, including formaldehyde, methylamine, and methanol, that can be incorporated into primary metabolic pathways (Fournier *et al.*, 2006; Sharp *et al*., 2006; Yoshinari and Shafer, 1990). Limited evidence for metabolism suggests that non-energy-generating transformations, such as co-metabolic oxidation reactions, play an important role in the biological attenuation of NDMA.

Rhodococci are soil heterotrophs of the order *Actinomycetales* with a noted diversity of functional enzymatic activities (Gurtler *et al.*, 2004; Larkin *et al.*, 1998). Collectively, this order is biologically and economically significant for the production of a diverse array of enzymes involved in the production of commercial secondary metabolites, antibiotics, and the metabolism of xenobiotic compounds for environmental and industrial applications (O'Keefe and Harder, 1991). Global genomic, transcriptomic, and functional

analyses of *Rhodococcus jostii* RHA1 reveal tremendous enzymatic diversity with the potential to grow on a wide variety of aromatic compounds, carbohydrates, nitriles, and steroids as the sole carbon and energy sources (see McLeod *et al.*, 2006 and references therein). Indeed, the genome of strain RHA1 is predicted to encode over 200 oxygenases, including both PrMO and AlkMO gene clusters. The genetic blueprint provided by the annotated genome and the development of a corresponding global microarray facilitate the identification of genes responsible for physiological traits of strain RHA1, especially for growth and enzymatic activity. For this reason, strain RHA1 was selected as a model organism to better understand the genetics and biochemistry of NDMA transformation.

To gain insight into NDMA degradation by strain RHA1, we analyzed the effects of propane on gene expression and NDMA removal. Here, we report the first experimental evidence for NDMA degradation by a PrMO. First, the kinetics associated with NDMA degradation in strain RHA1 was characterized. Then, the candidate genes associated with propane and NDMA oxidation were identified and quantified through differential expression, as assayed by global transcriptional microarray analysis and reverse transcriptase-quantitative PCR (RT-qPCR). Finally, targeted gene disruption was used to confirm the role of PrMO in NDMA degradation and exclude the role of AlkMO in the observed degradation.

6.2. Materials and Methods.

6.2.1. Strains and plasmids.

The bacterial strains and plasmids that were used or made in this study are listed in Table 6.1.

6.2.2. Cellular growth and harvest conditions.

All strains were grown aerobically in batch flasks at 30°C and 150 rpm to ensure viability, enzyme activity, and adequate mixing unless otherwise noted. For maintaining strain RHA1 and raising cells for degradation assays, cells were incubated in Luria-Bertani (LB) medium (Becton Dickinson, Sparks, MD) in equilibrium with the atmosphere. Minimal salts medium (Sharp *et al.*, 2005) was amended with 23 mM pyruvate (Fisher Scientific, Fair Lawn, NJ) or a 20% headspace volume addition of 99.5% purity propane (Matheson, Newark, CA) in sealed flasks containing from 12% to 15% (vol/vol) growth medium. The quantity of electron donors was appropriate to prevent oxygen limitation. Deionized water, produced from a Barnstead Nanopure II water-purifying system, was used for preparation of stock solutions, buffer, and medium. Culture growth phase was determined by monitoring optical density at 600 nm (OD_{600}). Cells were harvested from culture medium in the late exponential phase of growth at OD_{600} of ~0.7 and 1.5 for cells grown, respectively, on propane or liquid organics. Cells were harvested with a Beckman Avanti J-301 centrifuge (Palo Alto, CA) at 15,000 × *g* for 5 min. Cells exposed to propane were centrifuged and transferred to a new container to remove residual propane. The resulting pellet was then suspended in 0.1 M phosphate buffer (pH 7) solution. For cells grown with liquid substrates, this washing process was repeated two more times. Washed cells were suspended with buffer to a target density to optimize measurement of transformation rates (adjusted OD_{600} of between 0.1 and 7.0), and a fraction of the cells were frozen for future protein analysis.

6.2.3. Quantification of NMDA removal.

Cell suspensions to evaluate and quantify the biodegradation of NDMA were incubated in 125-ml bottles sealed with Teflon-lined Miniert valves (Altech, Deerfield, IL). These flasks contained washed cells suspended in phosphate buffer as described above. NMDA (99+%) was purchased from Acros Organics (Geel, Belgium), and additions to experimental cultures, controls, and standards have been described previously (Sharp *et al.*,

2005). NDMA extractions were performed by removing 2-ml samples at each time point followed by equilibration with an equal volume of high-purity methylene chloride (EM Science, Darmstadt, Germany). Methylene chloride extracts containing NDMA were analyzed by previously described methods (Mitch and Sedlak, 2002; Sharp *et al.*, 2005) involving tandem mass spectrometry. The detection limit for the liquid-liquid extraction as determined by standard curve was approximately 5 µg NDMA liter^{-1}.

NDMA degradation rates as a function of protein density were obtained by monitoring initial loss during cellular incubations in phosphate buffer (Sharp, 2006). Each rate consisted of an average of four linearly spaced time points run over 2 h. Biomass was quantified as mass of cellular protein, and there was no significant change in cell density during the course of these incubations. Cellular pellets from frozen 1.5-ml samples were suspended in 210 µl of 48 mM NaOH. The mixture was sheared by bead beating for 5 min and boiled at 100°C for 20 min. The digest was then centrifuged in an Eppendorf 5417C (Hamburg, Germany) for 10 min at 10,000 × *g* to remove cellular debris from the supernatant. Protein mass per volume was quantified from 50 µl or appropriate dilutions of the supernatant using the Coomassie Plus protein assay reagent kit with bovine serum albumin as the standard (Pierce Biotechnology, Rockford, IL). Degradation rates were graphed as a function of substrate concentration to determine the interdependence of these variables. An iterative best fit for nonlinear regression with 95% confidence intervals was applied to the Monod kinetic model for a constant cell density (Equation 6.1):

$$V_C = \frac{V_{max,n}C}{K_n + C}$$

(Equation 6.1)

Components of this equation were defined as follows. V_c is the reaction velocity (µg NDMA mg protein^{-1} h^{-1}) at NDMA concentration C (µg NDMA liter^{-1}), $V_{max,n}$ represents the maximum reaction velocity (µg NDMA mg protein^{-1} h^{-1}), and K_n is the half-saturation constant (µg NDMA liter^{-1}).

6.2.4. Analysis of global gene expression using spotted microarrays.

For transcriptomic analysis, 65-ml or 80-ml liquid cultures of *R. jostii* RHA1 were grown in sealed 1-liter flasks containing minimal medium amended, respectively, with 23 mM pyruvate or atmospheric air containing 20% gaseous bulk propane (+99.5% purity) as the sole electron donor and carbon source. Triplicate cultures for each condition were harvested in late-exponential growth. OD_{600} of approximately 0.7 and 1.3, respectively, were selected to correspond to 70% of the maximal OD_{600} reached in these incubations as determined by prior growth curves. Upon achieving the target density, 1/10 volumes of "stop solution" (5% phenol [pH 5] in ethanol) were added (Bernstein *et al.*, 2002). Cells were collected by centrifugation at 4,900 × *g* for 10 min at 4°C, suspended in 1.0 ml of

the supernatant plus 2.0 ml RNAprotect (QIAGEN), and incubated for 5 min at room temperature. Cells were then centrifuged at 13,000 × *g* for 2 min at room temperature. Pellets representing 40 ml of culture were each frozen on dry ice and stored at 80°C. RNA extraction was performed on the harvested pellets by adapting previous methods (Gonçalves *et al.*, 2006). Total RNA isolation involved vortexing with glass beads, hot phenol, and sodium dodecyl sulfate at final concentrations of 14.3% and 0.9% (vol/vol), respectively. Debris was precipitated with acetate followed by the addition of 4.0 ml phenolchloroform (1:1 [vol/vol]). Nucleic acids were precipitated with acetate plus isopropanol, treated with DNase, and purified with an RNeasy mini column (QIAGEN). Synthesis of cDNA from the extracted RNA, indirect Cy labeling, and microarray hybridizations were performed as described previously (Gonçalves *et al.*, 2006), with the following modifications. The cDNA synthesis mixture included 1.5 μg random hexamer primers (Invitrogen) per 6.0μg RNA, which was brought to 15.3 μl with diethyl pyrocarbonate-treated water. After RNA denaturation for 10 min at 70°C followed by cooling for 5 min on ice, cDNA synthesis components were added to final concentrations of 0.46 mM each dATP, dCTP, and dGTP; 0.19 mM dTTP; 0.28 mM aminoallyl-dUTP (Ambion); 0.01 M dithiothreitol; 10 U RNaseOUT (Invitrogen); and other ingredients as described previously (Gonçalves *et al.*, 2006). Equal amounts of differentially labeled cDNA, consisting of 50 million pixels measured by Image-Quant 5.2 (Molecular Dynamics), from propane- and pyruvate-grown cells were hybridized at 42°C for 17 h. After the automated washes, the slides were dipped in 0.2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and dried by centrifugation at 225 × g for 5 min at room temperature. For one of the three hybridizations, the Cy3 and Cy5 dyes were reversed (i.e., cDNA from the propane treatment was labeled with Cy5 rather than Cy3) to control for dye bias (Tseng *et al.*, 2001). The microarray contained duplicate 70-mer oligonucleotide probes for 8,213 RHA1 genes, representing 89% of the predicted genes (McLeod *et al.*, 2006). The probes were designed and synthesized by Operon Biotechnologies, Inc. (Huntsville, AL).

Microarray spot intensities were quantified using Imagene 6.0 (BioDiscovery, Inc.). Expression ratios were normalized using GeneSpring version 7.2 (Silicon Genetics) by the intensity-dependent Lowess method, with 20% of the data used for smoothing. Average normalized expression ratios were calculated for each gene. Significant differential expression on propane versus pyruvate was defined as absolute ratios of ≥4.0 and Student's *t* test $P < 0.05$.

Details of the microarray design, transcriptomic experimental design, and transcriptomic data have been deposited in the NCBI Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO Series accession no. GSE8480.

6.2.5. Quantification of gene expression by RT-qPCR.

The above extracted RNA was also used for RT-qPCR analysis. While trace genomic DNA contamination present after DNase/RNeasy mini column cleaning was acceptable for the cDNA-specific Cy labeling used in the microarray study, further removal of

contaminating DNA was conducted prior to reverse transcription. This was accomplished through two more rounds of DNase I treatments using the DNAfree kit (Ambion) followed by an additional cleanup step in a RNeasy MinElute Cleanup kit (QIAGEN) to remove any other impurities. All treated RNA was stored at 80°C prior to further use. The transcripts of two genes from the PrMO operon (*prmA* and *prmB*) and one gene from the AlkMO operon (*alkB*) were selected for quantification. TaqMan primer-probe sets labeled with 6-carboxyfluorescein (FAM) using 6-carboxytetramethylrhodamine (TAMRA) as a quencher were purchased from PE Applied Biosystems (Foster City, CA). The primer-probe set for *prmA*, *prmB*, *alkB*, and DNA polymerase IV (DNA pol IV) genes are listed in Table 6.2. The DNA pol IV gene was used as an internal reference (housekeeping gene) for quantification. Primers and probes were designed for quantitative PCR using ABI Prism Primer Express Software (Applied Biosystems). For each design, sequence specificity was confirmed using the NCBI BLAST algorithm optimized for short nucleotide sequences on the GenBank database (www.ncbi.nlm.nih.gov).

Differential expression of the target genes *prmA*, *prmB*, and *alkB* was quantified using a two-step RT-qPCR method adapted from a previous study (Johnson *et al.*, 2005). For the first step, cDNA was synthesized using the TaqMan reverse transcription reagents kit (Applied Biosystems). Each 10-µl PCR volume contained 2 µl of RNA (~0.001 ng total RNA) and 0.5 µM of each reverse primer. For the second step of the RT-qPCR method, the reverse-transcribed samples were amplified on an ABI Prism 7000 sequence detection system (Applied Biosystems). The target and housekeeping genes were quantified in triplicate. Each 25-µl qPCR volume contained 2 µl of the reverse-transcribed RNA samples, 12.5 µl of 2×TaqMan universal PCR master mix (Applied Biosystems), 0.2 µM of probe, and 0.7 µM of each primer (forward and reverse). Thermocycling conditions were as follows: 2 min at 50°C, 10 min at 95°C, and 40 cycles of 15 s at 95°C and 1 min at 60°C. Differential expression was calculated by the Pfaffl method (Pfaffl, 2001), which takes into account the amplification efficiency of qPCR for each target gene. The mass of DNA per volume was quantified using a NanoDrop ND-1000 spectrophotometer (Wilmington, DE), according to the manufacturer's instructions.

Concentrated plasmid DNA standards were synthesized by cloning separately the gene cluster for PrMO and AlkMO into *E. coli* TG1/pBS(Kan) (Canada *et al.*, 2002). This resulted in the creation of *E. coli* TG1/pBS(Kan)PrMO.RHA1 and *E. coli* TG1/pBS(Kan) AlkMO.RHA1 (Table 6.1). The 4.3-kb PrMO gene cluster containing *prmA* was PCR amplified from chromosomal DNA of *R. jostii* RHA1 using front primer R.PrMO.f.pBS and rear primer R.PrMO.r.pBS (Table 6.3). The front primer introduced a restriction site, a new ribosome binding site, a stop codon for the upstream *lacZα* gene, and an altered start codon for the first gene in the *R. jostii* PrMO gene cluster; the rear primer introduced an alternate restriction site downstream of the last gene in the cluster. PCR products were gel extracted prior to restriction digestion, and plasmid pBS(Kan) was dephosphorylated using Antarctic phosphatase (New England Biolabs) after digestion. Analogous methods and design were used for constructing pBS(Kan)AlkMO-RHA1, with the 2.9-kb AlkMO cluster containing *alkB*, except front primer R.AlkMO.f.pBS and rear primer R.AlkMO.r.pBS were used (Table 6.3). The plasmid inserts were both confirmed by DNA sequencing.

6.2.6. Construction of knockout mutants.

The *prmA* and *alkB* genes were separately deleted in frame (Table 6.1) using the *sacB* counterselection system essentially as described previously (van der Geize *et al.*, 2001). GeneRunner software was used to design oligonucleotides with appropriate restriction sites that amplified flanking regions of each gene (Table 6.3). The mutagenic plasmids were transformed into *E. coli* DH5α by electroporation, verified by PCR, and then transformed into S17 *E. coli* competent donor cells (Simon *et al.*, 1983) maintained in 25 µg ml^{-1} kanamycin (Table 6.1). Conjunctive plasmid transfer was achieved by co-culturing the donor and *R. jostii* RHA1 on selective LB peptone plates amended with 30 µg ml^{-1} nalidixic acid and 50 µg ml^{-1} kanamycin followed by *sacB* counterselection. Final confirmation of the removal of the target gene in kanamycin-sensitive, sucrose-resistant colonies was verified by colony PCR using a pair of primers that matched sequences flanking the target gene (Table 6.3).

Table 6.1 Bacterial strains and plasmids used in this study

Strain of plasmid	Characteristic(s)	Sources or reference
Strain		
Rhodococcus jostii		
RHA1	Wild type	17
RHA027	Δ*prmA* RHA1 with *prmA* deletion	This study
RHA028	Δ*alkB* RHA1 with *alkB* deletion	This study
Escherichia coli		
DH5α	Host used for cloning the mutagenic plasmid	Bethesda Research
S17-1	Donor strain for conjugation	27
TG1/pBS(Kan)PrMO.RHA1	*E. coli* host containing gene cluster for PrMO	This study
TG1/pBS(Kan)AlkMO.RHA1	*E. coli* host containing gene cluster for AlkMO	This study
Plasmids		
pK18mobsacB	5.7-kb mobilizable suicide vector used for triple ligation; *sacB alphII*	30
pΔ*prmA*	2.2-kb fusion PCR fragment flanking Δ*prmA* cloned into pK18mobsacB; used to make strain RHA027	This study
pΔ*alkB*	2.2-kb fusion PCR fragment flanking Δ*alkB* cloned into pK18mobsacB; used to make strain RHA028	This study

Table 6.2. RT-qPCR primer and probe set used in this study

Primer or probe[a]	Sequence
prmA	
Forward primer	5'-CGCGGCGAACATCTACCT-3'
Reverse primer	5'-TGGCTACGAACAGGGTGTTG-3'
Probe	5'-TGGTCGCCGAGACAGCGTTCA-3'
prmB	
Forward primer	5'-GGACGAGGATTGACGGATTTC-3'
Reverse primer	5'-CGGCGGGTCCATCGAT-3'
Probe	5'-CGTTCGTGGCCTGCCTCTCGG-3'
alkB	
Forward primer	5'-TCCCTCACACAGCTGGAACTC-3'
Reverse primer	5'-TCGCTGTGACGCTGCAA-3'
Probe	5'-ACCACATCGTGACCAATATCTTCCTGTACCA-3'
DNA pol IV	
Forward primer	5'-GACAACAAGTTACGAGCCAAGATC-3'
Reverse primer	5'-CCTCCGTCAGCCGGTAGAT-3'
Probe	5'-CGACGGACTTCGGCAAACCGC-3'

[a]All primer-probe sets used 6-carboxyfluorescein (FAM)-labeled TaqMan TAMRA probes.

Table 6.3 Cloning, gene deletion, and knockout confirmation screening PCR primers used in this study

Primer[a]	Sequence[b]	Added Restriction Site
Cloning		
PrMO cluster		
R.PrMO.f.pBS	5'-GCCGGTACCCGATTAAGGAGGCGCACAATGAGTAGGCAAAGCCTG-3'	KpnI
R.PrMO.r.pBS	5'-GTGTGGCTCTAGACGGCTGCGGTCTACTGCGCTGTGAGG-3'	XbaI
AlkMO cluster		
R.AlkMO.f.pBS	5'-CGGCGAATTCATAAGGAGGTTCGGATCATGACGACGTCGAATATC-3'	EcoRI
R.AlkMO.r.pBS	5'-GCGGTCGTCTAGAGACATGACCTCGATGCTAGCGG-3'	XbaI
Gene Deletion		
"Up" fragment of Δ*prmA*		
ΔprmAup-f	5'-GCTCTAGAATCGCCATCTGGTCCGGTGAGTCG-3'	XbaI
ΔprmAup-r	5'-CCCAAGCTTCGGATCCCATGACAGTTCGGTGATC-3'	HindIII
"Down" fragment of Δ*prmA*		
ΔprmAdn-f	5'-CCCAAGCTTTATGTCCGACGCCGAACGCAAC-3'	HindIII
ΔprmAdn-r	5'-ATGGATCCCGTGAAATCCGTCAATCCTCGTCC-3'	BamHI
"Up" fragment of Δ*alkB*		
ΔalkBup-f	5'-CATTGCATGCTGAAGATCGGCTGGCGACACGACG-3'	SphI
ΔalkBup-r	5'-ATTAAGCTTCAACCACAGGTAGCGCTTGCGGTC-3'	HindIII
"Down" fragment of Δ*prmA*		
ΔalkBdn-f	5'-ATTAAGCTTGTGAACATCCAACCCGGCAAGC-3'	HindIII
ΔalkBdn-r	5'-GCTCTAGAAATCGGCATCGGCCATCGACC -3'	XbaI
Screening		
prmA gene		
prmA-f	5'-GTGTGACGTGCTGATGGGCTGTG-3'	
prmA-r	5'-TTGAGCAGCTCGATGGTGCAGTC-3'	
alkB gene		
alkB-f	5'-GCACATTGCCGCGATGCTTCA-3'	
alkB-r	5'-ACAGGAAGTCCTTCGACACCGTCG-3'	

[a] Cloning primers were used to introduce monooxygenase sequences into the *E. coli* host. Screening primers were used for knockout confirmation.
[b] For cloning primers, the added restriction and start codon sites are underlined. For gene deletion primers, the added restriction sites are underlined

6.3. Results.

6.3.1. NDMA removal by resting wild-type cells.

To assess the constitutive NDMA removal activity of *R. jostii* RHA1, the cells were grown independently in (i) liquid LB medium, (ii) liquid soy broth, or (iii) liquid minimal salts medium amended with pyruvate as the sole organic substrate. While growth proved most rapid and robust in LB media, cells from each condition that were washed and suspended in phosphate buffer yielded similar NDMA removal rates. The average removal rate for cells grown under these three conditions and exposed to 200 µg NDMA liter^{-1} was 0.04 ± 0.01 µg NDMA mg protein^{-1} h^{-1}. In contrast, propane-grown *R. jostii* RHA1 cells that were similarly harvested exhibited removal rates that were approximately 500-fold higher (Figure 6.1).

Monod parameters for NDMA degradation by propane-grown *R. jostii* RHA1 were calculated by applying a nonlinear fit (R^2 = 0.91) of initial disappearance rates to the Monod equation (equation 1) measured at a fixed cellular density. Propane-grown RHA1 cells that were washed and exposed to NDMA in phosphate buffer exhibited a maximum NDMA removal rate ($V_{max,n}$) of 18 ± 3µg NDMA mg protein^{-1} h^{-1} and half-saturation constant (K_n) of 20 ± 17 µg NDMA liter^{-1}.

6.3.2. Effect of propane on gene expression.

The increased NDMA degradation rates observed after growth on propane were explored by investigating the effect of propane on gene expression. Specifically, we employed a microarray with probes for 8,213 of 9,225 predicted genes of *R. jostii* RHA1 to identify global transcriptional differences between triplicate batches grown on propane versus those grown on pyruvate. Table 6.4 lists genes with significant differential expression defined as absolute expression ratios no less than 4.0 and with 95% significance ($P < 0.05$) according to Student's t test.

A number of features of this data set are striking. First, growth on propane affects expression of a limited number of genes, many of which cluster in both proximity and function. More specifically, 45 genes were up-regulated in response to propane (Table 6.4), nine of which occur in a chromosomal gene cluster associated with a putative PrMO (Figure 6.2). Another nine genes up-regulated on propane (ro10135 to 10140 and ro10143 to 10145) encode components of an ethylbenzene dioxygenase. These genes are found on the linear plasmid pRHL2 and belong to two operons that were previously found to be coregulated (Gonçalves *et al.*, 2006). Thirty-six genes were down-regulated in response to propane (Table 6.4), of which 5 are in a putative operon associated with the tricarboxylic acid

cycle (ro08824 to ro08828). Several additional down-regulated genes are involved in the metabolism of simple sugars, including glucose and pyruvate.

A second notable feature of the data set is that the most highly up-regulated gene in response to propane, with an expression ration of 125, was *prmA*, encoding the large hydroxylase subunit of PrMO. The *prmA* gene is part of a 13-gene cluster (Figure 6.2), of which 7 additional genes had expression ratios greater than 10 (Table 6.4). One of the genes in the cluster, *prmB*, lacks a probe on the microarray. Finally, significant differential expression was not demonstrated for a cluster of genes encoding a putative AlkMO (ro02534 to ro02538), despite its predicted functional similarity to PrMO (Lopes Ferreira *et al.*, 2007).

6.3.3. RT-qPCR analysis of the expression of PrMO and AlkMO genes.

To better quantify the differential expression of genes encoding PrMO and AlkMO, we employed RT-qPCR. Specifically, three genes were targeted: *prmA*, encoding the large hydroxylase subunit of PrMO; *prmB*, encoding a reductase component of PrMO not represented by a probe on the microarray; and *alkB*, encoding the large subunit of AlkMO. RNA extracts used for the prior microarray experiment were also used for RT-qPCR. The relative amount of each gene transcript was normalized to that of a housekeeping gene coding for polymerase IV (DNA pol IV).

As shown in Figure 6.3, the RT-qPCR results are generally consistent with those from the microarray. Using the Pfaffl method (Pfaffl, 2001) of relative quantification, the *prmA* and *prmB* genes of the PrMO had propane/pyruvate expression ratios of 2,450 and 3,020, respectively. Conversely, the *alkB* gene had a much lower expression ratio of 3.2. The levels of expression of each of these genes were significantly different on the two substrates, as determined by Student's t test ($\alpha = 0.05$ and $n = 9$). For *prmA*, the RT-qPCR expression ratio is more than 10-fold greater than the microarray value. This is not unusual for such highly expressed genes and probably indicates that the change in expression exceeded the dynamic range of the microarray analysis (Gonçalves *et al.*, 2006). The results for *prmB* confirm that, like the other genes in the putative PrMO operon, it is also up-regulated on propane (Figure 6.2).

6.3.4. Deletion strains for monooxygenase genes.

To confirm that PrMO is the primary catalyst for NDMA oxidation in *R. jostii* RHA1, knockout mutant strains were generated with deletions in *prmA* and *alkB*, respectively. As shown in Table 6.1, this resulted in the mutant *R. josti* strains RHA027 (*prmA* mutant) and RHA028 (*alkB* mutant). Growth of the engineered mutants on both solid- and liquid-

phase LB media proved rapid and reproducible, and their morphology mirrored that of the wild-type strain. Both wild-type RHA1 and the *alkB* mutant grew robustly on liquid minimal medium with propane as the sole organic substrate. In contrast, the *prmA* mutant did not grow on propane.

The constitutive removal of NDMA (Figure 6.1) enabled screening of these knockout mutants. Parallel batch cultures of wild-type *R. jostii* RHA1 and the two mutants were grown in liquid LB medium and harvested in the late exponential phase of growth. The cells were washed, suspended in phosphate buffer containing 200 µg NDMA liter^{-1}, and assayed for NDMA removal (Figure 6,4). In less than 4 h, both the wild-type strain and the *alkB* mutant removed NDMA to below detection limits. In contrast, NDMA removal by the *prmA* mutant was indistinguishable from that of the abiotic control. After 19 h, an additional sample was analyzed and still no significant biological NDMA removal by the *prmA* mutant was detected (not shown).

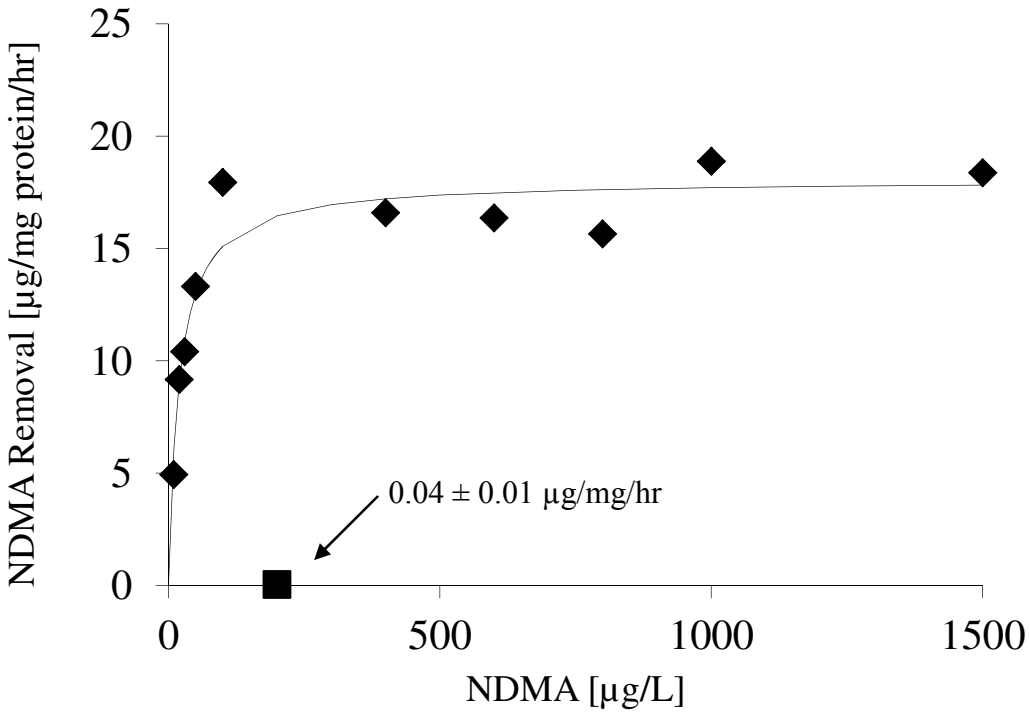


Figure 6.1. Constitutive removal of NDMA occurs at a fraction of the propane-induced rate. A Monod kinetic model (—) is fit to NDMA removal rates measured after *R. jostii* RHA1 is grown on propane (♦). The constitutive NDMA degradation rate at 200 µg/L (■) represents average removal after independent growth on three non-inducing substrates (pyruvate, LB, or soy broth).

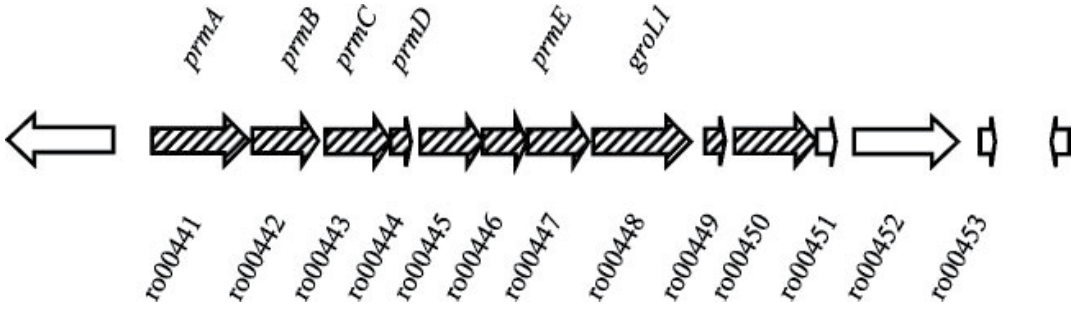


Figure 6.2. Putative operon containing prm genes. Gene annotations are available in Table 6.4. Hatched arrows represent genes that had significant expression ratios, as determined by microarray and/or RT-qPCR, indicating up-regulation on propane.

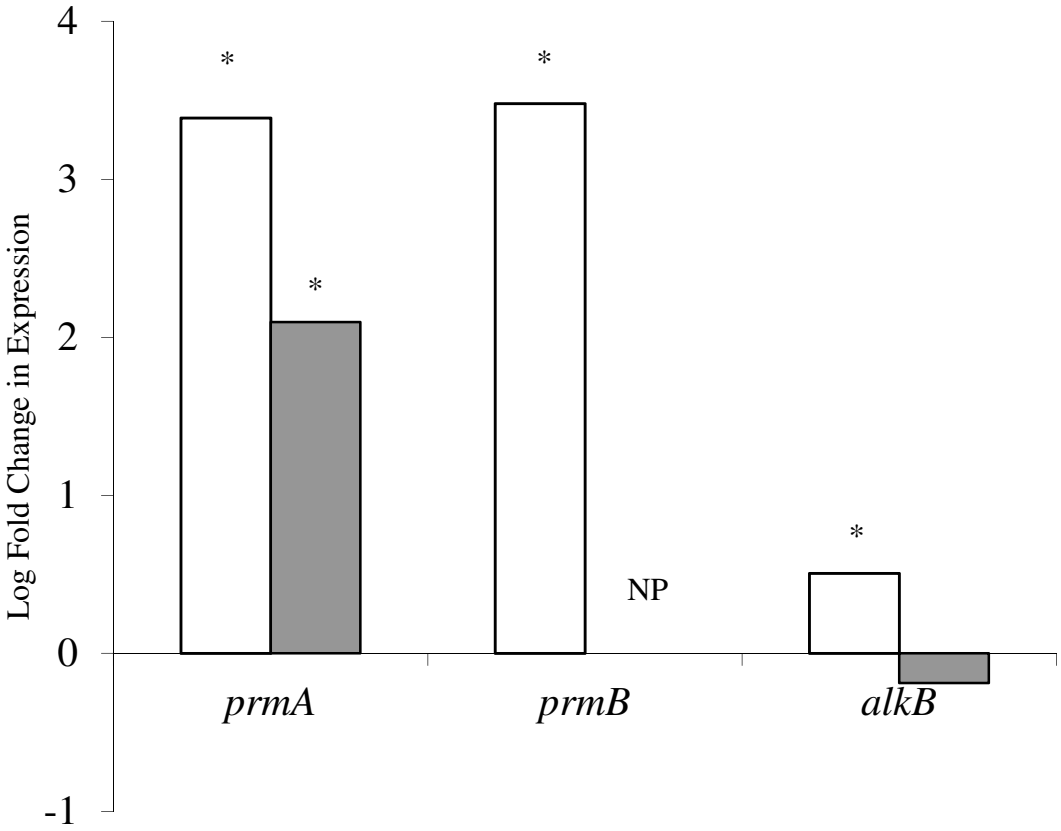


Figure 6. 3. Effect of propane on transcription of three aliphatic monooxygenase components as quantified by RT-qPCR. White (□), Pfaffl method and gray (■) spotted microarray. The microarray did not code for *prmB* (NP), preventing its quantification by that method. Asterisks denote that the propane-grown values are statistically different from those of the pyruvate-grown controls based upon Student's t-test ($P < 0.05$; $n = 9$ [3 analytical replicate measurements for each of 3 biological replicates] for RT-qPCR and $n = 6$ for microarray).

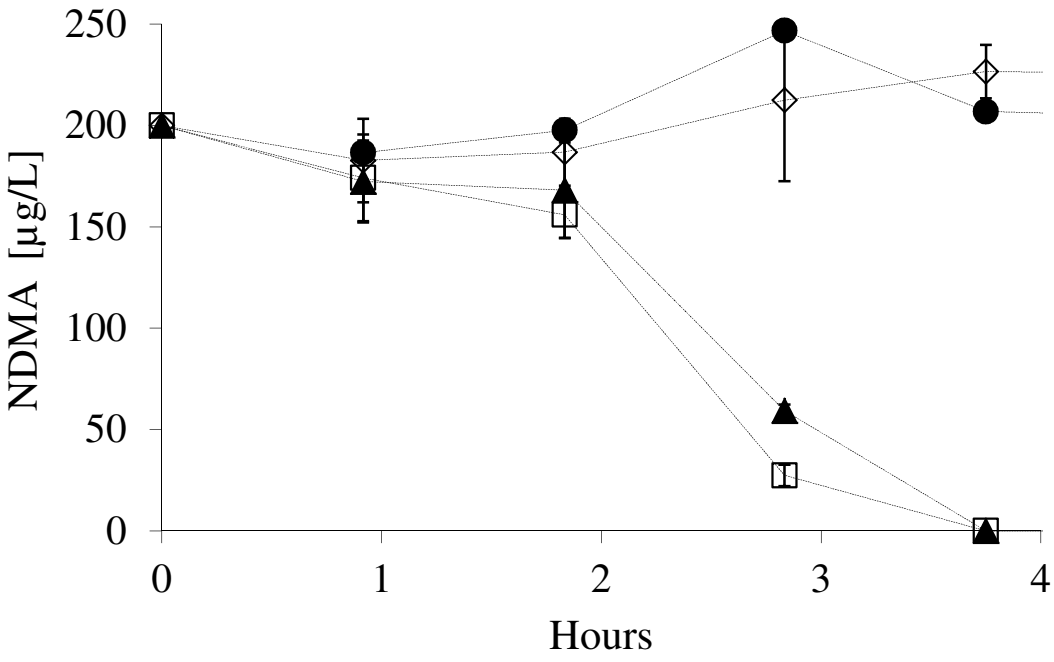


Figure 6.4. Excision of the *prmA* component of *R. jostii* RHA1 eliminates this strain's ability to transform NDMA. Where □ = wild-type RHA1; ▲ = knockout mutant RHA1Δ*alkB*; ◊ = knockout mutant RHA1Δ*prmA*; and ● = no cell control. Cells were grown in LB medium and harvested in the late exponential phase of growth. Cells were washed and then suspended in phosphate buffer to a cellular density of 510, 530, 730, and 0 mg protein L^{-1} respectively. 200 mg $NDMA^{-1}$ was added to each sample and NDMA monitored over time. Error bars portray the mean deviation of biological replicates.

Table 4. Differentially expresed genes for growth on propane relative to pyruvate

Gene ID[a]	Fold Change	Gene / Annotation
Upregulated		
ro00441	125	prmA / propane monooxygenase hydroxylase large subunit
ro00442	no probe	prmB / propane monooxygenase reductase
ro00443	13	prmC / propane monooxygenase hydroxylase small subunit
ro00444	21	prmD / propane monooxygenase coupling protein
ro00445	50	conserved hypothetical protein
ro00446	14	conserved hypothetical protein
ro00447	29	prmE / alcohol dehydrogenase
ro00448	13	groL1 / 60 kDa chaperonin GroEL
ro00449	4	conserved hypothetical protein
ro00450	8	probable glycolate oxidase FAD-linked subunit
ro00455	4	conserved hypothetical protein
ro00521	5	metabolite transporter, MFS superfamily
ro00636|ro00553	4	conserved hypothetical protein
ro01183	4	conserved hypothetical protein
ro02242	10	probable 2-pyrone-4,6-dicarboxylic acid hydrolase
ro02764	4	hypothetical protein
ro02795	4	mdlC / benzoylformate decarboxylase
ro03490	4	probable carbon monoxide dehydrogenase small subunit (ferredoxin)
ro03894	7	pcal2 / 3-oxoacid CoA-transferase alpha subuni
ro04027|ro06995|ro08131|ro08421|ro09095	5	probable triacylglycerol or secretory lipase or conserved hypothetical protein
ro04062	7	conserved hypothetical protein
ro04527	6	possible magnesium chelatase

ro04843	9	conserved hypothetical protein
ro04898	5	probable organic hydroperoxide resistance protein
ro05000	8	sensor kinase, two-component system
ro06099	6	citrate (pro-3S)-lyase
ro06305	8	rfbD / dTDP-4-dehydrorhamnose reductase
ro06664	4	non-ribosomal peptide synthetase
ro08036	8	hypothetical protein
ro08409|ro09134|ro08121	4	possible glycosyl hydrolase or metallopeptidase / glycoside hydrolase
ro09108	7	hypothetical protein
ro10116	8	bphG4 / acetaldehyde dehydrogenase
ro10126	11	bphB2 / cis-3-phenylcyclohexa-3,5-diene-1,2-diol dehydrogenase
ro10127	6	chnE / 6-oxohexanoate dehydrogenase
ro10135	31	etbC / 2,3-dihydroxybiphenyl 1,2-dioxygenase
ro10136	18	bphD1 / 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate hydrolase
ro10137	7	bphE2 / 2-oxopent-4-enoate hydratase
ro10138	10	bphF2 / 4-hydroxy-2-oxovalerate aldolase
ro10139	14	possible ketosteroid isomerase-related protein
ro10140	14	oxidoreductase
ro10143|ro10133	29	etbAa1 or etbAa2 / ethylbenzene dioxygenase alpha subunit
ro10144|ro10134	20	etbAa1 or etbAa2 / ethylbenzene dioxygenase alpha subunit
ro10145	12	etbAc / ethylbenzene dioxygenase, ferredoxin component
ro10147	5	transporter, MFS superfamily
ro10422|ro10416	5	hypothetical protein
ro11069	4	cytochrome P450 CYP257

Down-regulated

ro00995	-7	probable branched-chain amino acid ABC transporter binding protein
ro01066	-7	pyruvate dehydrogenase (cytochrome)
ro01361	-5	sugar transporter, MFS superfamily
ro02000	-6	conserved hypothetical protein
ro02071	-5	long-chain-fatty-acid--CoA ligase
ro02146	-6	groL2 / 60 kDa chaperonin GroEL
ro02448	-5	probable tellerium resistance protein
ro03152|ro08804	-5	conserved hypothetical protein
ro03258|ro03083|ro08112	-5	possible heat shock protein or MerR transcriptional regulator
ro04165	-7	possible vanillate monooxygenase oxygenase subunit
ro04524	-5	trpB2 / tryptophan synthase beta subunit
ro04557	-4	hisD2 / histidinol dehydrogenase
ro04739|ro04740	-4	hypothetical protein
ro05011	-4	acetyl-coenzyme A carboxylase carboxyl transferase alpha and beta subunits
ro05146	-4	hydrolase
ro05181	-5	hypothetical protein
ro05912	-5	probable NADH dehydrogenase subunit D
ro06034	-5	long-chain-fatty-acid--CoA ligase
ro06190	-5	chaperone protein
ro08019	-6	aldehyde dehydrogenase
ro08148	-8	possible transposase
ro08345	-4	dnaJ4 / chaperone protein
ro08348|ro03566	-12	heat shock protein
ro08449	-7	short chain dehydrogenase

ro08610	-9	probable integration host factor
ro08821	-6	hypothetical protein
ro08824	-5	fumarate hydratase, class I
ro08825	-5	probable succinate dehydrogenase hydrophobic membrane anchor protein
ro08826	-8	sdhC / succinate dehydrogenase cytochrome b subunit
ro08827	-6	succinate dehydrogenase flavoprotein subunit
ro08828	-7	sdhB3 / succinate dehydrogenase Fe-S protein subunit
ro08830	-10	metabolite transporter, MFS superfamily
ro08833	-5	epoxide hydrolase
ro10046	-4	conserved hypothetical protein
ro10375	-6	conserved hypothetical protein
ro11166	-5	probable glucose-6-phosphate 1-dehydrogenase

[a] Up-regulated, ≥4-fold change ($P < 0.05$); down-regulated, ≤4-fold chance ($P < 0.05$). Under circumstances in which there were multiple probes for a given gene, only the last one was selected for display.

6.4. Discussion.

In this study, we demonstrated that the PrMO gene cluster in *Rhodococcus jostii* RHA1 encodes both for growth on propane and removal of NDMA. *R. jostii* RHA1 has the capability to remove NDMA when grown on pyruvate, soy broth, LB medium, or propane. However, transformation rates ($V_{max,n}$) were enhanced by approximately 500-fold after growth on propane relative to these other non-inducing substrates (Figure 6.1 and 6.4). This pattern of NDMA removal and propane induction is mirrored in *Rhodococcus* sp. strain RR1 (GenBank accession no. DQ889725) and *Rhodococcus ruber* (DSM no. 43338) but not in all *Rhodococci* tested (Sharp, 2006).

The *prm* genes of RHA1 are part of a gene cluster (Figure 6.2) which appears to be conserved in other actinobacteria. The first eight genes of this cluster have homologs in *Gordonia* sp. strain TY-5 (Kotani *et al.*, 2003) and in *Mycobacterium smegmatis* (GenBank accession no. NC008596). The orders of the genes are identical in the three organisms, and the encoded proteins of *R. jostii* RHA1 are 64% to 93% identical to their homologs in the other two organisms. The *prmABCD* genes in TY-5 are part of a co-transcript induced by propane. Knockout mutagenesis in TY-5 showed that *prmB* and the seventh gene in the cluster, which we named *prmE*, are involved in propane catabolism. The *prmE* gene appears to encode a secondary alcohol dehydrogenase that catalyzes the second step of the catabolic pathway.

Our results provide both transcriptomic and phenotypic evidence for the involvement of the annotated PrMO in NDMA biotransformation. Combined oligonucleotide microarray and RT-qPCR demonstrate that transcripts from the *prm* gene cluster increased by orders of magnitude following growth on propane (Table 6.4 and Figure 6.3). Next, partial excision of *prmA* prevented an otherwise genetically identical bacterium from growing on propane and eliminated both its constitutive and induced capability to degrade NDMA (Figure 6.4). Despite the presumptive functional similarity between PrMO and AlkMO, deletion of *alkB*, encoding the large catalytic subunit of the latter enzyme, had no appreciable effect on either growth on propane or removal of NDMA (Figure 6.4). Accordingly, the *alk* operon was not significantly up-regulated during growth (Table 6.4 and Figure 6.3). These findings are consistent with observations of *Nocardioides* in which degradation of C_2-to-C_{16} *n*-alkanes was the result of two distinct systems which included one alkane hydroxylase with a homolog to *alkB* (65% nucleotide identity by pairwise alignment) that was active on alkanes larger than C_6 (Hamamura *et al.*, 2001). However, a survey of *alkB* expression in three strains of *Mycobacterium austroafricanum* has shown that expression of this gene can correlate with the transformation of smaller gaseous alkanes, including propane (Lopes Ferreira *et al.*, 2007). NDMA removal after growth on propane and the presumed expression of AlkMO was observed in one of these strains, *Mycobacterium vaccae* JOB5 (Chapter 7); however, the strain did not share RHA1's ability to constitutively remove NDMA. Though environmental NDMA degradation through induction on propane appears to extend beyond homologues of *prmA*, degradation and gene expression in systems not exposed to propane are less well understood.

Since the phenotype of the *prmA* deletion strain indicates that PrMO is solely responsible

for both propane and NDMA oxidation in *R. jostii* RHA1, the 20- to 30-fold up-regulation of *etb* genes on propane was surprising. Interestingly, *EtbA* has been implicated in the transformation of polychlorinated biphenyls, biphenyl, and ethylbenzene (Gonçalves *et al.*, 2006). Thus it is possible that propane, a comparatively inexpensive, benign, and mobile carbon source could serve as an effective alternative to ethylbenzene or biphenyl to induce expression of *EtbA* in environmental settings. It has been suggested (McLeod *et al.*, 2006) that large genomes with multiple broad-specificity catabolic enzymes such as those reported in *R. jostii* RHA1 could have a competitive advantage in constantly changing soil environments. Such metabolic diversity could result in bacteria that can sustain growth by simultaneously metabolizing an array of compounds present in trace quantities. The co-activation of multiple oxygenase enzymes, while a surprising allocation of biochemical resources, could contribute to such a strategy.

Rhodococci and other members of the *Actinomycetales* are common soil bacteria. Given the involvement of PrMO in NDMA degradation and the previously discussed identification of similar activity in related strains, quantification of genes such as *prmA* in uncharacterized communities could provide a proxy for NDMA transformation potential. Furthermore, PrMOs have been reported to degrade a diverse array of organic compounds, including chlorinated C_1-to-C_6 alkanes, vinyl chloride, chlorinated ethylenes, methyl and ethyl tertbutyl ether, and tert-amyl methyl ether (Shennan, 2006; Steffan *et al.*, 1997), suggesting broader applications.

Interestingly, a correlation between desiccation-induced cell stress and induction of the *prm* operon in *R. jostii* RHA1 was previously observed (LeBlanc *et al.*, 2008). The reason for up-regulation of *prmA* under these conditions is not obvious. However, other genes in the operon, such as *groEL* encoding a chaperone protein, may be part of a general stress response. Due to this response, stressed *R. jostii* RHA1 cells, such as those likely to occur in a subsurface vadose zone experiencing alternating wet and dry cycles, varying oxygen content, or periods of growth and starvation, could have increased activity toward low-concentration environmental contaminates such as NDMA. While it is unclear how common this feature of *prmA* regulation is, it is possible that attenuation strategies involving stressed biomass could hold promise for remediating aquifers containing analogous micropollutants without the introduction of exogenous inducers.

Chapter 7

Functional characterization of propane-enhanced *N*-Nitrosodimethylamine degradation by two *Actinomycetales*

7.1. Introduction.

N-nitrosodimethylamine (NDMA), a member of the mutagenic nitrosamines, is a confirmed animal carcinogen (IARC, 1987; Mitch *et al.*, 2003). Environmental releases have been linked to industrial sources (Sedlak *et al.*, 2005), aerospace facilities, (CalDHS, 2002; MacDonald, 2002) and to certain water-reuse scenarios (Njam and Trussell, 2001; OCWD, 2000). NDMA's persistence in groundwater aquifers has led to the closure of wells in contact with these waters, and state and provincial agencies in the US and Canada have set advisory levels at 10 and 9 ng/L in water, respectively (CalDHS, 2002; MOE, 2003).

NDMA's chemical properties render it mobile in subsurface aquifers and resistant to abiotic attenuation (Mirvish, 1975; Oliver, 1979; Thomas, 1982); however, microorganisms can play a role in the degradation of NDMA in undefined microcosms (Gunnison *et al.*, 2000; Kaplan and Kaplan, 1985; Yang *et al.*, 2005) and in pure strains (Fournier *et al.*, 2006, 2009; Rowland and Grasso, 1975; Sharp *et al.*, 2005, 2007; Yoshinari and Shafer, 1990). Though the potential for biological degradation exists, variability of NDMA removal in wastewater treatment systems (Sedlak *et al.*, 2005) suggests a dependence upon unidentified conditions associated with the expression and activity of enzymes involved in NDMA biodegradation.

Environmental NDMA concentrations are typically orders of magnitude lower than most water contaminants targeted for bioremediation. Analyses of municipal effluent have demonstrated that treated wastewater typically contains <1,000 ng NDMA L^{-1} (Gan *et al.*, 2006; Sedlak *et al.*, 2005) while groundwater associated with fuel spills can have concentrations 3–4 orders of magnitude higher (CalDHS, 2002; WateReuse, 2005). Even though investigations of the

NDMA biodegradation pathway have revealed intermediates that could be used as a carbon or nitrogen source (*i.e.*, methanol, formaldehyde, methylamine, and nitrate), strains and consortia have not been shown to grow on NDMA as a sole carbon and energy source (Fournier *et al.*, 2006; Fournier *et al*., 2009; Gunnison *et al.*, 2000; Kaplan and Kaplan, 1985; Sharp *et al.*, 2005; Sharp *et al.*, 2007; Tate and Alexander, 1975). Consequently, it is plausible that NDMA biotransformation may predominantly occur via co-metabolic reactions with little direct benefit to the transforming cells. In this scenario, degradation could be controlled by the presence of inducing substrates that promote enzymes capable of oxidizing NDMA, while at the same time compete for active sites.

Bacteria possessing monooxygenases, including propane monooxygenase, have been shown to co-metabolically degrade NDMA with rapid rates of biodegradation in Chapter 6 and other studies (Fournier *et al.*, 2006; Fournier *et al.,* 2009; Sharp *et al.*, 2005; Sharp *et al.,* 2007; Yoshinari and Shafer, 1990). Propane has previously been studied as an inducing substrate for the remediation of xenobiotics such as TCE (Wackett *et al.*, 1989) and MTBE (Smith *et al.*, 2003), and has demonstrated promise in field tests evaluating the enhanced remediation of these compounds (Steffan *et al.*, 2000; Tovanabootr *et al.*, 2001). Most reports on propanotroph isolates, which could be biased by ease of culti-

vability, tend to focus on Gram-positive, GC rich bacteria of the order *Actinomycetales* which include the *Corynebacterium*, *Nocardia*, *Mycobacterium*, and *Rhodococcus* genera (Ashraf *et al.*, 1994).

In this study, two laboratory strains of the genus *Rhocococcus* and *Mycobacterium* that grow on propane and degrade NDMA were examined (Sharp *et al.*, 2005). A detailed kinetic analysis of NDMA degradation by *Rhodococcus* sp. RR1 and *Mycobacterium vaccae* JOB5 was conducted to compare degradation rates and extent of NDMA degradation. Inhibition studies investigate the role of competition between propane and NDMA during co-metabolism, while genomic and expression investigations were used to query for functional genes involved in NDMA degradation and propane metabolism.

Table 7.1 Molecular primers used for gene amplification and comparative expression

Purpose	Primer	Sequence	Source
Degenerate primers targeting *prmA*			
	deg.prmA.F	GCTCCTACTTCCCGATGGArsarga-raarg	This Study
	deg.prmA.R	GCTGGGCGATCAGGgtyttnccrtc	This Study
Degenerate primers targeting *prmA*			
	Rhose2	ACGGSCCAYTTCTACRTCG	Lopes-Ferreira (2007)
	Roas1	CCGTARTGYTCGAGRTAG	Lopes-Ferreira (2007)
qPCR	qPCR.RR1. alkB.F	TGCGTAACGACGTCCTCAAC	This Study
	qPCR.RR1. alkB.R	AGGATCAGGAACGGGATGATC	This Study
qPCR	qPCR.RR1. prmA.F	AACCTCAAGAAGCTCTACATGAA-CAA	This Study
	qPCR.RR1. prmA.R	GAAGCCCTCCCCGAACTG	This Study
qPCR	qPCR.JOB5. alkB1.F	TCGCCACACCCGAGGAT	This Study
	qPCR.JOB5. alkB1.R	AACTGGAACGTGTAAATGCTCT-CA	This Study
qPCR	qPCR.JOB5. alkB2.F	TGCTCTACGGCGCATTGA	This Study
	qPCR.JOB5. alkB2.R	GAGATCAGGATGTACGGGATCAG	This Study

7.2. Materials and Methods.

7.2.1. Cellular Growth and Harvest.

Unless noted, reagents and equipment were identical to those described in prior work (Sharp *et al.*, 2005, 2007). The following bacteria were used: *Rhodococcus* sp. RR1 (GenBank DQ889725); *Rhodococcus jostii* RHA1 (NCBI Taxonomy ID 101510); and *M. vaccae* JOB5 (supplied by Daniel Arp and recently reclassified as *M. austroafricanum* JOB5 ATCC 29678). Strains were grown in aerobic batch culture in sealed 250 or 1,000 mL glass flasks containing 30 or 150 mL of growth medium, respectively (150 rpm and 30°C). Culture growth was determined by measuring optical density at 600nm (OD_{600}) using a Milton Roy Spectronic 20D+ visible light spectrophotometer. Basal salts medium (BSM) was amended with propane by injecting 30% (v/v headspace) of filter sterilized gas into the sealed liquid culture flasks; filter-sterilized pyruvate (20mM) was added to BSM for comparative expression studies (Sharp *et al.*, 2007). Tryptic soy broth (15 mg/L) was used to promote cellular growth while not inducing for a specific oxygenase enzyme.

Cells were harvested from culture medium late in the exponential phase of growth (OD_{600} ~ 0.6-2.0). Residual propane was removed by bubbling with nitrogen gas (300mL min^{-1} for 2 min). A bacterial pellet was isolated by centrifugation at 15,000*g* for 5 min followed by suspension

in 0.1M phosphate buffer (pH 7). After two subsequent washing steps, cell density targeted optimal visualization of transformation rates (adjusted OD_{600} between 0.02 and 3.0).

Subsamples were frozen for future protein analysis via the Pierce Coomassie Plus Protein Assay Kit (Rockford, IL). Cellular digestion was accomplished by NaOH addition with bead beating for 2 min followed by boiling for 15 min.

7.2.2. Biodegradation and Kinetic Assays.

Experiments to evaluate the biodegradation kinetics of NDMA, including standards and controls, were conducted in 125mL amber incubation bottles sealed with Teflon-lined Miniert valves (Altech, Deerfield, IL). Kinetic studies of propane degradation were conducted in 14mL serum bottles capped with butyl rubber stoppers and aluminum crimp seals at neutral pressure (Wheaton Scientific, Millville, NJ). Aqueous phase concentrations of propane were calculated by using the dimensionless Henry's constant (air/water at 25°C) of $K_H = 26.6$ (Schwarzenbach *et al.*, 1990). Where appropriate, a 10-min intermediate incubation with 5% (v/v headspace) acetylene gas was used for monooxygenase

inactivation (Sharp *et al.*, 2005).

Degradation rates were obtained by monitoring initial disappearance of propane or NDMA by bacterial assays and controls in phosphate buffer. Additional exogenous reductants were not added at this stage. Linear degradation curves contained 3–5 data points collected over the first 4 h of incubation when growth was negligible. Best fits to the Monod equation (Equation 7.1), which relates the rate of removal to the concentration of the substrate at a constant cell density

(Shuler and Kargi, 2002), were determined using nonlinear regression with 95% confidence intervals. The solution was generated using the iterative solver program embedded in Microsoft Excel where v_c is the reaction velocity (mg substrate (mg protein)$^{-1}$ h^{-1}) at substrate concentration

C (µg substrate L^{-1}), v_{max} represents the maximum reaction velocity (µg substrate (mg protein)$^{-1}$ h^{-1}), and K_s the half-saturation constant (µg substrate L^{-1}). The resultant constants for propane and NDMA, respectively, are $v_{max,p}$, $K_{s,p}$, $v_{max,n}$, and $K_{s,n}$.

$$v_c = \frac{v_{max} C}{K_S + C}$$

(Equation 7.1)

The inhibitory effect of propane on the NDMA degradation rate was quantified using Equation 2 with varying initial propane concentrations and a constant initial NDMA addition. A similar approach has been used to describe the effect of propane on MTBE degradation in strain JOB5 (Smith *et al.*, 2003).

$$\frac{v_{n,p}}{v_n} = \frac{C_p}{K_{inh} + C_p}$$

(Equation 7.2)

In Equation 7.2, $v_{n,p}$ is the reaction velocity (µg NDMA (mg protein)$^{-1}$h^{-1}) at aqueous propane concentration C_p (mg propane L^{-1}), v_n represents the reaction velocity in the absence of propane (µg NDMA (mg protein)$^{-1}$h^{-1}), and K_{inh} represents the propane concentration (mg propane L^{-1}) where NDMA degradation is slowed by 50%.

7.2.3. Analytical Methods.

For experiments involving an initial NDMA concentration above 10 µg/L, extractions were performed by removing 2 mL of solution from incubations at each time point of interest. Samples and standards were extracted into methylene chloride as described pre-

viously (Sharp *et al.*, 2005). Analysis of lower NDMA concentrations involved an adaptation of a batch solid phase extraction (Choi and Valentine, 2003; Mitch and Sedlak, 2002) where 100mL sub-samples were exposed to 0.01% sodium azide. The addition of 4μL of a 2.5 ng/μL d_6-NDMA methanol stock (Cambridge Isotope Laboratories; Andover, MA) provided an internal standard for correction of extraction recoveries. Subsequently, 200 mg of Ambersorb 572 resin was equilibrated with active mixing for 3 h, separated by filtration, and then dried overnight. 550 μL of methylene chloride was added to the dried resin and the sample was mixed briefly. Following an equilibration of 30 min, 250 μL of the methylene chloride was removed for analysis by tandem mass spectroscopy.

Methylene chloride extracts containing NDMA and the deuterated standard were analyzed using previously described GC MS/MS methods (Mitch and Sedlak, 2002; Sharp *et al.*, 2005). The detection limit for the liquid–liquid extraction was ~5μg/L as determined by the standard curve (44 and 47 daughter ions). The limit of detection (LOD) for the resin/dichloromethane technique was 20 ng/L. This LOD was determined from replicate NDMA-free blanks and was

calculated as the mean plus three standard deviation units (Anderson, 1989; Skoog and West, 1980).

Headspace measurements were analyzed by withdrawing 200 μL headspace samples from sealed bottles with a Hamilton gas-tight syringe (Reno, NV). The gas was then injected (250°C) into a Hewlett-Packard 5890 gas chromatograph with flame ionization detector (300°C) and a GS-GASPRO capillary column (30m long with 0.32mm I.D., Agilent JW Scientific, Santa Clara, CA). Gas chromatography temperature conditions were as follows: 35°C (hold time 1 min) ramping at 30°C/min to 180°C. The retention times for acetylene, propane, and acetaldehyde were 1.4, 1.6, and 5.8 min, respectively.

7.2.4. Degenerate Primer Design.

In order to assess the presence of propane monooxygenase (PrMO) enzymes in bacterial strains, degenerate primers were designed to target homologues of the large (alpha) subunit gene, *prmA* based on the amino acid sequences of proteins from *Gordonia* TY-5, *Mycobacterium* TY-6, *Pseudonocardia* TY-7, *Mycobacterium smegmatis* MC2155, *R. jostii* RHA1, and *Methylibium petroleiphilum* PM1 (respective NCBI accession: BAD03956, BAF34294, BAF34308, ABK75704, ABG92277, and YP_001020147). The CODEHOP (Consensus Degenerate Hybrid Oligonculeotide Primers) program was used (Rose *et al.*, 1998) with the input of a multiple sequence alignment (MSA) of the six amino acid sequences produced from CLUSTAL X Version 2.0 (Larkin *et al.*, 2007), to generate a pair of *prmA* degenerate primers, deg.prmA.F and deg.prmA.R (Table 7.1). Amplification of degenerate primer polymerase chain reaction (PCR) products from *R.jostii* genomic DNA (gDNA) was visualized on 1% agarose electrosphoresis gels and on HP Agilent's Bioanalyzer (Santa Clara, CA).

Alkane monooxygenase (*alkB*) degenerate primers, Rhose2 and Rhoas1 (Table 7.1), were developed in a previous study (Lopes Ferreira *et al.*, 2007).

7.2.5. Gene Expression and Sequence Analysis.

Analysis of gene expression was determined by quantitative reverse transcriptase PCR (qRT-PCR). Real-time PCR primers for the *prmA* and *alkB* genes (Table 7.1) were designed according to the sequence of the gene fragment produced by the degenerate primers using PerlPrimer (Marshall, 2004). Fast SYBR Green Master Mix (Applied Biosystems, Foster City, CA) was used for quantification of genes and of complimentary DNA (cDNA) produced from transcripts. Differential expression ratios were calculated based on a standard curve method where cDNA copy numbers were normalized to gDNA copy numbers for each condition (Pfaffl, 2001).

Total RNA for analysis of gene expression was extracted and purified from *Rhodococcus* sp. RR1 and strain JOB5 under propane- and pyruvate-growth conditions, in quadruplicate, using Qiagen's RNeasy Mini Kit (Valencia, CA). Each RNA sample was treated with Ambion's DNA-free Kit (Applied Biosystems) to remove any contaminating gDNA, until the CT value was>35. This RNA was used to synthesize cDNA using Roche's Reverse Transcriptase (Mannheim, Germany). gDNA was purified using MO-BIO's Ultra-Clean Microbial DNA Isolation Kit (Carlsbad, CA).

DNA sequencing was carried out at the University of California Berkeley DNA Sequencing Facility using an Applied Biosystems 96 capillary 3730xI DNA Analyzer. PCR amplification products from the degenerate primers were ligated into *Sma*I digested and phosphorylated pUC19 linearized plasmids (Fermentas, Burlington, ON, Canada) and cloned into electrocompetent *Escherichia coli* cells (New England Biolabs, Ipswich, MA) for sequencing.

7.3. Results.

7.3.1. Kinetics of NDMA Removal.

Propane-grown cells of *Rhodococcus* sp. RR1 and strain JOB5 were independently harvested, washed, and suspended in phosphate buffer. The cleaned cells were then exposed to varying concentrations of NDMA to assess biodegradation rates. Abiotic and azide-killed controls revealed no measurable loss of NDMA. Substrate toxicity as determined by agreement with Monod $v_{max,n}$ was not observed for either strain RR1 or JOB5 during the transformation of up to 10,000 µg/L NDMA.

Resulting Monod constants (Equation 7.1) associated with NDMA degradation for the two bacteria as well as prior literature values for a related *Rhodococcus* species are listed in Table 7.2. Model fits employed no fewer than 10 rates measured over a range of kinetically relevant concentrations. Best-fit analysis revealed a threefold difference in maximum transformation rates ($v_{max,n}$) between studied strains ranging from 15 to 49 µg NDMA (mg protein)$^{-1}$ h^{-1}, while the half saturation constants ($K_{s,n}$) for the two *Rhodococci* were statistically similar with an average value of 28 µg NDMA L^{-1}. Propane grown cells of strain JOB5 exhibited a two-orders of magnitude higher half-saturation constant for NDMA (2,200 ± 1,000 µg NDMA L^{-1}).

Although *Rhodococcus* sp. RR1 can constitutively degrade NDMA after growth on the complex medium soy broth, the maximum transformation rate of 0.14 ± 0.01 µg NDMA (mg protein)$^{-1}$ h^{-1} attained by these cells was <1% of the rate achieved by the same cells when grown on propane. However, a calculated half saturation constant of 45 ± 10 µg NDMA L^{-1} for NDMA degradation by soy broth grown cells overlaps with the $K_{s,n}$ derived for propane grown cells (Table 7.2). In contrast, no measurable NDMA degradation occurred when strain JOB5 was grown on soy broth (Sharp *et al.*, 2005).

7.3.2. Biodegradation of Trace Quantities of NDMA.

The potential to degrade trace quantities of NDMA with lower concentrations of cells was assessed. Propane-grown *Rhodococcus* sp. RR1 cells (2 mg protein L^{-1}) removed 100 ng NDMA L^{-1} to below the LOD (20 ng/L). An approximate rate of 0.2 µg NDMA (mg protein)$^{-1}$ h^{-1} was calculated for this removal, which closely matches the predicted rate of 0.14 µg NDMA (mg protein)$^{-1}$ h^{-1} derived from the calculated Monod kinetic constants (Table 7.2). Complete NDMA removal was also observed for strain RR1 after growth on soy broth (110 mg protein L^{-1}). In contrast, cells deactivated with 0.01% sodium azide exhibited minimal NDMA removal (92 ± 7 ng NDMA L^{-1} remaining). Similarly, propane-grown strain JOB5 (17 mg protein L^{-1}) degraded 150 ng NDMA L^{-1} to below detection

limits. Since acetylene has been shown to be an effective inhibitor of monooxygenase activity in strain JOB5, in contrast with *Rhodococcus* sp. RR1 (Sharp *et al.*, 2005), it was used as an enzymatic inhibitor for NDMA-oxidizing activity of strain JOB5. This control exhibited no significant degradation (140 ± 17 ng NDMA L^{-1}).

7.3.3. Competition Between Propane and NDMA.

In order to investigate whether the propane-induced enzyme involved in NDMA degradation is inhibited by the presence of propane, the kinetics of propane degradation by these bacterial isolates was first characterized. Specifically, propane degradation rates by propane-grown cells of *Rhodococcus* sp. RR1 and strain JOB5 that were degassed and washed to remove residual propane and subsequently exposed to defined concentrations of propane were quantified and fitted to Equation 7.1 (Table 7.2). Significant differences in both $K_{s,p}$ and $v_{max,p}$ values between the two strains were apparent. In particular, RR1 exhibited kinetic constants that were an order of magnitude higher than those for strain JOB5.

Next, the effects of propane on NDMA degradation rates were measured over a range of propane concentrations. Although propane inhibited NDMA degradation (100–500 µg NDMA L^{-1}) in proportion to concentration (0–20,000 µg propane L^{-1}) for both strains, graphical interpretation using the double-reciprocal (Lineweaver–Burke) approach revealed complex inhibition kinetics that do not correspond with classic enzymatic models (data not shown). Alternatively, an approach that has previously been applied to quantify the inhibition of propane on MTBE degradation in strain JOB5 (Smith *et al.*, 2003) yielded a quantifiable comparison between the inhibition patterns of the two strains. When the NDMA degradation rate in the presence of a given propane concentration ($v_{n,p}$) is normalized by the NDMA degradation rate in the absence of propane (v_n), an inhibitory constant can be calculated (Equation 2). As shown in Figure 7.1, the degradation of 200 µg NDMA L^{-1} by strain RR1 was inhibited by 50% (K_{inh}) at 7,700 µg propane L^{-1} (R^2 = 0.9669) while strain JOB5 required approximately 1/30th of that value (120 µg propane L^{-1}; R^2 = 0.9895) for 50% inhibition. Consistent with these values, the presence of 1,400 µg propane L^{-1}, or 5% (v/v) in the headspace, had little effect on NDMA degradation by RR1 while it exerted an ~80% decrease for strain JOB5.

7.3.4. Presence of Propane Monooxygenase.

At the time of this study, only six amino acid sequences existed in the NCBI Entrez Protein database (see Materials and Methods Section) with the annotation of PrMO large (alpha) subunit, *prmA*. These sequences were used to develop the *prmA* degenerate primer set, deg.prmA.F and deg.prmA.R (Table 7.1). Based upon the nucleotide sequence of the *prmA* gene in *R. jostii* RHA1 (accession NC_008268), these primers were calculated to

produce a PCR amplification product of 1,333 bp or 90.1% of the total gene length. The efficacy of the PCR primers was verified by producing a 1,333 bp fragment derived from RHA1 gDNA that was found to be identical to the *prmA* gene deposited in NCBI GenBank for this strain.

These *prmA* degenerate primers were then applied to gDNA derived from Rhodococcus sp. RR1 and strain JOB5. Of these two propanotrophs, only RR1 produced a PrMO homologue with the primers (Figure 7.2). The nucleotide sequence of the partial *prmA* gene fragment from RR1 (1,318 bp) was found to be 91% identical to the *prmA* fragment of RHA1, while the corresponding amino acid sequences were 96% identical (NCBI's blastx-2 sequences tool). The partial nucleotide sequence for the RR1 *prmA* gene has been deposited as GenBank accession HM209445.

7.3.5. Presence of Alkane Monooxygenases.

Previously developed *alkB* degenerate primers (Table 7.1) were used to test for the presence of an alkane monoxygenase (AlkMO) in both *Rhodococcus* sp. RR1 and strain JOB5. PCR amplification using the *alkB* degenerate primers produced an expected 343 bp fragment from both strains; sequence analyses revealed that *Rhodococcus* sp. RR1 possessed one *alkB* homologue while strain JOB5 contained at least two distinct copies of an *alkB* gene, which share ~60% nucleotide identity.

7.3.6. Monooxygenase Expression in Pyruvate- Versus Propane-Grown Cells.

Analysis by RT-qPCR confirmed that the PrMO gene in *Rhodococcus* sp. RR1 was induced in the presence of propane. The mRNA levels of *prmA* were 70-fold higher (*p*-value = 0.00055) when grown on propane versus pyruvate as the sole carbon source (Figure 7.3). In contrast, *alkB* transcription levels were not significantly higher after growth on propane (< 5-fold). Similarly, analysis of expression of *alkB* genes found in strain JOB5 indicated that neither was differentially up-regulated (< 5-fold).

Table 7.2. Monod parameters associated with NDMA and propane biodegradation by propane-grown cells. Monod constants were calculated by fitting data to Eq.1 using non-linear regression with 95% confidence intervals. The concentration of propane in the dissolved solution was adjusted using Henry's constant to account for liquid phase partitioning**.**

Species	Substrate	$v_{max,s}$ [µg substrate (mg protein)$^{-1}$ (hr)$^{-1}$]	K_s [µg substrate (L)$^{-1}$]
Rhodococcus sp. RR1	NDMA	44 ± 5	36 ± 10
	Propane	190 ± 65	6200 ± 400
Rhodococcus jostii RHA1*	NDMA	18 ± 3	20 ± 17
	Propane	NA	NA
Mycobacterium vaccae JOB5	NDMA	28 ± 3	2200 ± 1000
	Propane	33 ± 4	790 ± 400

* Monod constants for NDMA degradation by *R. jostii* RHA1 are from Chapter 6.
NA = data not available.

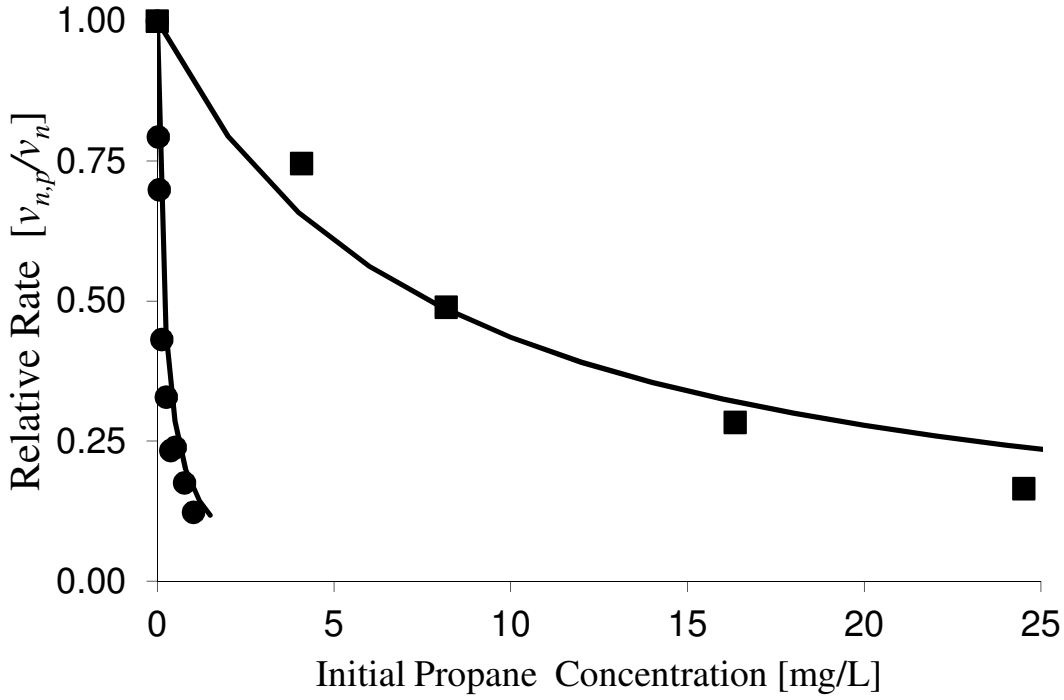


Figure 7.1: Inhibitory effect of propane on NDMA degradation. Initial relative rates for the degradation of 200 mg/L NDMA was normalized as degradation rate ($v_{n,p}$) in the presence of a given propane concentration divided by the rate (v_n) in the absence of propane. The concentration of propane in the dissolved solution was adjusted by the Henry's constant to account for liquid phase partitioning. ■ = relative rate of NDMA degradation for strain *Rhodococcus* sp. RR1; ● = relative rate of NDMA degradation for strain *Mycobacterium vaccae* JOB5. Data were fit as solid lines to equation 2 using non-linear regression.

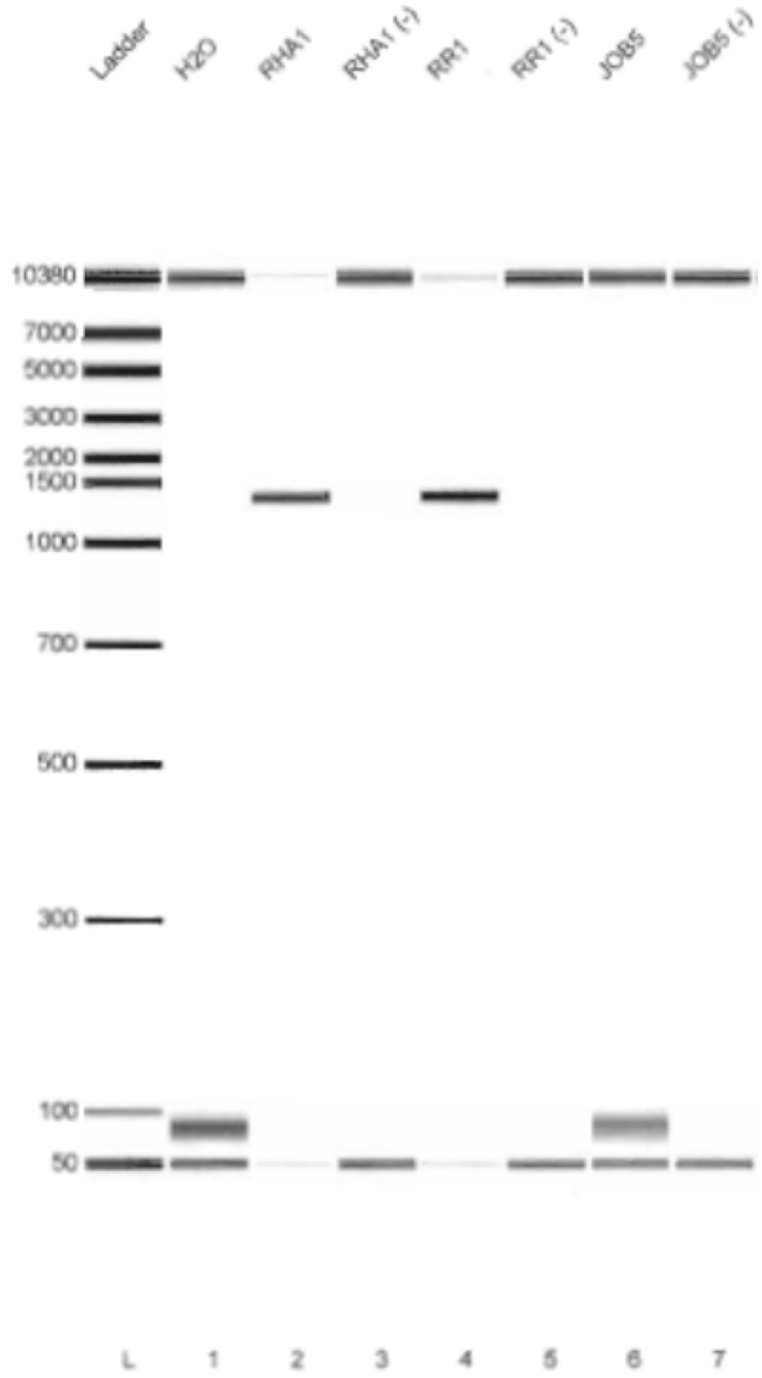


Figure 7.2. Identification of propane monooxygenase genes. PCR products of primer set deg.prmA.F and deg.prmA.R on genomic DNA from *Rhodococcus jostii* RHA1 'RHA1', *Rhodococcus* RR1 'RR1', and *Mycobacterium vaccae* JOB5 'JOB5'. Negative control wells 'RHA1 (-)', 'RR1 (-)', and 'JOB5 (-)' contain template genomic DNA without the degenerate primers. 'H2O' lane is a control containing no template DNA. Samples are run on Agilent Technologies 2100 Bioanalyzer using DNA 7500 Assay Kit. Image produced with Agilent Technologies Bioanalyzer 2100 Biosizing Software. Numbers to the left of the ‹Ladder› represent nucleotide length.

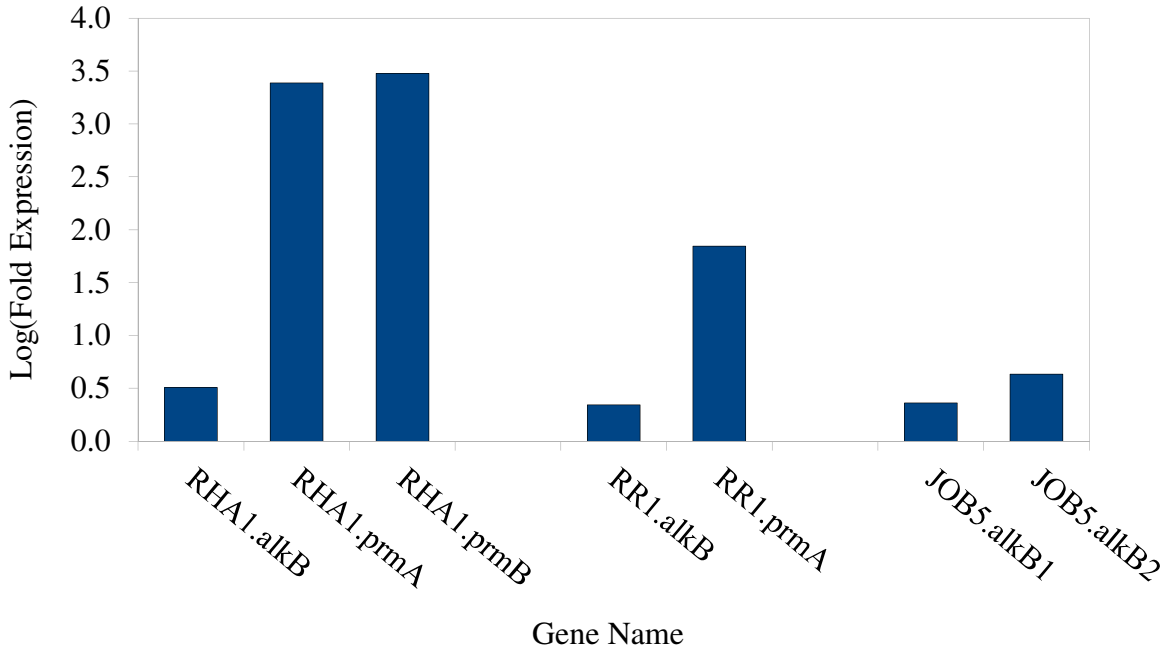


Figure 7.3: Difference in expression for candidate propane oxidizing genes when contrasting propane and pyruvate grown cultures. Expression data for *R. jostii* RHA1 is from Chapter 6.

7.4. Discussion.

This study characterizes kinetic parameters associated with the co-metabolic degradation of NDMA by two different strains of bacteria capable of growth on propane. It also investigates differences in candidate enzymes between the strains to help interpret kinetic variability. The *Rhodococcus* and *Mycobacterium* tested in this study are common soil bacteria of the order *Actinomycetales* that have previously been shown to metabolize propane (Ashraf *et al.*, 1994 and references within). Increases in xenobiotic degradation rates after growth on propane suggests the involvement of a propane monooxygenase (Fournier *et al.*, 2006, 2009; Sharp *et al.*, 2005, 2007; Smith *et al.*, 2003); however, our results demonstrate that the propane-induced monooxygenase differs significantly between the strains with one found to lack homology to other annotated PrMOs.

7.4.1. Kinetic Analysis.

The potential for NDMA transformation following growth on complex, common organics is supported by the observations of others (Gunnison *et al.*, 2000; Sedlak *et al.*, 2005; Yang *et al.*, 2005). However, orders of magnitude differences in transformation rates between constitutive and promoted conditions reported here and in prior studies (Fournier *et al.*, 2009; Sharp *et al.*, 2007), suggest that the addition of propane as an inducing substrate could substantially enhance the bioremediation of NDMA in contaminated soils. Furthermore, measurable constitutive activity is not a characteristic of all propanotrophs, suggesting that propane amendment could be necessary to achieve effective biodegradation rates.

A comparison of half-saturation constants associated with NDMA degradation by axenic cultures indicates considerable variability between species (Table 7.2). Although the maximum rate constants for *Rhodococcus* sp. RR1 and strain JOB5 differs by < 50%, *Rhodococcus* sp. RR1 exhibited dramatically higher enzymatic affinity for NDMA as reflected by $K_{s,n}$ (36 µg/L vs. 2,200 µg/L). Kinetic variables derived for *Rhodococcus* sp. RR1 after growth on propane were similar to those reported for *R. jostii* RHA1 with a statistically identical $K_{s,n}$ (Table 7.2). When characterizing NDMA transformation by the methanotroph *M. trichosporium* OB3b, Yoshinari and Shafer (1990) generated a rate-to concentration ratio that was constant from 2,200 to 740,000 µg/L and corresponded to linear increases in rates from 0.02 to 7 µg/mg/h. While they were unable to generate specific maximum rate constants, this result corresponds to a $K_{s,n}$ above 740,000 µg/L for that species, which is approximately 400- and 20,000-times the half saturation value for strain JOB5 and *Rhodococcus* sp. RR1, respectively. Thus, propanotrophs may have a significantly higher affinity for NDMA than methanotrophs. Given that groundwater contamination levels are commonly <1 µg/L NDMA (OCWD, 2000; Sedlak *et al.*, 2005; WateReuse, 2006), it is plausible that strains with a lower $K_{s,n}$, such as these propanotrophs, will play a more prominent role in environmental NDMA degradation. Collectively, the orders of magnitude variability in Monod constants exhibited by these three bacteria indicates

that NDMA degradation kinetics in environmental communities will be a function of bacterial composition and induction conditions.

7.4.2. Impact of NDMA Concentration on Biodegradation.

Advisory levels of ~10 ng NDMA L^{-1} (CalDHS, 2002; MOE, 2003) are nearly a thousand-fold lower than regulations set for more traditional water contaminants targeted for bioremediation. Therefore, an understanding of NDMA biodegradation at concentrations in the nanograms-per-liter range is crucial for predicting the fate of environmentally relevant concentrations and their potential for attenuation. In this study, both of the tested propanotrophs demonstrated the ability to transform parts-per trillion levels of NDMA to concentrations below the experimental level of detection. Others have also reported that biological processes can remove NDMA at similar concentrations in pure culture (Fournier *et al.*, 2009) and wastewater treatment systems (Sedlak *et al.*, 2005), suggesting that different organisms show promise for participation in bioremediation strategies that approach the low NDMA threshold for acceptable water quality.

At the high end of the concentration spectrum, the presence of concentrations up to 10 mg NDMA L^{-1} appears to have little toxic effect on the tested strains. Since NDMA is rarely found at or above this concentration in contaminated environments (CalDHS, 2002), it seems that substrate toxicity will not play a prominent role in inhibiting NDMA biodegradation in the field. Similar effects have been observed by others where toxic effects on NDMA degradation rates were not observed for concentrations as high as 740 mg NDMA L^{-1} with a methanotroph (Yoshinari and Shafer, 1990) and for concentrations as high as 10,000 mg $NDMAL^{-1}$ with undefined consortia (Kaplan and Kaplan, 1985).

7.4.3. Inhibition of NDMA Degradation.

The inhibition of NDMA degradation by strain JOB5 after exposure to acetylene is consistent with mechanistic inactivation of certain monooxygenases. This phenomenon has been documented for enzymes including soluble methane and toluene monooxygenases but is not absolute for all monooxygenases relevant to bioremediation applications (Prior and Dalton, 1985; Sharp *et al.*, 2005; Smith *et al.*,2003). In the Sharp et al. (2005) study, the propane-induced PrMO in RR1 is not irreversibly inhibited by acetylene. Differences in prosthetic groups and active hydroxylase units associated with short-chain alkane monooxygenases have been cited as potential causes for the different inhibition patterns (Ortiz de Montellano and Reich, 1986; Shanklin *et al.*, 1997). The presence of a propane-induced PrMO in *Rhodococcus* sp. RR1 and the absence of an identifiable PrMO in strain JOB5 (Figs. 2 and 3) may explain the differences in inhibition characteristics.

It appears that propane, which serves as an enzyme inducer in both of the studied strains, competes with NDMA for the active enzyme (Figure 7.1). The K_{inh} values, which mark a propane concentration that results in 50% inhibition of the NDMA degradation rate, are the same order of magnitude as the respective $K_{s,p}$ values for both strains (Table 7.2). This suggests that the same enzyme is involved in both processes. The K_{inh} value for strain JOB5 observed in this study is similar to one previously reported for propane inhibition (244–325 µg propane L^{-1}) of MTBE degradation by strain JOB5 (Smith *et al.*, 2003). While our results did not identify the responsible enzyme in strain JOB5, they demonstrate that AlkMOs in strain JOB5 are not involved in either NDMA transformation or propane metabolism. The presence of distinct AlkMO's in strain strain JOB5 is supported by functional assays that implicate two separate AlkMO's during growth on C_5–C_{14} *n*-alkanes (House and Hyman, 2010).

An interspecies comparison of $K_{s,n}$ and $K_{s,p}$ values indicates pronounced differences in the level of inhibition between these two strains (Table 7.2). Specifically, while the kinetic and affinity constants exhibited by strain JOB5 were similar for both propane and NDMA, *Rhodococcus* sp. RR1 exhibited a strong enzymatic preference for NDMA over propane (i.e., $K_{s,n} << K_{s,p}$) despite the requirement for induction by propane. This enzymatic preference for NDMA is consistent with the finding that additions of 5% (v/v) propane exerted no significant inhibition on NDMA degradation by RR1 while significantly inhibiting strain strain JOB5. These results suggest that the extent of inhibition will be a function of the characteristics of the active organisms, and specifically the enzymes involved.

7.4.4. Gene Presence and Expression.

The promotion of *prmA* gene expression in *Rhodococcus* sp. RR1 after growth on propane is consistent with the behavior observed for *R. jostii* RHA1 (Sharp *et al.*, 2007), except that differential expression of propane-induced *prmA* in RR1 was only 70-fold versus > 2,000-fold for RHA1 (Figure 7.3). This discrepancy may be explained both by biological variability and by differences in transcriptional measurement methods involving both primers/probe combinations (SYBR-green vs. Taqman) and reference normalization methods (DNA copy number vs. housekeeping gene) between the study of *Rhodococcus* sp. RR1 and *R. jostii*. Through a series of transcriptional, biodegradation, and gene knockout studies, the *R. jostii* report conclusively demonstrated that the PrMO was involved in both NDMA and propane degradation and that PrMO was constitutively expressed (Chapter 6). Similarities in phylogeny, PrMO composition, and kinetic behavior suggest highly homologous enzymes are involved in NDMA and propane degradation in both *Rhodococci*.

7.4.5. Environmental Implications.

This report presents pertinent kinetic constants associated with the biodegradation of NDMA, and it collectively expands upon findings that rapid and nearly complete NDMA degradation can be achieved by different propanotrophs even at nanogram-per-liter concentrations (Fournier *et al.*, 2009; Sharp *et al.*, 2007). Different characteristics associated with competition between propane and NDMA in these species is presumably due to different enzymes. Our findings suggest that even after functional biostimulation (*i.e.*, propane amendment), the type of propane-induced enzymes present in the microbial community could dictate the effectiveness of a remediation strategy. To this end, the degenerate primers designed in this study could be used as biomarkers to assess the presence and expression of PrMOs in environmental systems.

Chapter 8

Conclusions

8.1. Summary and conclusions.

Although 1,4-dioxane and *N*-nitrosodimethylamine (NDMA) have rarely been the primary pollutants of concern at contaminated groundwater sites, awareness of the prevalence and persistence of these probable human carcinogens in contaminated groundwater plumes, especially where conventional treatment strategies have been or are being applied, has risen (Mitch *et al*., 2003; Mohr, 2010). Biological transformation of 1,4-dioxane and NDMA have been investigated to determine the potential of natural and enhanced bioremediation processes for removal of these emerging contaminants from water supplies. The doctoral works of Shaily Mahendra (2007) and Jonathan O. Sharp (2006) led to the discovery of monooxygenase-expressing bacteria capable of aerobically degrading these contaminants (Sharp *et al*., 2005; Mahendra and Alvarez-Cohen, 2006) and the identification of intermediates of their oxidative biotransformation (Mahendra *et al*., 2007). Although prior research implicated monooxygenase enzymes as catalysts for the initial oxidation of 1,4-dioxane and NDMA, at the onset of this research, no molecular information was known about the specific genes and enzymes involved in the biodegradation pathways of these compounds.

The course of research described in this dissertation sprung from the rare ability of *Pseudonocardia dioxanivorans* CB1190 to grow on 1,4-dioxane as a sole carbon and energy source (Parales *et al*., 1994; Mahendra and Alvarez-Cohen, 2005) and the power to enhance NDMA degradation rates in the actinomycetes *Rhodococcus* sp. RR1 and *Mycobacterium vaccae* JOB5, by propane stimulation (Sharp *et al*., 2005). In order to gain more insight into the genetic basis of the metabolism of 1,4-dioxane and the overal physiology of *Pseudonocardia*, the genome of *P. dioxanivorans* CB1190 was sequenced, assembled, and annotated in a joint effort with the Department of Energy Joint Genome Institute (Sales *et al*., 2011). Before this sequencing effort started, no genome in the genus of *Pseudonocardia* had ever been sequenced and only a few genomes with such high G+C content (73%) had been sequenced. Although a draft sequence is now available for *Pseudonocardia* sp. P1, which exists in mutualism with leaf-cutter ants (Barke *et al*., 2010), the genome sequence of *P. dioxanivorans* CB1190 is the first genome of a free-living *Pseudonocardia* species and the first finished genome of any member of its genus (Chapter 3). A total of eight bacterial multicomponent monooxygenases (MO), including a propane MO, four aromatic (toluene) MOs, a phenol MO, a 4-hydroxyphenylacetate MO, and a tetrahydrofuran MO, were discovered in the genome sequence of *P. dioxanivorans* CB1190, any of which could potentially be involved the degradation of 1,4-dioxane. In addition, the genome sequence revealed a myriad of pathways that *P. dioxanivorans* CB1190 could use for nitrogen and carbon metabolism. A complete Calvin-Benson-Bassham CO_2 fixation pathway was identified and a number of carboxylase genes found in other well-characterized CO_2 fixation pathways were identified. The genome was also searched for protein-encoding genes involved in transport systems, signal transduction systems, secretion systems, and heavy metal and antibiotic resistance. Although the genome sequence revealed genes potentially capable of catalyzing steps for complete mineralization of 1,4-dioxane, further studies were needed to confirm and characterize the involvement of these genes in the 1,4-dioxane metabolic pathway.

A combination of results from genome-driven transcriptional analyses, enzymatic assays, and phenotypic experiments, described in Chapters 4 and 5, enhanced the understanding of the 1,4-dioxane metabolic pathway in *P. dioxanivorans* CB1190. An expression microarray targeting the whole draft genome assembly and annotation of the *P. dioxanivorans* CB1190 genome was used to determine differentially expressed genes induced during the degradation of 1,4-dioxane. A degradation pathway (Figure 2.3) was previously proposed based upon intermediates identified during 1,4-dioxane degradation in monooxygenase-expressing bacteria, including *P. dioxanivorans* CB1190 (Mahendra *et al*., 2007). The four-carbon cyclic ether structure of 1,4-dioxane was proposed to undergo enzyme-catalyzed oxidation steps that opens the ring and produce two-carbon intermediates that can enter central carbon metabolic pathways, such as glycolate. Therefore, the premise of the research described in Chapter 4 was to use whole genome expression microarrays to identify differentially expressed genes among the transcriptomes of 1,4-dioxane-, glycolate-, and pyruvate-grown *P. dioxanivorans* CB1190. Based upon the microarray results, the 1,4-dioxane degradation pathway was revised and annotated to include the enzymes of up-regulated genes that are involved in each transformation step (Figure 4.1). The results in Chapter 4 from amino acid ^{13}C isotopic tracer analysis and enzyme assays performed with cell-free extracts from *P. dioxanivorans* CB1190 and *Rhodococcus jostii* RHA1 clones expressing the two putative glyoxylate carboligase pathway genes (Psed_3890 to Psed_3889) strengthen the likelihood that the revised pathway (Figure 4.1) is accurate. The additional transcriptional analyses and functional studies with *R. jostii* expression clones, described in Chapter 5, confirmed the role of the THFMO in catalyzing the initial hydroxylation of 1,4-dioxane. One of the significant findings in Chapter 4 was biochemical and genetic evidence that the THFMO is not responsible for the transformation of β-hydroxyethoxyacetic acid (HEAA), an identified 1,4-dioxane degradation intermediate. Further studies are needed to identify the enzyme that leads to the cleavage of the ether bond in the four-carbon compound HEAA to produce the two-carbon compounds: glyoxal, ethylene glycol, and glycolate.

In addition to identifying the role of the THFMO in 1,4-dioxane degradation, its role in THF degradation was examined in Chapter 5. Transcriptional analyses of THF- and succinate-grown *P. dioxanivorans* CB1190 cells elucidated, for the first time, the genes and enzymes involved in the metabolic pathway of THF degradation in *P. dioxanivorans* CB1190. The up-regulation of the entire THFMO cluster *thm* is in agreement with Northern blot analysis of *P. tetrahydrofuran* that detected the expression of a nearly identical cluster during utilization of THF (Thiemer *et al*., 2004). The transcriptomic microarray data revealed, for the first time, an alcohol dehydrogenase (Psed_0131) gene that is induced during growth on THF and that potentially catalyzes the conversion of 2-hydroxytetrahydrofuran to γ-butyrolactone in the metabolic degradation of THF. Although the *R. jostii* clones expressing *thmADBC* confirmed the role of the THFMO in the hydroxylation of THF, additional studies are needed to identify the intermediates of THF degradation and to confirm the enzymatic function of the protein-encoding genes described to be involved in the proposed metabolic pathway for THF degradation (Section 5.4).

Much like 1,4-dioxane, the molecular basis of propane-enhanced degradation of NDMA was not known at the onset of this research. Although multiple lines of evidence pointed

to the involvement of a MO enzyme in propane-grown *Rhodococcus* sp. RR1 and *Mycobacterium vaccae* JOB5 (Sharp *et al*., 2005), the molecular sequence identity of the propane-induced MO enzyme was not known. At the onset of this study, other than 16s rRNA DNA sequences deposited for each strain, no gene sequence information for *Rhodococcus* sp. RR1 and *M. vaccae* JOB5 existed. However, the genome sequence of *Rhodococcus jostii* RHA1 was available (McLeod *et al*., 2006). Fortuitously, the genome of *R. jostii* RHA1 contained genes encoding an alkane MO as well as a propane MO that was recently characterized in *Gordonia* sp. TY-5 (Kotani *et al*., 2003). After verification that propane-grown *R. jostii* can degrade NDMA co-metabolically, transcriptional analyses using qRT-PCR and whole genome expression microarrays designed for *R. jostii* demonstrated that the propane MO gene cluster (and not the alkane MO gene cluster) was up-regulated during growth on propane relative to pyruvate (Chapter 6). Furthermore, genetic knockouts of the *prmA* and *alkB* genes demonstrated that a complete propane monooxygenase gene cluster was required for growth on propane and degradation of NDMA.

After identifying the role of a propane MO in NDMA degradation in *R. jostii*, a study (described in Chapter 7) was performed to characterize the kinetics, the biochemical nature, and genetic basis of propane-enhanced NDMA degradation by *Rhodococcus* sp. RR1 and *M. vaccae* JOB5. A number of differences were observed between these two propanotrophs. First, *Rhodoccocus* sp. RR1 is capable of degrading NDMA constitutively after growth on the complex medium soy broth, while no measurable NDMA degradation was observed with *M. vaccae* JOB5. Second, the Monod kinetic constant ($K_{s,n}$) for half-saturation determined for each strain showed that *Rhodococcus* sp. RR1 has a stronger affinity for NDMA at lower concentrations than *M. vaccae* JOB5. Third, the competition between propane and NDMA was found to be stronger in *M. vaccae* JOB5 than *Rhodococcus* sp. RR1. A search for propane MO and alkane MO gene sequence in the two isolates using degenerate oligonucleotide primers suggested a genetic basis for the differences in the kinetics and biochemical nature of propane-enhanced degradation of NDMA between the two strains. A gene sequence was discovered in *Rhodococcus* sp. RR1 that was homologous to the propane monooxygenase α-subunit gene *prmA* in *R. jostii*. However, no propane MO homolog was found in *M. vaccae* JOB5 and, although alkane MO sequences were found in both isolates, only the *prmA* in *Rhodococcus* sp. RR1 was up-regulated during growth on propane.

The extremely mobile and persistent emerging contaminants 1,4-dioxane and NDMA are susceptible to biotransformation by bacterial multicomponent MOs. Although the substrate specificities of the 1,4-dioxane-degrading THF monooxygenase in *P. dioxanivorans* CB1190 and the NDMA-degrading propane MOs in *R. jostii* and *Rhodococcus* sp. RR1 are different, the three MOs are closely related. Unusual among bacterial multicomponent MOs, the THF and propane monooxygenases contain four subunits found in this gene order: a large oxygenase α-subunit, a reductase component, a small oxygenase β-subunit, and a coupling protein. Only an alkene MO, described in *Nocardia corallina* B-276, shares this type of gene order (Seaki *et al*., 1999). Furthermore, comparison of the amino acid and nucleotide sequences of the THF and propane MOs in *P. dioxanivorans* CB1190 (Table 3.8) indicate that they are homologous.

While the role of MO enzymes in catalyzing the first step in the biodegradation of

1,4-dioxane and NDMA was proven as part of this research, the studies in this dissertation demonstrated that the biological fate of these contaminants in natural or engineered systems is determined by a network of genes and enzymes. For 1,4-dioxane, these studies have revealed that co-metabolism and metabolism of 1,4-dioxane is not due to different types of MOs catylizing the transformation, as is indicated by the presence and expression of the *thm* cluster in both *P. dioxanivorans* CB1190 and *P. tetrahydrofuranoxydans*, but likely another enzyme involved in the transformation of an intermediate. For NDMA, it was discovered in this work and others that the type of MO enzyme involved in the initial oxidation step can determine the products of degradation. From the studies in this dissertation, the propane-induced enzyme that led to the production of nitrate, N_2, methylamine, formate and CO_2 as major metabolites (Sharp, 2006; Fournier *et al*., 2009) was proven to be a propane MO. In contrast, when a toluene-4-monooxygenase enzyme is responsible for NDMA degradation the major products are *N*-nitromethylamine (NTMA) and formate (Fouriner *et al*., 2006). All of the studies in this dissertation are aided by genomic information, which ultimately demonstrates the power and usefulness of molecular biology and functional genomics to improve the ability to predict, monitor, and optimize the performance of bioremediation systems.

8.2. Suggestions for future research.

High-throughput techniques, such as genomics and transcriptomics, produce a wealth of data. Bioinformatics and computational biology tools are available to gain valuable knowledge from large amounts of data generated by these "omic" techniques. However, as evidenced by the genomic and transcriptomic results in Chapters 3 to 6, analysis of high-throughput data on its own cannot give definitive answers to the original inquiries posed, and often the analysis leads to the development of newer, unexpected questions. For instance, the functions of most of the protein-encoding genes in *P. dioxanivorans* CB1190 are putative and only based upon weak homology to sequences in databases, whose own functions are usually not verified (Brenner, 1999). Even from the results produced in this dissertation, the function of only a few of the genes have been tested and proven. With transcriptional data, although the induction of gene expression is required for the production of the enzyme it encodes, the presence of transcripts does not necessarily ensure enzymatic activity. Therefore, the function of genes cannot be definitely determined by differential expression alone. This realization should not be viewed as a problem of high-throughput techniques but rather as an indication of the importance of marrying "omic" techniques with biochemical and microbial experiments. This marriage would allow results produced from analysis of high-throughput data to guide laboratory and field research and vice versa.

The transcriptomic microarray analyses performed in Chapters 4 and 5 revealed a potential set of genes in *P. dioxanivorans* CB1190 involved in the biodegradation of 1,4-dioxane and THF. However, experiments were performed on only a few genes to elucidate and confirm the function of the enzymes they encode. Therefore, future research can be performed to determine the specific genes that function in additional specific transforma-

tion steps in the revised 1,4-dioxane biodegradation pathway (Figure 4.1). For example, the specific secondary alcohol dehydrogenase that catalyzes the conversion of 2-hydroxy-1,4-dioxane to 1,4-dioxane-2-one and the aldehyde dehydrogenase involved in the transformation of 2-hydroxyethoxyacetaldehyde to 2-hydroxyethoxyacetic acid (HEAA) are still unknown. With respect to THF degradation, further research can be conducted to verify the functions of the up-regulated alcohol dehydrogenase gene that converts 2-hydroxytetrahydrofuran to γ-butyrolactone and to identify the enzyme responsible for oxidizing 4-hydroxybutyrate to succinate semialdehyde (Chapter 5). The molecular cloning system developed for expressing *P. dioxanivorans* CB1190 genes in *R. jostii* RHA1 (Section 4.2.5 and 5.2.9) could be used to test the function of these genes. Additional research efforts should also focus on developing a genetic mutation system for *P. dioxanivorans* CB1190, which could lead to the development of genetic knockout libraries that aid in determining the function of over 6,900 genes.

Development and application of analytical chemistry techniques that can identify and monitor metabolites produced by *P. dioxanivorans* would also be a useful research path. Amino acid istopomer analysis of 1,4-[U-^{13}C]dioxane grown *P. dioxanivorans* (Chapter 4) demonstrated the ability to use isotopic tracer analysis to experimentally confirm the central metabolic pathway used during 1,4-dioxane growth. Similar stable isotope analyses with *E. coli* (Yuan *et al*., 2008), *Geobacter metallireducens* (Tang *et al*., 2007), *Shewanella oneidensis* (Yang *et al*., 2006), and *Desulfovibrio vulgaris* Hildenborough(Tang *et al*., 2007) have been used to determine the fluxes through metabolic pathways of these organisms under certain conditions. Advances in high resolution mass spectrometry measurements would aid in validating the function of genes by providing evidence of enzyme activity and physiology and in identifying bottlenecks in pathways for degradation of contaminants or production of valuable metabolites (*i.e.*, biofuels or antibiotics) (Tang *et al*., 2008). In regards to THF degradation, other than the detection of γ-butyrolactone in *Graphium* sp. as biodegradation intermediate (Skinner *et al*., 2009), intermediates of THF degradation have never been identified. Therefore, the identification of THF degradation intermediates using mass spectrometry techniques would be a possible project for future research.

Although *P. dioxanivorans* is of interest for its pollutant degradation capacity, *Pseudonocardia* species are also being studied because of their ability to synthesize potentially useful natural products. A research group led by Cameron Currie at the University of Wisconsin at Madison is sequencing a number of *Pseudonocardia* species isolated from a community of microorganisms that exist in colonies of leaf-cutter ants (Cafaro and Currie, 2005). This group is subjecting agarose plugs containing *Pseudonocardia* cells to high resolution mass spectrometry analysis to determine natural products, such as the antifungal agent dentigerumycin (Oh *et al*, 2009), that are produced from these ant-associated organisms. Since many members of the family *Pseudonocardiaceae* are known to produce secondary metabolites of interest (see Chapter 3), the secondary metabolites produced by *P. dioxanivorans* could be investigated to determine if they may be industrially or medically important.

Interesting physiological characteristics of *P. dioxanivorans* include its ability to fix carbon dioxide (Parales *et al*, 1994) and nitrogen (Mahendra and Alvarez-Cohen, 2005).

The genome sequence of *P. dioxanivorans* CB1190 showed that it encodes for a complete Calvin-Benson-Bassham CO_2 fixation pathway. The genome also contained a variety of genes encoding for carboxylases, that have been shown to catalyze important reactions in other proposed CO_2 fixation pathways (Section 3.3.5). In regards to nitrogen fixation, the genome lacked a homolog of known nitrogenase enzymes. Since the genome was sequenced from a culture grown on 1,4-dioane in a minimal medium containing ammonia, it is possible that the nitrogenase sequences were lost. Another possibility is that the *P. dioxanivorans* possesses a unique system to fix nitrogen. Studies aimed at answering the unknowns about these fixation pathways are another potential avenue of future research. Characterization of these CO_2 and nitrogen fixation pathways could play an important factor in applying *P. dioxanivorans* as a biodegradation tool in organic-starved and nitrogen-limiting environments.

In terms of NDMA biodegradation, this research identified the propane-induced MOs in *R. jostii* RHA1 and *Rhodococcus* sp. RR1. However, the propane-induced MO in *M. vaccae* JOB5 is still unknown. Since advances in high-throughput sequencing have made it a more cost-feasible technique, genome sequencing or RNA-seq (transcriptomic) analysis of *M. vaccae* JOB5 could be a future direction of research. In addition to being able to degrade 1,4-dioxane and NDMA, *M. vaccae* JOB5 has been reported to degrade trichloroethene (Burback and Perry, 1993; Vanderberg *et al*., 1995a), chloroform (Hamamura *et al*., 1989), 2,4,6-trinitrotoluene (Vanderberg *et al*., 1995b), and methyl tert-butyl ether (Smith *et al*., 2003; Johnson *et al*., 2004). Rather than sequencing the genome or transcriptome of *M. vaccae* JOB5, the already sequenced genome of *M. vanbaalenii* PYR-1 could be used much like the genome of *R. jostii* RHA1 was used to identify the propane MO in *Rhodococcus* sp. RR1. In addition to both being members of the genus *Mycobacterium*, the two alkane MOs found in *M. vaccae* JOB5 (Chapter 7) are significantly similar to two alkane monooxygenases annotated in the *M. vanbaalenii* genome. This research should first determine the ability of *M. vanbaalenii* PYR-1 to grow on propane and subsequently degrade NDMA. If propane induces NDMA degradation in *M. vanbaalenii* PYR-1, the genomic, proteomic, and metabolomics technologies that have been developed to elucidate the fluoranthene degradation pathway in *M. vanbaalenii* (Kweon *et al*., 2007) could be used. Within time, more genomes will be sequenced of organisms able to degrade environmental contaminants, therefore functional genomics approaches that marry the genome sciences, microbiology, molecular biology, and analytical chemistry will continue to be a driver for research in advancing our understanding of bioremediation in natural and engineered systems.

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Appendices

Appendix 1

One-component signal transduction proteins in *P. dioxanivorans* CB1190

Appendix 1. One-component signal transduction proteins in *P. dioxanivorans* CB1190

Gene	Input Domains	Output Domains	Total Domain Architecture			
Psed_0023		GntR	GntR			
Psed_0038		HTH_5	HTH_5			
Psed_0066		Trans_reg_C	Trans_reg_C	BTAD	NB-ARC	
Psed_0069		HTH_5	HTH_5	LMWPc		
Psed_0077	IcIR	HTH_IclR	HTH_IclR	IclR		
Psed_0084	AsnC_trans_reg	HTH_11	HTH_11	AsnC_trans_reg		
Psed_0086	GAF	ANTAR	GAF	ANTAR		
Psed_0091		MerR	MerR			
Psed_0098		GntR	GntR	FCD		
Psed_0104		HTH_11	HTH_11			
Psed_0114		Pkinase	Pkinase	PASTA	PASTA	PASTA	PASTA
Psed_0115		Pkinase	Pkinase			
Psed_0124	IcIR	HTH_IclR	IclR			
Psed_0147		MarR	MarR			
Psed_0200		HTH_5	HTH_5			
Psed_0202		GerE	GerE	LuxR_C		
Psed_0213		MarR	MarR			
Psed_0222		TetR_N	TetR_N			
Psed_0238		TetR_N	TetR_N			
Psed_0243		GerE, HD	HD	GerE		
Psed_0259		HTH_IclR	HTH_IclR	Methyltransf_2		
Psed_0263	IclR	HTH_IclR	HTH_IclR	IclR		
Psed_0311		TetR_N	TetR_N			
Psed_0321	TetR_C	TetR_N	TetR_N	TetR_C		

Appendix 1. One-component signal transduction proteins in *P. dioxanivorans* CB1190

Psed_0346		GntR	GntR	FCD	
Psed_0351	LysR_substrate	HTH_1	HTH_11	LysR_substrate	
Psed_0357		HTH_3	HTH_3		
Psed_0373		TetR_N	TetR_N		
Psed_0375		TetR_N	TetR_N		
Psed_0421	LysR_substrate	HTH_1	HTH_1	LysR_substrate	
Psed_0440	PAS	EAL, GGDEF	PAS_3	GGDEF	EAL
Psed_0444		GntR	GntR		
Psed_0449		FUR	FUR		
Psed_0459		Trans_reg_C	Trans_reg_C		
Psed_0469		GGDEF	GGDEF		
Psed_0477	Aminotran_1_2	GntR	GntR	Aminotran_1_2	
Psed_0490		HTH_8	HTH_8		
Psed_0495		TetR_N	TetR_N		
Psed_0497		HTH_5	HTH_5		
Psed_0507		TetR_N	TetR_N		
Psed_0518		TetR_N	TetR_N		
Psed_0533		HTH_5	HTH_5		
Psed_0569		TetR_N	TetR_N		
Psed_0577		MarR	MarR		
Psed_0579		TetR_N	TetR_N		
Psed_0588		HTH_3	HTH_3		
Psed_0616		TetR_N	TetR_N		
Psed_0620		GntR	GntR	FCD	
Psed_0628		HTH_8	HTH_8		
Psed_0647		MerR	MerR		

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Psed_0651		ANTAR	ANTAR		
Psed_0667	LysR_substrate	HTH_1	HTH_1	LysR_substrate	
Psed_0674		GGDEF	GGDEF		
Psed_0675		HxlR	HxlR		
Psed_0680		HxlR	HxlR		
Psed_0684		GntR	GntR	FCD	
Psed_0694	LysR_substrate	HTH_1	HTH_1	LysR_substrate	
Psed_0700		GerE	GerE		
Psed_0704		SpoIIE	SpoIIE		
Psed_0719		TetR_N	TetR_N		
Psed_0730		Rrf2	Rrf2		
Psed_0732		LytTR	LytTR		
Psed_0754		HTH_5	HTH_5		
Psed_0772	LysR_substrate	HTH_1	HTH_1	LysR_substrate	
Psed_0780	LysR_substrate	HTH_1	HTH_1	LysR_substrate	
Psed_0792	LysR_substrate	HTH_1	HTH_1	LysR_substrate	
Psed_0807		HTH_AraC	AraC_N	HTH_AraC	HTH_AraC
Psed_0820		HTH_AraC	AraC_N	HTH_AraC	HTH_AraC
Psed_0837		PadR	PadR		
Psed_0843		TetR_N	TetR_N		
Psed_0846		Trans_reg_C	Trans_reg_C	BTAD	
Psed_0873		HTH_3	HTH_3		
Psed_0905	LysR_substrate	HTH_1	HTH_1	LysR_substrate	
Psed_0912	GAF	ANTAR	GAF	ANTAR	
Psed_0917		HTH_5	HTH_5		
Psed_0927		TetR_N	TetR_N		

Appendix 1. One-component signal transduction proteins in *P. dioxanivorans* CB1190

Psed_0957		EAL	EAL	
Psed_0976		TetR_N	TetR_N	
Psed_0983		HTH_5	HTH_5	
Psed_1038		GntR	GntR	FCD
Psed_1042		MarR	MarR	
Psed_1053		GntR	GntR	FCD
Psed_1075		TetR_N	TetR_N	
Psed_1079		TetR_N	TetR_N	
Psed_1092	HEM4	Trans_reg_C	HEM4	Trans_reg_C
Psed_1132	SKI	GntR	GntR	SKI
Psed_1161	LysR_substrate	HTH_1	HTH_1	LysR_substrate
Psed_1163		HTH_5	HTH_5	
Psed_1176		MerR, MerR-DNA-bind	MerR	MerR-DNA-bind
Psed_1184		PadR	PadR	
Psed_1195		GntR	GntR	FCD
Psed_1291		MerR, MerR-DNA-bind	MerR	MerR-DNA-bind
Psed_1295		TetR_N	TetR_N	
Psed_1311		MerR, MerR-DNA-bind	MerR	MerR-DNA-bind
Psed_1314		HTH_15	HTH_15	MerB
Psed_1316		HTH_5	HTH_5	
Psed_1318		MerR, MerR-DNA-bind	MerR	MerR-DNA-bind
Psed_1324		HTH_3	HTH_3	

Appendix 1. One-component signal transduction proteins in *P. dioxanivorans* CB1190

Psed_1338	LysR_substrate	HTH_1	HTH_1	LysR_substrate	
Psed_1340		TetR_N	TetR_N		
Psed_1342		GntR	GntR	FCD	
Psed_1348		GntR	GntR	FCD	
Psed_1358		GntR	GntR	FCD	
Psed_1370	Peripla_BP_1	LacI	LacI	Peripla_BP_1	
Psed_1372		TetR_N	TetR_N		
Psed_1393		TetR_N	TetR_N		
Psed_1415	LysR_substrate	HTH_1	HTH_1	LysR_substrate	
Psed_1421		FUR	FUR		
Psed_1423		TetR_N	TetR_N		
Psed_1432		HTH_AraC	AraC_N	HTH_AraC	HTH_AraC
Psed_1445		HTH_3	HTH_3		
Psed_1465		GerE	GerE		
Psed_1480		HD	HD		
Psed_1481		HTH_AraC	DJ-1_PfpI	HTH_AraC	
Psed_1485		TrmB	TrmB	Regulator_TrmB	
Psed_1501		HTH_AraC	DJ-1_PfpI	HTH_AraC	
Psed_1527		Sigma70_r2, Sigma70_r3, Sigma70_r4	Sigma70_r2	Sigma70_r3	Sigma70_r4
Psed_1543		GntR	GntR	FCD	
Psed_1557		HxlR	HxlR		
Psed_1563	IclR	HTH_IclR	HTH_IclR	IclR	
Psed_1568		TetR_N	TetR_N		
Psed_1579		TetR_N	TetR_N		
Psed_1597		TetR_N	TetR_N		

Appendix 1. One-component signal transduction proteins in *P. dioxanivorans* CB1190

Psed_1628		Trans_reg_C	Trans_reg_C	BTAD
Psed_1632		MarR	MarR	
Psed_1634		Trans_reg_C	Trans_reg_C	
Psed_1636		HTH_3	Cupin_2	
Psed_1659		HTH_AraC	HTH_AraC	
Psed_1673		TetR_N	TetR_N	
Psed_1699		TetR_N	TetR_N	
Psed_1704		HTH_11	HTH_11	
Psed_1706		GerE	GerE	LuxR_C
Psed_1718		MarR	MarR	
Psed_1739		GerE	GerE	
Psed_1743	LysR_substrate	HTH_1	HTH_1	LysR_substrate
Psed_1782		TetR_N	TetR_N	
Psed_1802	Peripla_BP_1	LacI	LacI	Peripla_BP_1
Psed_1831	IclR	HTH_IclR	HTH_IclR	IclR
Psed_1900		Pkinase	Pkinase	
Psed_1921		HTH_AraC	Cupin_2	HTH_AraC
Psed_1925	LysR_substrate	HTH_1	HTH_1	LysR_substrate
Psed_1932		TetR_N	TetR_N	
Psed_1937		HD	HD	
Psed_1938		HTH_AraC	DJ-1_PfpI	HTH_AraC
Psed_1996		HTH_5	HTH_5	
Psed_1999		Trans_reg_C	Trans_reg_C	BTAD
Psed_2002		TetR_N	TetR_N	
Psed_2044		HTH_5	HTH_5	
Psed_2048		TetR_N	TetR_N	

Appendix 1. One-component signal transduction proteins in *P. dioxanivorans* CB1190

Psed_2059	Peripla_BP_1	LacI	LacI	Peripla_BP_1	
Psed_2060		TetR_N	TetR_N		
Psed_2078		TetR_N	TetR_N		
Psed_2081		HTH_8	HTH_8		
Psed_2106		HTH_1	HTH_1		
Psed_2122		LexA_DNA_bind	LexA_DNA_bind	Peptidase_S24	
Psed_2164	Peripla_BP_1	LacI	LacI	Peripla_BP_1	
Psed_2171		TetR_N	TetR_N		
Psed_2197	LysR_substrate	HTH_1	HTH_1	LysR_substrate	
Psed_2217		GerE	GerE		
Psed_2226		GerE	GerE		
Psed_2228		GerE	NACHT	GerE	
Psed_2245		HTH_8	HTH_8		
Psed_2246		GntR	GntR	FCD	
Psed_2267		MarR	MarR		
Psed_2269		HTH_11	HTH_11		
Psed_2278		GerE	GerE	LuxR_C	
Psed_2304		MarR	MarR		
Psed_2307		MarR	MarR		
Psed_2318		MerR	MerR		
Psed_2323	IclR	HTH_IclR	HTH_IclR	IclR	
Psed_2338		ANTAR	ANTAR		
Psed_2350		Sigma70_r2, Sigma70_r3, Sigma70_r4	Sigma70_r2	Sigma70_r3	Sigma70_r4
Psed_2351		HTH_3	HTH_3		

Appendix 1. One-component signal transduction proteins in *P. dioxanivorans* CB1190

Psed_2357		TetR_N	TetR_N		
Psed_2376		ANTAR	ANTAR		
Psed_2377	GAF	ANTAR	GAF	ANTAR	
Psed_2380		Sigma70_r2, Sigma70_r3, Sigma70_r4	Sigma70_r2	Sigma70_r3	Sigma70_r4
Psed_2409		HTH_5	HTH_5		
Psed_2412		HTH_5	HTH_5	Acetyltransf_1	
Psed_2425		Sigma70_r2, Sigma70_r3, Sigma70_r4	Sigma70_r2	Sigma70_r3	Sigma70_r4
Psed_2432		HTH_3	HTH_3		
Psed_2442		HTH_8	HTH_8		
Psed_2450		GntR	GntR	UTRA	
Psed_2469		GntR	GntR	FCD	
Psed_2471		TetR_N	TetR_N		
Psed_2474		TetR_N	TetR_N		
Psed_2485		TetR_N	TetR_N		
Psed_2487	LysR_substrate	HTH_1	HTH_1	LysR_substrate	
Psed_2490		TetR_N	TetR_N		
Psed_2501		GntR	GntR	FCD	
Psed_2505		Sigma70_r2, Sigma70_r3, Sigma70_r4	Sigma70_r2	Sigma70_r3	Sigma70_r4
Psed_2507	LysR_substrate	HTH_1	HTH_1	LysR_substrate	
Psed_2518	IclR	HTH_IclR	HTH_IclR	IclR	
Psed_2525	IclR	HTH_IclR	HTH_IclR	IclR	
Psed_2530	IclR	HTH_IclR	HTH_IclR	IclR	
Psed_2532		TetR_N	TetR_N		

Appendix 1. One-component signal transduction proteins in *P. dioxanivorans* CB1190

Psed_2534		TetR_N	TetR_N			
Psed_2548		GntR	GntR	FCD		
Psed_2577		GntR	GntR	FCD		
Psed_2609		TrmB	TrmB			
Psed_2614		MarR	MarR			
Psed_2615		TetR_N	TetR_N			
Psed_2626		GntR	GntR	FCD		
Psed_2629		MarR	MarR			
Psed_2637		Pkinase	Pkinase			
Psed_2639	LysR_substrate	HTH_1	HTH_1	LysR_substrate		
Psed_2660		TetR_N	TetR_N			
Psed_2673		TetR_N	TetR_N			
Psed_2695		TetR_N	TetR_N			
Psed_2699		TetR_N	TetR_N			
Psed_2711		TetR_N	TetR_N			
Psed_2713		FUR	FUR			
Psed_2735		TetR_N	TetR_N			
Psed_2744		HTH_3	HTH_3			
Psed_2747	IclR	HTH_IclR	HTH_IclR	IclR		
Psed_2750		HTH_AraC	DJ-1_PfpI	HTH_AraC	HTH_AraC	
Psed_2753		GntR	GntR	FCD		
Psed_2779		Pkinase	Pkinase	PASTA	PASTA	PASTA
Psed_2834		PaaX	PaaX	PaaX_C		
Psed_2861		TetR_N	TetR_N			
Psed_2886		GGDEF	GGDEF			
Psed_2887		GGDEF	GGDEF			

Appendix 1. One-component signal transduction proteins in *P. dioxanivorans* CB1190

Psed_2941		MarR	MarR			
Psed_2942	TetR_C	GntR, TetR_N	GntR	TetR_N	TetR_C	
Psed_2949		HTH_AraC	HTH_AraC			
Psed_2955	IclR	HTH_IclR	HTH_IclR	IclR		
Psed_2969		GerE	GerE			
Psed_2971	IclR	HTH_IclR	HTH_IclR	IclR		
Psed_2978		Sigma70_r2, Sigma70_r3, Sigma70_r4	Sigma70_r1_2	Sigma70_r2	Sigma70_r3	Sigma70_r4
Psed_2980		HTH_3	HTH_3			
Psed_2985		HTH_5	HTH_5			
Psed_3029		GntR	GntR	FCD		
Psed_3040	FeoA	Fe_dep_repr_C, Fe_dep_repress	Fe_dep_repress	Fe_dep_repr_C	FeoA	
Psed_3045		MarR	MarR			
Psed_3051		Sigma70_r2, Sigma70_r3, Sigma70_r4	Sigma70_r1_2	Sigma70_r2	Sigma70_r3	Sigma70_r4
Psed_3091		Trans_reg_C	Trans_reg_C	BTAD		
Psed_3119		TetR_N	TetR_N			
Psed_3138		DUF742	DUF742			
Psed_3147		Sigma70_r2, Sigma70_r3, Sigma70_r4	Sigma70_r2	Sigma70_r3	Sigma70_r4	
Psed_3170		MarR	MarR			
Psed_3172		Pkinase	Pkinase			
Psed_3176		HxlR	HxlR			
Psed_3187		GntR	GntR	FCD		
Psed_3203		MerR, MerR-DNA-bind	MerR	MerR-DNA-bind		

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Psed_3209	IclR	HTH_IclR	HTH_IclR	IclR			
Psed_3215		TetR_N	TetR_N				
Psed_3226		TetR_N	TetR_N				
Psed_3227		TetR_N	TetR_N				
Psed_3228		GntR	GntR	FCD			
Psed_3239		HTH_5	HTH_5				
Psed_3253		PadR	PadR				
Psed_3268		HTH_AraC	HTH_AraC	HTH_AraC			
Psed_3309		MarR	MarR				
Psed_3316		GerE	GerE				
Psed_3338		Pencillinase_R	Pencillinase_R				
Psed_3384		TetR_N	TetR_N				
Psed_3392		Guanylate_cyc	Guanylate_cyc				
Psed_3399		HTH_5	HTH_5				
Psed_3420		HTH_WhiA	WhiA_N	HTH_WhiA			
Psed_3478		Sigma70_r2, Sigma70_r3, Sigma70_r4	Sigma70_r2	Sigma70_r3	Sigma70_r4		
Psed_3529		HTH_3	HTH_3				
Psed_3547		TetR_N	TetR_N				
Psed_3552		TetR_N	TetR_N				
Psed_3578	ACT	HD	HD	RelA_SpoT	TGS	ACT	
Psed_3605		HTH_3	HTH_3	Cupin_2			
Psed_3622		MerR	MerR	TipAS			
Psed_3623	GAF, PAS	SpoIIE	PAS	PAS_4	PAS_3	GAF	SpoIIE
Psed_3644		MarR	MarR				
Psed_3655		TetR_N	TetR_N				

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Psed_3666		DeoR, HTH_DeoR	HTH_DeoR	DeoR
Psed_3671		GntR	GntR	FCD
Psed_3690		MarR	MarR	
Psed_3701		GntR	GntR	FCD
Psed_3713		HTH_AraC	HTH_AraC	
Psed_3717	IclR	HTH_IclR	HTH_IclR	IclR
Psed_3735	IclR	HTH_IclR	HTH_IclR	IclR
Psed_3746	IclR	HTH_IclR	HTH_IclR	IclR
Psed_3758	IclR	HTH_IclR	HTH_IclR	IclR
Psed_3766		GntR	GntR	FCD
Psed_3772		HTH_5	HTH_5	
Psed_3779		PadR	PadR	Vir_act_alpha_C
Psed_3793		GerE	Abhydrolase_1	GerE
Psed_3796		HxlR	HxlR	
Psed_3802		MarR	MarR	
Psed_3827		MerR, MerR-DNA-bind	MerR	MerR-DNA-bind
Psed_3831		Arg_repressor	Arg_repressor	Arg_repressor_C
Psed_3859	LysR_substrate	HTH_1	HTH_1	LysR_substrate
Psed_3860		TetR_N	TetR_N	
Psed_3887	IclR	HTH_IclR	HTH_IclR	IclR
Psed_3892		GntR	GntR	UTRA
Psed_3897		TetR_N	TetR_N	
Psed_3902		PadR	PadR	
Psed_3908	LysR_substrate	HTH_1	HTH_1	LysR_substrate
Psed_3919		GntR	GntR	FCD

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Psed_3931	AsnC_trans_reg	HTH_IclR	HTH_IclR	AsnC_trans_reg	
Psed_3935		GntR	GntR	FCD	
Psed_3983	FeoA	Fe_dep_repr_C, Fe_dep_repress	Fe_dep_repress	Fe_dep_repr_C	FeoA
Psed_3997		TetR_N	TetR_N		
Psed_4001		MarR	MarR		
Psed_4011		MarR	MarR		
Psed_4027		MarR	MarR		
Psed_4029			GntR	GntR	FCD
Psed_4046		SpoIIE	SpoIIE	SpoIIE	
Psed_4050	LysR_substrate	HTH_1	HTH_1	LysR_substrate	LysR_substate
Psed_4058		HTH_11	HTH_11		
Psed_4081	PAS	EAL, GGDEF	PAS	GGDEF	EAL
Psed_4135		TetR_N	TetR_N		
Psed_4140		Trans_reg_C	trans_reg_C	BTAD	
Psed_4151		GntR	GntR	FCD	
Psed_4165		GntR	GntR	FCD	
Psed_4176		GntR	GntR	FCD	
Psed_4177	IclR	HTH_IclR	HTH_IclR	IclR	
Psed_4185	IclR	HTH_IclR	HTH_IclR	IclR	
Psed_4197	IclR	HTH_IclR	HTH_IclR	IclR	
Psed_4211		TetR_N	TetR_N		
Psed_4223		HxlR	HxlR		
Psed_4233		SpoIIE	SpoIIE		
Psed_4235		Pkinase	Pkinase		
Psed_4240		SpoIIE	SpoIIE		

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Psed_4243		PaaX	PaaX	PaaX_C
Psed_4256		GerE	GerE	
Psed_4257		GerE	GerE	
Psed_4278		HD	HD	
Psed_4286	IclR	HTH_IclR	HTH_IclR	IclR
Psed_4296		TetR_N	TetR_N	
Psed_4312		TetR_N	TetR_N	
Psed_4315		GerE	GerE	
Psed_4319		GerE	GerE	
Psed_4328		GerE, HD	HD	GerE
Psed_4345		HTH_5	HTH_5	
Psed_4354	Rhodanese	HTH_5	HTH_5	Rhodanese
Psed_4377		HTH_5	HTH_5	
Psed_4392		GntR	GntR	GntR
Psed_4393		GntR	Phage_integrase	GntR
Psed_4405	TetR_C	TetR_N	TetR_N	TetR_C
Psed_4410		MarR	MarR	
Psed_4418		TetR_N	TetR_N	
Psed_4422		TetR_N	TetR_N	TetR_C_4
Psed_4426		HTH_AraC	Cupin_2	HTH_AraC
Psed_4427		TetR_N	TetR_N	
Psed_4430		HTH_AraC	HTH_AraC	
Psed_4442		MarR	MarR	
Psed_4445		TetR_N	TetR_N	
Psed_4457		HD	HD	
Psed_4470		HTH_5	HTH_5	

Appendix 1. One-component signal transduction proteins in *P. dioxanivorans* CB1190

Psed_4473		HTH_5	HTH_5			
Psed_4474		FUR	FUR			
Psed_4494		HTH_DeoR	HTH_DeoR	HrcA		
Psed_4529			LepA	lepA_II	lepA_C	LepA_C
Psed_4552		TetR_N	TetR_N			
Psed_4576	TetR_C	TetR_N	TetR_N	TetR_C		
Psed_4595	LysR_substrate	HTH_1	HTH_1	LysR_substrate		
Psed_4601		TetR_N	TetR_N			
Psed_4611		TetR_N	TetR_N			
Psed_4621		HTH_3	HTH_3			
Psed_4631		HTH_IclR	HTH_IclR			
Psed_4636		GntR	GntR	FCD		
Psed_4650		GntR	GntR	UTRA		
Psed_4716		MarR	MarR			
Psed_4731		Sigma70_r2, Sigma70_r3, Sigma70_r4	Sigma70_rw	Sigma70_r3	Sigma70_r4	
Psed_4752		HD	GlnD_UR_Utase	HD		
Psed_4787		GntR	GntR	FCD		
Psed_4810			PP2C			
Psed_4812		GntR	Phage_integrase	GntR		
Psed_4834		Pencillinase_R	Pencillinase_R			
Psed_4851	PAS	ANTAR	PAS_4	ANTAR		
Psed_4868		Pencillinase_R	Pencillinase_R			
Psed_4879		MarR	MarR			
Psed_4886		GerE	GerE			
Psed_4912		GerE	GerE			

Appendix 1. One-component signal transduction proteins in *P. dioxanivorans* CB1190

Psed_4913		GntR	GntR	FCD	
Psed_4938		MarR	MarR		
Psed_4943		TetR_N	TetR_N		
Psed_4945		HxlR	HxlR		
Psed_4947		HTH_5	HTH_5		
Psed_4974		Rrf2	Rrf2		
Psed_4979		MarR	MarR		
Psed_5008		TetR_N	TetR_N		
Psed_5011	LysR_substrate	HTH_1	HTH_1	LysR_substrate	
Psed_5026		TetR_N	TetR_N		
Psed_5035		TetR_N	TetR_N		
Psed_5049		GntR	GntR	FCD	
Psed_5051	LysR_substrate	HTH_1	HTH_1	LysR_substrate	
Psed_5062	cNMP_binding	HTH_CodY	cNMP_binding	HTH_CodY	
Psed_5069		PaaX	PaaX	PaaX_C	
Psed_5074	IclR	HTH_IclR	HTH_IclR	IclR	
Psed_5104		MarR	MarR		
Psed_5124		PadR	PadR		
Psed_5127		HTH_8	HTH_8		
Psed_5129		PadR	PadR		
Psed_5134	PAS	EAL, GGDEF	PAS_4	GGDEF	EAL
Psed_5164		Pkinase	Pkinase		
Psed_5165		TetR_N	TetR_N		
Psed_5177		MarR	MarR	Hydrolase	
Psed_5190		TetR_N	TetR_N		
Psed_5236		TetR_N	TetR_N		

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Psed_5245		TetR_N	TetR_N	
Psed_5254		TetR_N	TetR_N	
Psed_5256		LytTR	LytTR	
Psed_5283		TetR_N	TetR_N	
Psed_5286		Pkinase	Pkinase	
Psed_5290		TetR_N	TetR_N	
Psed_5309		Pkinase	Pkinase	
Psed_5319		TetR_N	TetR_N	
Psed_5321		MerR, MerR-DNA-bind	MerR	MerR-DNA-bind
Psed_5349		RHH_1	RHH_1	
Psed_5386		HTH_3	HTH_3	Cupin_2
Psed_5459	Aminotran_1_2	GntR	GntR	Aminotran_1_2
Psed_5472		GerE	GerE	
Psed_5487		HTH_3	HTH_3	Cupin_2
Psed_5493		TetR_N	TetR_N	
Psed_5494		TetR_N	TetR_N	
Psed_5496		MerR	MerR	
Psed_5511		HTH_AraC	HTH_AraC	
Psed_5516		FUR	FUR	
Psed_5541		HTH_AraC	HTH_AraC	
Psed_5578		GerE	GerE	
Psed_5594		ANTAR	ANTAR	
Psed_5596		TetR_N	TetR_N	
Psed_5604		MerR	MerR	

Appendix 1. One-component signal transduction proteins in *P. dioxanivorans* CB1190

Psed_5620		Put_DNA-bind_N	Put_DNA-bind_N	CoA_binding
Psed_5641		MarR	MarR	
Psed_5717		HTH_11	HTH_11	
Psed_5726		DUF742	DUF472	
Psed_5819		MerR	MerR	
Psed_5850		Trans_reg_C	Trans_reg_c	BTAD
Psed_5855	Aminotran_1_2	GntR	GntR	Aminotran_1_2
Psed_5877		HTH_5	HTH_5	
Psed_5885		TetR_N	TetR_N	
Psed_5887		HTH_11	HTH_11	
Psed_5889		GerE	GerE	
Psed_5896		TetR_N	TetR_N	
Psed_5909		GntR	GntR	
Psed_5935		PadR	PadR	Vir_act_alpha_C
Psed_5937		TetR_N	TetR_N	
Psed_5956		HTH_5	HTH_5	
Psed_5970		GntR	GntR	UTRA
Psed_5975		HxlR	HxlR	
Psed_5977		HTH_AraC	AraC-N	HTH_AraC
Psed_5980		HTH_8	HTH_8	
Psed_5990		GerE	GerE	
Psed_5997		HTH_8	HTH_8	
Psed_6000		HTH_3	HTH_3	
Psed_6003		MarR	MarR	
Psed_6010	AsnC_trans_reg	HTH_5	HTH_5	AsnC_trans_reg

Appendix 1. One-component signal transduction proteins in *P. dioxanivorans* CB1190

Psed_6038		HTH_AraC	HTH_AraC	
Psed_6068	IclR	HTH_IclR	HTH_IclR	IclR
Psed_6086		GntR	Phage_integrase	GntR
Psed_6093	Peripla_BP_1	LacI	LacI	Peripla_BP_1
Psed_6147		MarR	MarR	
Psed_6159		TetR_N	TetR_N	
Psed_6163		HTH_5	HTH_5	
Psed_6170		MerR	MerR	
Psed_6171		Guanylate_cyc	HAMP	Guanylate_cyc
Psed_6175		TetR_N	TetR_N	
Psed_6187		PadR	PadR	Vir_act_alpha_C
Psed_6195		TetR_N	TetR_N	
Psed_6209		GntR	GntR	FCD
Psed_6222		TetR_N	TetR_N	
Psed_6231		HTH_3	HTH_3	Cupin_2
Psed_6252	LysR_substrate	HTH_1	HTH_1	LysR_substrate
Psed_6254		GerE	GerE	
Psed_6264		GntR	GntR	
Psed_6270		TetR_N	TetR_N	
Psed_6275		TetR_N	TetR_N	
Psed_6282		Trans_reg_C	Trans_reg_C	BTAD
Psed_6297		HxlR	HxlR	
Psed_6299		GerE	GerE	
Psed_6307		HTH_5	HTH_5	
Psed_6310		PadR	PadR	
Psed_6316		GerE	Abhydrolase_1	GerE

Appendix 1. One-component signal transduction proteins in *P. dioxanivorans* CB1190

Psed_6320		HTH_AraC	HTH_AraC	
Psed_6325		Trans_reg_C	Trans_reg_c	BTAD
Psed_6327	LysR_substrate	HTH_1	HTH_1	LysR_substrate
Psed_6331		GerE	GerE	
Psed_6334		TetR_N	TetR_N	
Psed_6359		HTH_AraC	HTH_AraC	HTH_AraC
Psed_6397		TetR_N	TetR_N	
Psed_6398		TetR_N	TetR_N	
Psed_6404		GerE	GerE	
Psed_6429		TetR_N	TetR_N	
Psed_6437		GerE	GerE	
Psed_6447		HTH_AraC	HTH_AraC	
Psed_6454		TetR_N	TetR_N	
Psed_6457		GntR	GntR	FCD
Psed_6482		HTH_AraC	HTH_AraC	
Psed_6507	cNMP_ b+C2inding	Crp	cNMP_binding	Crp
Psed_6530	IclR	HTH_IclR	HTH_IclR	IclR
Psed_6552		MerR	MerR	
Psed_6574		TetR_N	TetR_N	
Psed_6598		GerE	GerE	
Psed_6621		HTH_11	HTH_11	
Psed_6622	LysR_substrate	HTH_1	HTH_1	LysR_substrate
Psed_6625		TetR_N	TetR_N	
Psed_6627		TetR_N	TetR_N	
Psed_6663		PadR	PadR	

Appendix 1. One-component signal transduction proteins in *P. dioxanivorans* CB1190

Psed_6711	HTH_5	HTH_5	
Psed_6721	HTH_5	HTH_5	
Psed_6727	GcrA	GcrA	
Psed_6731	GntR	FCD	
Psed_6786	GntR	GntR	FCD
Psed_6816	HTH_3	HTH_3	

Appendix 1. One-component signal transduction proteins in *P. dioxanivorans* CB1190

Appendix 2

Signal transduction proteins with only input (sensory) domains

Appendix 2. Signal transduction proteins with only input (sensory) domains

Gene	Input Domains	Total Domain Architecture	
Psed_0119	FHA	FHA	
Psed_0120	FHA		FHA
Psed_0708	CBS	CBS_pair	
Psed_1276	cNMP_binding	cNMP_ binding	
Psed_1285	cNMP_binding	cNMP_ binding	
Psed_1329	GAF	GAF	
Psed_1538	STAS	STAS	
Psed_1693	SBP_bac_3	SBP_bac_3	
Psed_1697	SBP_bac_3	SBP_bac_3	
Psed_1898	CBS	CBS	
Psed_2083	GAF	GAF	
Psed_2216	cNMP_binding, Pyr_redox_2	cNMP_ binding	Pyr_re- dox_2
Psed_2284	CBS	CBS	CBS
Psed_2363	CBS	CBS	
Psed_2459	GAF	GAF	
Psed_3160	FHA	FHA	
Psed_3359	GAF	GAF	
Psed_3456	cNMP_binding, Pyr_redox_2	cNMP_ binding	Pyr_re- dox_2
Psed_3650	STAS	STAS	
Psed_3961	SBP_bac_3	SBP_bac_3	
Psed_4132	GAF	GAF	
Psed_4840	CBS	CBS	
Psed_4931	SBP_bac_3	SBP_bac_3	
Psed_6601	CBS	CBS	
Psed_6612	SBP_bac_3	SBP_bac_3	

Appendix 3

Bacterial σ factors, Extracytoplasmic function (ECF) σ factors, and anti-σ-factors in P. *dioxanviroans* CB1190

Appendix 3. Bacterial σ factors, Extracytoplasmic function (ECF) σ factors, and anti-σ-factors in P. *dioxanviroans*

Gene	Protein	Domains
Psed_0095	RNA polymerase sigma factor, sigma-70 family	ECF_42
Psed_0255	RNA polymerase sigma-70 factor	ECF_41
Psed_0291	RNA polymerase sigma factor, sigma-70 family	ECF_1
Psed_0376	RNA polymerase sigma factor, sigma-70 family	ECF_42
Psed_0426	RNA polymerase sigma-70 factor, sigma-E family	ECF_39
Psed_0659	RNA polymerase sigma factor, sigma-70 family	ECF_17
Psed_0699	RNA polymerase sigma factor, sigma-70 family	ECF_42
Psed_0774	RNA polymerase sigma factor, sigma-70 family	ECF_41
Psed_0935	RNA polymerase sigma factor, sigma-70 family	ECF_42
Psed_0936	RNA polymerase sigma factor, sigma-70 family	ECF_40
Psed_1028	RNA polymerase sigma factor, sigma-70 family	ECF_14
Psed_1140	RNA polymerase sigma factor, sigma-70 family	ECF_41
Psed_1267	RNA polymerase sigma factor, sigma-70 family	ECF_26
Psed_1308	RNA polymerase sigma factor, sigma-70 family	ECF_26
Psed_1456	RNA polymerase sigma factor, sigma-70 family	ECF_999
Psed_1650	RNA polymerase sigma factor, sigma-70 family	ECF_41
Psed_1727	RNA polymerase sigma factor, sigma-70 family	ECF_999
Psed_1918	RNA polymerase sigma-70 factor	ECF_999
Psed_1959	RNA polymerase sigma factor, sigma-70 family	ECF_1
Psed_2039	RNA polymerase sigma factor, sigma-70 family	ECF_1
Psed_3546	RNA polymerase sigma factor, sigma-70 family	ECF_40
Psed_3658	RNA polymerase sigma-70 factor	ECF_999
Psed_3774	RNA polymerase sigma factor, sigma-70 family	ECF_42

Appendix 3. Bacterial σ factors, Extracytoplasmic function (ECF) σ factors, and anti-σ-factors in P. *dioxanviroan* CB1190

Psed_3869	RNA polymerase sigma factor, sigma-70 family	ECF_999		
Psed_3955	RNA polymerase sigma factor, sigma-70 family	ECF_42		
Psed_4117	RNA polymerase sigma factor, sigma-70 family	ECF_42		
Psed_4275	RNA polymerase sigma factor, sigma-70 family	ECF_1		
Psed_4454	RNA polymerase sigma factor, sigma-70 family	ECF_41		
Psed_5156	RNA polymerase sigma-70 factor	ECF_12		
Psed_5354	RNA polymerase sigma factor, sigma-70 family	ECF_40		
Psed_5625	RNA polymerase sigma factor, sigma-70 family	ECF_12		
Psed_5845	RNA polymerase sigma factor, sigma-70 family	ECF_36		
Psed_6524	RNA polymerase sigma-70 factor, sigma-E family	ECF_38		
Psed_6676	RNA polymerase sigma factor, sigma-70 family	ECF_27		
Psed_1527	RNA polymerase sigma-70 factor, sigma-B/F/G subfamily	Sigma70_r2	Sigma70_r3	Sigma70_r4
Psed_2350	RNA polymerase sigma factor, sigma-70 family	Sigma70_r2	Sigma70_r3	Sigma70_r4
Psed_2380	RNA polymerase sigma-70 factor, sigma-B/F/G subfamily	Sigma70_r2	Sigma70_r3	Sigma70_r4
Psed_2425	RNA polymerase sigma factor, sigma-70 family	Sigma70_r2	Sigma70_r3	Sigma70_r4
Psed_2505	RNA polymerase sigma-70 factor, sigma-B/F/G subfamily	Sigma70_r2	Sigma70_r3	Sigma70_r4
Psed_2978	RNA polymerase sigma factor, sigma-70 family	Sigma70_r2	Sigma70_r3	Sigma70_r4
Psed_3051	RNA polymerase sigma factor RpoD	Sigma70_r2	Sigma70_r3	Sigma70_r4
Psed_3147	RNA polymerase sigma-70 factor, sigma-B/F/G subfamily	Sigma70_r2	Sigma70_r3	Sigma70_r4
Psed_3478	RNA polymerase sigma-70 factor, sigma-B/F/G subfamily	Sigma70_r2	Sigma70_r3	Sigma70_r4
Psed_4731	RNA polymerase sigma factor, FliA/WhiG family	Sigma70_r2	Sigma70_r3	Sigma70_r4
Psed_0240	RNA polymerase, sigma-24 subunit, ECF subfamily			
Psed_2245	sigma-54 factor interaction domain-containing protein	HTH_8		
Psed_2442	sigma-54 factor interaction domain-containing protein	HTH_8		
Psed_5980	sigma-54 factor interaction domain-containing protein	HTH_8		
Psed_5997	sigma-54 factor interaction domain-containing protein	HTH_8		

Appendix 3. Bacterial σ factors, Extracytoplasmic function (ECF) σ factors, and anti-σ-factors in P. *dioxanviroan* CB1190

Psed_0312	Sulfate transporter/antisigma-factor antagonist STAS	STAS
Psed_0660	Anti-sigma-K factor RskA	
Psed_1528	anti-sigma factor	
Psed_1531	anti-anti-sigma factor	
Psed_1538	anti-anti-sigma factor	
Psed_2040	putative transmembrane anti-sigma factor	
Psed_2310	Sulfate transporter/antisigma-factor antagonist STAS	STAS
Psed_2382	Sulfate transporter/antisigma-factor antagonist STAS	STAS
Psed_2627	Sulfate transporter/antisigma-factor antagonist STAS	STAS
Psed_2628	Sulfate transporter/antisigma-factor antagonist STAS	STAS
Psed_2775	Putative anti-sigma regulatory factor, serine/threonine protein kinase	
Psed_3156	Sulfate transporter/antisigma-factor antagonist STAS	STAS
Psed_3488	Sulfate transporter/antisigma-factor antagonist STAS	STAS
Psed_3649	Sulfate transporter/antisigma-factor antagonist STAS	STAS
Psed_3650	Sulfate transporter/antisigma-factor antagonist STAS	STAS
Psed_4203	Sulfate transporter/antisigma-factor antagonist STAS	STAS
Psed_4239	Sulfate transporter/antisigma-factor antagonist STAS	STAS
Psed_5155	anti-sigma factor	

Appendix 3. Bacterial σ factors, Extracytoplasmic function (ECF) σ factors, and anti-σ-factors in P. *dioxanviroan* CB1190

Appendix 4

Transport proteins in *P. dioxanivorans* CB1190

Appendix 4. Transport proteins in *P. dioxanivorans* CB1190

Locus Tag	Protein	Putative Substrate/ Mechanism	Transporter Classification	% ID	E-Value
Psed_0424	Ion transport 2 domain protein	K+	1.A.1.1.1	43.59	2E-18
Psed_1101	TrkA-N domain protein	K+	1.A.1.13.2	27.4	1E-14
Psed_1276	cyclic nucleotide-binding	K+	1.A.1.24.1	29.71	0.0000004
Psed_6507	cyclic nucleotide-binding	K+	1.A.1.25.1	29.46	5E-10
Psed_1137	Ion transport 2 domain protein	K+	1.A.1.3.4	42.55	0.000007
Psed_4755	ammonium transporter	ammonia	1.A.11.1.3	49.11	9E-107
Psed_0619	Monosaccharide-transporting ATPase	K+	1.A.2.2.2	34.11	6E-39
Psed_0794	large conductance mechanosensitive channel protein	ion	1.A.22.1.2	49.32	2E-28
Psed_3470	MscS Mechanosensitive ion channel	ion	1.A.23.2.1	26.02	0.00000002
Psed_4800	MscS Mechanosensitive ion channel	ion	1.A.23.3.1	37.2	1E-29
Psed_2779	Calcium/calmodulin-dependent protein kinase	PPD	1.A.26.1.1	25.7	5E-15
Psed_5164	Serine/threonine protein kinase-related	PPD	1.A.26.1.1	28.78	6E-17
Psed_5286	Serine/threonine protein kinase-related	PPD	1.A.26.1.1	22.29	3E-12
Psed_3048	AmiS/UreI transporter	urea/amide	1.A.29.1.2	57.58	5E-43
Psed_1830	Heat shock protein 70	Hsp70	1.A.33.1.2	29.77	3E-32
Psed_3986	Peptidase M23	GJ-CC	1.A.34.1.1	29.25	0.000008
Psed_5334	Peptidase M23	GJ-CC	1.A.34.1.1	31.68	0.000006
Psed_1060	magnesium and cobalt transport protein CorA	Mg2+/Co2+/Ni2+	1.A.35.3.1	19.09	6E-16
Psed_2341	Mg2 transporter protein CorA family protein	Mg2+/Co2+/Ni2+	1.A.35.3.1	22.39	3E-16
Psed_4761	magnesium and cobalt transport protein CorA	Mg2+/Co2+/Ni2+	1.A.35.3.1	20.71	7E-16
Psed_0096	Uncharacterised protein family UPF0126	TRIC	1.A.62.2.1	26.5	5E-17

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Psed_3785	Uncharacterised protein family UPF0126	TRIC	1.A.62.2.1	30.81	5E-27
Psed_2301	Uncharacterised protein family UPF0126	TRIC	1.A.62.3.1	32.57	4E-25
Psed_2045	major intrinsic protein	MIP	1.A.8.12.3	38.74	3E-35
Psed_2223	MIP family channel protein	MIP	1.A.8.9.1	42.49	6E-48
Psed_5075	MIP family channel protein	MIP	1.A.8.9.1	41.18	1E-43
Psed_1645	ATP-dependent Clp protease proteolytic subunit	Autotransporter	1.B.12.4.1	59.9	3E-58
Psed_1963	ATP-dependent Clp protease proteolytic subunit	Autotransporter	1.B.12.4.1	69.14	9E-66
Psed_1964	ATP-dependent Clp protease proteolytic subunit	Autotransporter	1.B.12.4.1	52.63	3E-47
Psed_5788	peptidase S14 ClpP	Autotransporter	1.B.12.4.1	27.69	0.000005
Psed_1186	cell division ATP-binding protein FtsE	Bam Complex	1.B.33.1.3	47.69	3E-47
Psed_3816	hypothetical protein	AFB-OM	1.B.50.1.1	43.04	1E-47
Psed_5552	OmpA/MotB domain protein	OOP	1.B.6.1.3	37.27	0.00000004
Psed_5461	ribosomal protein L1	drugs	1.C.82.1.1	54.46	4E-61
Psed_5379	protein of unknown function DUF909	ESAT-6	1.C.95.1.1	38.18	0.000008
Psed_1965	ATP-dependent Clp protease ATP-binding subunit clpX	SVF-Pore	1.F.1.1.1	37.74	0.000001
Psed_3490	major facilitator superfamily MFS_1	sugar	2.A.1.1.15	32.13	3E-20
Psed_4042	major facilitator superfamily MFS_1	sugar	2.A.1.1.15	30.4	6E-22
Psed_5250	major facilitator superfamily MFS_1	glucose	2.A.1.1.57	36.84	0.0000002
Psed_4352	major facilitator superfamily MFS_1	rhamnose	2.A.1.1.74	27.97	0.000001
Psed_5050	major facilitator superfamily MFS_1	xylose/fructose	2.A.1.1.75	31.01	0.000006
Psed_1967	major facilitator superfamily MFS_1	oxalate/formate	2.A.1.11.1	26.39	1E-18
Psed_2051	major facilitator superfamily MFS_1	oxalate/formate	2.A.1.11.1	27.2	0.0000001

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Psed_2410	major facilitator superfamily MFS_1	oxalate/formate	2.A.1.11.1	23.43	0.0000005
Psed_3759	major facilitator superfamily MFS_1	sialic acid	2.A.1.12.1	26.01	4E-34
Psed_6197	major facilitator superfamily MFS_1	lactate/pyruvate	2.A.1.12.2	27.41	1E-36
Psed_6774	major facilitator superfamily MFS_1	glucarate	2.A.1.14.1	27.7	4E-27
Psed_3070	major facilitator superfamily MFS_1	tartrate	2.A.1.14.3	51.51	3E-117
Psed_0071	major facilitator superfamily MFS_1	4-hydroxybenzoate/protocatachuate	2.A.1.15.1	34.64	5E-64
Psed_3691	major facilitator superfamily MFS_1	benzoate	2.A.1.15.5	22.86	0.00000009
Psed_4280	major facilitator superfamily MFS_1	vanillate	2.A.1.15.6	35.78	4E-77
Psed_6459	major facilitator superfamily MFS_1	niacin/nicotinamide	2.A.1.15.7	28.23	6E-44
Psed_2620	major facilitator superfamily MFS_1	4-methylmuconolactone	2.A.1.15.9	28.01	4E-31
Psed_1011	major facilitator superfamily MFS_1	cyanate	2.A.1.17.1	27.45	3E-17
Psed_4062	major facilitator superfamily MFS_1	cyanate	2.A.1.17.1	31.03	2E-21
Psed_0478	major facilitator superfamily MFS_1	drugs	2.A.1.19.1	30.36	0.0000002
Psed_3530	major facilitator superfamily MFS_1	arabinose	2.A.1.2.14	33.6	1E-49
Psed_1190	major facilitator superfamily MFS_1	drugs	2.A.1.2.24	46.25	1E-79
Psed_4210	major facilitator superfamily MFS_1	drugs	2.A.1.2.24	27.73	0.00000003
Psed_1424	major facilitator superfamily MFS_1	tetracycline	2.A.1.2.39	34.27	0.000001
Psed_0339	drug resistance transporter, Bcr/CflA subfamily	drugs	2.A.1.2.7	30.17	6E-29
Psed_4022	drug resistance transporter, Bcr/CflA subfamily	drugs	2.A.1.2.7	29.62	4E-38
Psed_6185	drug resistance transporter, Bcr/CflA subfamily	drugs	2.A.1.2.7	33.7	9E-46
Psed_3340	drug resistance transporter, EmrB/QacA subfamily	drugs	2.A.1.3.10	34.95	5E-48

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Psed_5318	drug resistance transporter, EmrB/QacA subfamily	drugs	2.A.1.3.10	33.25	2E-43
Psed_0838	drug resistance transporter, EmrB/QacA subfamily	drugs	2.A.1.3.12	34.48	3E-56
Psed_1390	major facilitator superfamily MFS_1	drugs	2.A.1.3.12	32.55	2E-41
Psed_4128	major facilitator superfamily MFS_1	drugs	2.A.1.3.12	29.89	6E-12
Psed_4157	major facilitator superfamily MFS_1	drugs	2.A.1.3.12	32	0.0000008
Psed_4406	major facilitator superfamily MFS_1	drugs	2.A.1.3.12	34.18	2E-46
Psed_5865	major facilitator superfamily MFS_1	drugs	2.A.1.3.12	29.19	8E-26
Psed_4010	major facilitator superfamily MFS_1	rifamycin	2.A.1.3.15	29.66	1E-14
Psed_5984	major facilitator superfamily MFS_1	rifamycin	2.A.1.3.15	31.65	6E-32
Psed_1803	major facilitator superfamily MFS_1	drugs	2.A.1.3.16	28.24	1E-14
Psed_3851	major facilitator superfamily MFS_1	drugs	2.A.1.3.17	31.63	7E-42
Psed_2111	major facilitator superfamily MFS_1	drugs	2.A.1.3.26	35.65	5E-60
Psed_0146	drug resistance transporter, EmrB/QacA subfamily	tetracycline	2.A.1.3.29	43.14	3E-110
Psed_6624	major facilitator superfamily MFS_1	drugs	2.A.1.3.3	47.48	2E-100
Psed_2661	drug resistance transporter, EmrB/QacA subfamily	drugs	2.A.1.3.30	27.56	2E-47
Psed_2662	major facilitator superfamily MFS_1	drugs	2.A.1.3.33	54.64	2E-122
Psed_2616	drug resistance transporter, EmrB/QacA subfamily	landomycin	2.A.1.3.34	51.81	1E-117
Psed_3036	major facilitator superfamily MFS_1	landomycin	2.A.1.3.34	32.95	2E-21
Psed_0276	major facilitator superfamily MFS_1	drugs	2.A.1.3.35	28.64	2E-40
Psed_3993	major facilitator superfamily MFS_1	drugs	2.A.1.3.36	30.11	8E-10
Psed_0281	hypothetical protein	methecillin	2.A.1.3.37	21.79	3E-19
Psed_3073	hypothetical protein	methecillin	2.A.1.3.37	19.62	2E-18

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Psed_3896	lysyl-tRNA synthetase	methecillin	2.A.1.3.37	19.47	1E-10
Psed_3225	major facilitator superfamily MFS_1	drugs	2.A.1.3.39	29.23	1E-14
Psed_4002	major facilitator superfamily MFS_1	drugs	2.A.1.3.4	25.42	1E-28
Psed_5084	major facilitator superfamily MFS_1	drugs	2.A.1.3.4	25.52	2E-33
Psed_1580	major facilitator superfamily MFS_1	drugs	2.A.1.3.5	29.26	2E-25
Psed_2473	major facilitator superfamily MFS_1	drugs	2.A.1.3.5	29.52	1E-30
Psed_3385	drug resistance transporter, EmrB/QacA subfamily	drugs	2.A.1.3.5	38.77	5E-67
Psed_4944	major facilitator superfamily MFS_1	drugs	2.A.1.3.5	33.03	4E-57
Psed_1391	major facilitator superfamily MFS_1	cephamycin	2.A.1.3.8	42.04	7E-59
Psed_0602	major facilitator superfamily MFS_1	abietane	2.A.1.30.1	32.05	2E-37
Psed_4356	major facilitator superfamily MFS_1	Ni2+/Co2+	2.A.1.31.1	49.21	2E-100
Psed_2884	major facilitator superfamily MFS_1	Co2+	2.A.1.31.2	26.27	0.00000002
Psed_3328	major facilitator superfamily MFS_1	Co2+	2.A.1.32.2	25	4E-14
Psed_1922	major facilitator superfamily MFS_1	drugs	2.A.1.35.1	25.54	3E-16
Psed_0594	major facilitator superfamily MFS_1	acriflavin	2.A.1.36.1	23.86	5E-16
Psed_0689	major facilitator superfamily MFS_1	acriflavin	2.A.1.36.1	23.12	5E-11
Psed_4412	major facilitator superfamily MFS_1	acriflavin	2.A.1.36.1	37.77	1E-63
Psed_0612	major facilitator superfamily MFS_1	enterobactin	2.A.1.38.2	26.76	3E-26
Psed_2688	major facilitator superfamily MFS_1	glucose-6-P	2.A.1.4.6	21.69	4E-12
Psed_0601	Xanthine/uracil/vitamin C permease	pyrimidine/purine	2.A.1.40.2	38.95	1E-79
Psed_2774	phospholipid/glycerol acyltransferase	lysophospholipid	2.A.1.42.2	28.89	0.000000004
Psed_3804	phospholipid/glycerol acyltransferase	lysophospholipid	2.A.1.42.2	31.22	4E-10
Psed_2222	major facilitator superfamily MFS_1	putative	2.A.1.46.1	47.85	3E-95
Psed_5895	protein of unknown function DUF1228	putative	2.A.1.51.1	35.65	1E-31
Psed_3928	major facilitator superfamily MFS_1	putative	2.A.1.54.1	24.82	6E-20

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Psed_0635	major facilitator superfamily MFS_1	proline	2.A.1.6.4	41.19	9E-99
Psed_0663	General substrate transporter	4-methyl-o-phthalate	2.A.1.6.5	37.67	9E-69
Psed_3760	major facilitator superfamily MFS_1	4-methyl-o-phthalate	2.A.1.6.5	26.42	4E-17
Psed_0481	General substrate transporter	shikimate	2.A.1.6.6	36.45	8E-66
Psed_2833	General substrate transporter	shikimate	2.A.1.6.6	42.79	4E-89
Psed_4222	major facilitator superfamily MFS_1	shikimate	2.A.1.6.6	39.58	4E-79
Psed_5282	major facilitator superfamily MFS_1	shikimate	2.A.1.6.6	41.98	7E-81
Psed_6443	major facilitator superfamily MFS_1	shikimate	2.A.1.6.6	38.33	5E-73
Psed_6941	major facilitator superfamily MFS_1	shikimate	2.A.1.6.6	42.56	2E-89
Psed_3332	major facilitator superfamily MFS_1	rhizopine	2.A.1.60.1	30.73	2E-32
Psed_4417	major facilitator superfamily MFS_1	rhizopine	2.A.1.60.1	24.46	2E-11
Psed_6506	major facilitator superfamily MFS_1	putative	2.A.1.67.1	31.66	9E-24
Psed_1087	nitrite transporter	nitrate/nitrite	2.A.1.8.11	40.37	4E-81
Psed_2587	major facilitator superfamily MFS_1	nitrate/nitrite	2.A.1.8.11	27.76	0.000000003
Psed_4299	major facilitator superfamily MFS_1	nitrate/nitrite	2.A.1.8.11	32.92	4E-43
Psed_3274	major facilitator superfamily MFS_1	nitrate/nitrite	2.A.1.8.9	26.51	0.000008
Psed_2936	protein of unknown function DUF81	4-toluenesulfonate	2.a.102.3.	29.66	6E-12
Psed_3173	protein of unknown function DUF81	4-toluenesulfonate	2.a.102.3.	27.08	0.000003
Psed_6854	protein of unknown function DUF81	putative	2.a.102.4.	32.95	4E-18
Psed_4122	L-lactate transport	lactate	2.A.14.1.2	40.53	1E-107
Psed_1993	ATP-dependent DNA helicase, RecQ family	dicarboxylate	2.A.16.2.2	23.12	0.0000001
Psed_3150	ATP-dependent DNA helicase RecQ	dicarboxylate	2.A.16.2.2	29.82	2E-18
Psed_6201	amino acid/peptide transporter	oligopeptide	2.A.17.1.1	41.11	8E-95
Psed_2916	phosphate transporter	phosphate	2.A.20.1.2	35.59	2E-27
Psed_5834	phosphate transporter	phosphate	2.A.20.1.2	35	2E-21
Psed_1281	phosphate transporter	phosphate	2.A.20.2.6	29.29	1E-10

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Psed_3667	SSS sodium solute transporter superfamily	glucose/galactose	2.A.21.3.2	30.23	8E-55
Psed_6617	Na+/solute symporter	monocarboxylate	2.A.21.4.1	54.05	2E-121
Psed_0089	SSS sodium solute transporter superfamily	pyruvate/acetate/propionate	2.A.21.7.3	60.61	3E-162
Psed_0155	Na+/solute symporter	pyruvate/acetate/propionate	2.A.21.7.3	27.89	2E-30
Psed_0156	Na+/solute symporter	pyruvate/acetate/propionate	2.A.21.7.3	25.77	4E-25
Psed_0435	ATP-binding region ATPase domain protein	SSS	2.A.21.9.1	30.87	5E-12
Psed_0748	ATP-binding region ATPase domain protein	SSS	2.A.21.9.1	28.51	8E-13
Psed_0786	ATP-binding region ATPase domain protein	SSS	2.A.21.9.1	31.62	6E-14
Psed_0995	ATP-binding region ATPase domain protein	SSS	2.A.21.9.1	30.47	1E-10
Psed_1863	ATP-binding region ATPase domain protein	SSS	2.A.21.9.1	31.48	2E-12
Psed_3958	ATP-binding region ATPase domain protein	SSS	2.A.21.9.1	25.32	0.00000009
Psed_4018	ATP-binding region ATPase domain protein	SSS	2.A.21.9.1	41.28	3E-15
Psed_4822	ATP-binding region ATPase domain protein	SSS	2.A.21.9.1	26.05	0.0000002
Psed_5029	ATP-binding region ATPase domain protein	SSS	2.A.21.9.1	32.2	1E-17
Psed_5639	ATP-binding region ATPase domain protein	SSS	2.A.21.9.1	30.64	4E-19
Psed_6021	ATP-binding region ATPase domain protein	SSS	2.A.21.9.1	32.48	5E-16
Psed_6329	ATP-binding region ATPase domain protein	SSS	2.A.21.9.1	41.28	2E-13
Psed_6412	ATP-binding region ATPase domain protein	SSS	2.A.21.9.1	28.7	7E-10
Psed_2324	PAS sensor protein	SSS	2.A.21.9.2	26.3	2E-20
Psed_2890	ATP-binding region ATPase domain protein	SSS	2.A.21.9.2	28.41	1E-12
Psed_4144	Osmosensitive K channel His kinase sensor	SSS	2.A.21.9.2	33.19	4E-22
Psed_5142	ATP-binding region ATPase domain protein	SSS	2.A.21.9.2	29.01	3E-14
Psed_5183	ATP-binding region ATPase domain protein	SSS	2.A.21.9.2	29.45	2E-15

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Psed_5945	ATP-binding region ATPase domain protein	SSS	2.A.21.9.2	31.87	8E-15
Psed_6430	ATP-binding region ATPase domain protein	SSS	2.A.21.9.2	31.35	8E-26
Psed_3747	amino acid permease-associated region	Putrescine	2.A.3.1.13	24.72	7E-12
Psed_3748	amino acid permease-associated region	Putrescine	2.A.3.1.13	23.36	8E-16
Psed_6427	amino acid permease-associated region	Putrescine	2.A.3.1.13	25	2E-18
Psed_6143	amino acid permease-associated region	proline	2.A.3.1.6	27.09	0.00000009
Psed_3064	hypothetical protein	APC	2.a.3.14.1	67.02	0
Psed_5193	amino acid permease-associated region	APC	2.A.3.3.2	37.98	4E-63
Psed_3792	amino acid permease-associated region	amino acid	2.A.3.6.1	30.11	1E-33
Psed_4562	amino acid permease-associated region	amino acid	2.A.3.6.1	26.42	2E-20
Psed_2733	Na(+)/H(+) antiporter nhaA	Na+/K+/H+	2.A.33.1.1	40.21	5E-68
Psed_0416	Na(+)/H(+) antiporter nhaA	Na+/K+/H+	2.A.33.1.2	37.7	3E-65
Psed_3117	Na+/H+ antiporter	Na+/K+/H+	2.A.36.3.1	29.45	1E-42
Psed_4896	Na+/H+ antiporter	Na+/K+/H+	2.A.36.3.1	27.21	4E-38
Psed_0239	sodium/hydrogen exchanger	Na+/K+/H+	2.A.36.4.3	25.63	3E-14
Psed_5902	sodium/hydrogen exchanger	Na+/K+/H+	2.A.36.4.3	25.82	4E-18
Psed_2825	sodium/hydrogen exchanger	Na+/K+/H+	2.A.36.6.4	33.13	1E-61
Psed_2445	sodium/hydrogen exchanger	Na+/K+/H+	2.A.37.4.2	27	2E-13
Psed_0828	sodium/hydrogen exchanger	Na+/K+/H+	2.A.37.5.2	33.33	6E-43
Psed_0829	TrkA-C domain protein	Na+/K+/H+	2.A.37.5.2	27.74	3E-12
Psed_1025	glycogen synthase	K+	2.A.38.4.5	23.89	2E-10
Psed_1742	glycosyl transferase group 1	K+	2.A.38.4.5	25.83	9E-11
Psed_1878	glycosyl transferase group 1	K+	2.A.38.4.5	27.52	1E-12
Psed_1881	glycosyl transferase group 1	K+	2.A.38.4.5	38.83	0.000000005
Psed_2335	glycosyl transferase group 1	K+	2.A.38.4.5	27.32	0.0000004
Psed_2765	glycosyl transferase group 1	K+	2.A.38.4.5	28.99	0.00000003

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Psed_2830	glycosyl transferase group 1	K+	2.A.38.4.5	26.76	8E-11
Psed_3663	glycosyl transferase group 1	K+	2.A.38.4.5	25.45	0.000000005
Psed_5572	glycosyl transferase group 1	K+	2.A.38.4.5	28.89	1E-13
Psed_5573	glycosyl transferase group 1	K+	2.A.38.4.5	27.55	2E-14
Psed_6279	glycosyl transferase group 1	K+	2.A.38.4.5	25	0.0000002
Psed_3082	permease for cytosine/purines uracil thiamine allantoin	pyrimidine/purine	2.A.39.1.1	29.18	2E-19
Psed_4378	cation diffusion facilitator family transporter	Cd2+/Zn2+/Co2+	2.A.4.1.1	44.03	2E-60
Psed_5925	cation diffusion facilitator family transporter	Cd2+/Zn2+	2.A.4.1.3	33.01	8E-51
Psed_1953	cation diffusion facilitator family transporter	heavy metal	2.A.4.6.1	36.68	1E-41
Psed_5197	cation diffusion facilitator family transporter	heavy metal	2.A.4.6.1	34.43	2E-39
Psed_1769	xanthine permease	pyrimidine/purine	2.A.40.3.1	44.84	2E-100
Psed_2935	Citrate transporter	arsenite	2.A.45.1.1	24.65	2E-26
Psed_6414	Cl- channel voltage-gated family protein	Cl-	2.A.49.4.1	24.76	0.0000001
Psed_2034	Cl- channel voltage-gated family protein	Cl-	2.A.49.5.1	29.81	8E-15
Psed_4980	Cl- channel voltage-gated family protein	Cl-	2.A.49.5.1	29.1	8E-18
Psed_2268	Cl- channel voltage-gated family protein	Cl-	2.A.49.6.1	28.8	2E-40
Psed_2284	CBS domain containing protein	Cl-	2.A.49.6.1	25.62	0.0000002
Psed_5351	inosine-5'-monophosphate dehydrogenase	Cl-	2.A.49.6.1	33.33	0.0000004
Psed_0054	zinc/iron permease	heavy metal	2.A.5.5.1	29.32	0.0000006
Psed_2328	high-affinity nickel-transporter	Ni2+/Co2+	2.A.52.1.1	40.74	2E-65
Psed_6872	high-affinity nickel-transporter	Ni2+/Co2+	2.A.52.1.1	39.52	5E-64
Psed_4266	sulfate transporter	sulfate	2.A.53.4.1	34.84	2E-78
Psed_4993	sulphate transporter	sulfate	2.A.53.4.1	29.32	4E-58
Psed_0157	Rhodanese-like protein	sulfate	2.A.53.9.1	38.67	0.000000009
Psed_6850	Rhodanese-like protein	sulfate	2.A.53.9.1	37	0.000000003

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Psed_6856	Rhodanese-like protein	sulfate	2.A.53.9.1	36.11	6E-10
Psed_0041	natural resistance-associated macrophage protein	Mn2+/Zn2+/Fe2+	2.A.55.3.1	26.94	9E-16
Psed_2709	Mn2+/Fe2+ transporter, NRAMP family	Mn2+/Zn2+/Fe2+	2.A.55.3.1	44.17	1E-87
Psed_4375	Manganese transport protein mntH	Mn2+/Zn2+/Fe2+	2.A.55.3.1	47.12	8E-92
Psed_6982	Mn2+/Fe2+ transporter, NRAMP family	Mn2+/Zn2+/Fe2+	2.A.55.3.1	45.86	8E-90
Psed_0621	TRAP transporter, 4TM/12TM fusion protein	TRAP-T	2.A.56.1.6	25.16	7E-14
Psed_2408	arsenical-resistance protein	arsenite	2.A.59.1.2	52.8	9E-103
Psed_4344	Bile acid:sodium symporter	putative	2.A.59.2.1	42.86	6E-50
Psed_5509	bile acid:sodium symporter	putative	2.A.59.2.1	38.46	7E-35
Psed_1663	diaminopimelate decarboxylase	drugs	2.A.6.2.28	21.59	3E-13
Psed_4582	acriflavin resistance protein	acriflavin	2.A.6.3.1	29.75	1E-106
Psed_0525	protein-export membrane protein SecD	RND	2.A.6.4.1	33.5	7E-55
Psed_0703	MMPL domain protein	actinorhodin	2.A.6.5.1	48.38	1E-158
Psed_4776	monovalent cation/proton antiporter, MnhG/ PhaG subunit	Na+/K+/H+	2.A.63.1.4	41.76	1E-13
Psed_4779	NADH dehydrogenase (quinone)	Na+/K+/H+	2.A.63.1.4	36.31	5E-77
Psed_4780	NADH-ubiquinone oxidoreductase chain 4L	Na+/K+/H+	2.A.63.1.4	36.84	4E-16
Psed_1031	Sec-independent protein translocase protein tatB-like protein	TAT	2.A.64.1.1	27.08	0.00000005
Psed_3286	Sec-independent protein translocase, TatC subunit	TAT	2.A.64.3.2	30.97	2E-27
Psed_4668	MATE efflux family protein	drugs	2.A.66.1.1	24.42	2E-19
Psed_1882	polysaccharide biosynthesis protein	polysaccharide	2.A.66.2.2	28.75	6E-16
Psed_6698	polysaccharide biosynthesis protein	polysaccaride	2.A.66.2.2	24.92	1E-20
Psed_5298	integral membrane protein MviN	putative	2.A.66.4.1	27.64	1E-23

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Psed_5645	UDP-N-acetylglucosamine	exopolysacharide	2.A.66.6.2	25.48	0.000000006
Psed_0337	small multidrug resistance protein	drugs	2.A.7.1.4	55.88	4E-26
Psed_5480	small multidrug resistance protein	drugs	2.A.7.1.8	51.92	3E-21
Psed_3858	protein of unknown function DUF6 transmembrane	drugs	2.A.7.3.2	42.91	5E-51
Psed_4602	protein of unknown function DUF6 transmembrane	drugs	2.A.7.3.2	26.54	1E-10
Psed_4658	protein of unknown function DUF6 transmembrane	putative	2.A.7.3.4	25.25	0.0000002
Psed_0669	protein of unknown function DUF6 transmembrane	drugs	2.A.7.3.6	32.06	0.0000007
Psed_2994	protein of unknown function DUF6 transmembrane	drugs	2.A.7.3.6	33.21	5E-25
Psed_5554	RarD protein, DMT superfamily transporter	chloramphenicol	2.A.7.7.1	28.96	3E-14
Psed_5861	Low affinity potassium transport system protein kup	K+	2.A.72.1.1	43.45	3E-146
Psed_6623	Lysine exporter protein (LYSE/YGGA)	lysine	2.A.75.1.1	37.38	2E-30
Psed_0083	Lysine exporter protein (LYSE/YGGA)	lysine	2.A.76.1.1	27.18	0.0000008
Psed_3964	Lysine exporter protein (LYSE/YGGA)	lysine	2.A.76.1.1	36.49	8E-26
Psed_1946	Lysine exporter protein (LYSE/YGGA)	lysine	2.A.76.1.3	29.02	3E-15
Psed_3604	AzlC family protein	amino acid	2.A.78.1.2	30.73	0.000000006
Psed_4026	AzlC family protein	methionine	2.A.78.1.3	25.32	2E-10
Psed_6090	protein of unknown function DUF1212	threonine/serine	2.A.79.1.1	36.28	1E-54
Psed_3768	hypothetical protein	putative	2.A.85.1.1	30.95	0.000001
Psed_0064	hypothetical protein	putative	2.A.85.1.3	31.08	7E-13
Psed_4259	protein of unknown function UPF0118	putative	2.A.86.1.2	31.27	2E-32
Psed_5219	protein of unknown function UPF0118	putative	2.A.86.1.2	27.27	9E-34

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Psed_6646	protein of unknown function UPF0118	putative	2.A.86.1.2	25.9	1E-17
Psed_1196	protein of unknown function DUF125 transmembrane	Fe2+	2.A.89.3.1	43.95	1E-35
Psed_4120	protein of unknown function DUF125 transmembrane	Fe2+	2.A.89.3.1	38.99	1E-49
Psed_1558	protein of unknown function DUF125 transmembrane	Fe2+	2.A.89.3.2	27.14	4E-13
Psed_4875	protein of unknown function DUF125 transmembrane	Fe2+	2.A.89.3.2	39.49	1E-32
Psed_4613	membrane protein insertase, YidC/Oxa1 family	Oxa1	2.A.9.3.1	23.91	7E-18
Psed_6689	membrane protein insertase, YidC/Oxa1 family	Oxa1	2.A.9.3.1	24.15	2E-21
Psed_0350	Bile acid:sodium symporter	putative	2.A.93.1.4	50.3	1E-75
Psed_1420	hypothetical protein	glyoxylate	2.A.96.1.2	23.63	0.000005
Psed_6024	hypothetical protein	acetate	2.A.96.1.3	24.52	0.00000002
Psed_5192	Uncharacterised protein family UPF0324	sulfate	2.A.98.1.3	29.75	2E-25
Psed_1200	peptidase S9 prolyl oligopeptidase active site domain protein	energy transduction	2.C.1.2.1	24.12	0.00000005
Psed_3206	Polyamine-transporting ATPase	carbohydrate	3.A.1.1.16	49.58	1E-65
Psed_1049	extracellular solute-binding protein family 1	carbohydrate	3.A.1.1.17	28.06	9E-25
Psed_1050	ABC-type transporter, integral membrane subunit	carbohydrate	3.A.1.1.25	41.44	1E-48
Psed_1051	ABC-type transporter, integral membrane subunit	carbohydrate	3.A.1.1.25	45.67	4E-52
Psed_1052	Glycerol-3-phosphate-transporting ATPase	maltose	3.A.1.1.26	55.1	5E-105
Psed_2634	glycogen debranching enzyme GlgX	cyclodextrin	3.A.1.1.6	25.85	0.0000002

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Psed_2878	malto-oligosyltrehalose trehalohydrolase	cyclodextrin	3.A.1.1.6	27.01	3E-12
Psed_2879	malto-oligosyltrehalose synthase	cyclodextrin	3.A.1.1.6	26.32	2E-13
Psed_2880	glycogen debranching enzyme GlgX	cyclodextrin	3.A.1.1.6	25	6E-10
Psed_3396	FeS assembly ATPase SufC	carbohydrate	3.A.1.1.8	31.25	4E-17
Psed_6640	extracellular solute-binding protein family 1	Fe3+	3.A.1.10.1	34.28	7E-48
Psed_6641	ABC-type transporter, integral membrane subunit	Fe3+	3.A.1.10.3	33.33	1E-71
Psed_6642	Fe(3+)-transporting ATPase	Fe3+	3.A.1.10.4	39.02	9E-58
Psed_1978	Polyamine-transporting ATPase	lipooligosaccharide	3.A.1.102.	48.62	2E-64
Psed_1979	ABC-2 type transporter	lipooligosaccharide	3.A.1.102.	29.02	7E-16
Psed_1980	ABC-2 type transporter	lipooligosaccharide	3.A.1.102.	28.44	3E-20
Psed_4465	Sulfate-transporting ATPase	lipooligosaccharide	3.A.1.102.	33.44	5E-35
Psed_0207	Teichoic-acid-transporting ATPase	lipopolysaccharide	3.A.1.103.	36.87	6E-41
Psed_0208	ABC-2 type transporter	lipopolysaccharide	3.A.1.103.	22.41	0.00000009
Psed_0199	Sulfate-transporting ATPase	drugs	3.A.1.105.	43.69	5E-40
Psed_0322	Polyamine-transporting ATPase	drugs	3.A.1.105.	49.68	9E-77
Psed_0323	ABC-2 type transporter	drugs	3.A.1.105.	32.93	6E-25
Psed_0584	daunorubicin resistance ABC transporter ATPase subunit	drugs	3.A.1.105.	55.49	7E-90
Psed_0585	ABC-2 type transporter	drugs	3.A.1.105.	32.4	3E-31
Psed_0844	daunorubicin resistance ABC transporter ATPase subunit	drugs	3.A.1.105.	56.44	1E-88
Psed_0845	ABC-2 type transporter	drugs	3.A.1.105.	44.14	9E-58
Psed_3401	Fe(3+)-transporting ATPase	drugs	3.A.1.105.	37.66	9E-47
Psed_3645	Polyamine-transporting ATPase	drugs	3.A.1.105.	44.55	4E-46
Psed_3646	ABC-2 type transporter	drugs	3.A.1.105.	27.8	2E-16

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Psed_4617	ABC-2 type transporter	drugs	3.A.1.105.	25.29	0.000000007
Psed_4618	Phosphonate-transporting ATPase	drugs	3.A.1.105.	35.9	4E-42
Psed_4964	daunorubicin resistance ABC transporter ATPase subunit	drugs	3.A.1.105.	46.03	8E-72
Psed_4965	ABC-2 type transporter	drugs	3.A.1.105.	34.98	3E-27
Psed_6089	ABC-2 type transporter	drugs	3.A.1.105.	29.81	1E-27
Psed_6569	Sulfate-transporting ATPase	drugs	3.A.1.105.	37.46	2E-40
Psed_2630	Xenobiotic-transporting ATPase	drugs	3.A.1.106.	35.51	5E-102
Psed_3106	Xenobiotic-transporting ATPase	drugs	3.A.1.106.	35.25	3E-93
Psed_4515	Xenobiotic-transporting ATPase	drugs	3.A.1.106.	34.18	1E-87
Psed_5110	Xenobiotic-transporting ATPase., Peptide-transporting ATPase	drugs	3.A.1.106.	45.03	0
Psed_6193	Xenobiotic-transporting ATPase	drugs	3.A.1.106.	31.16	6E-88
Psed_4060	Xenobiotic-transporting ATPase	drugs	3.A.1.111.	23.99	4E-46
Psed_4061	Xenobiotic-transporting ATPase	drugs	3.A.1.111.	24.04	6E-47
Psed_0250	Xenobiotic-transporting ATPase	drugs	3.A.1.119.	55.17	2E-49
Psed_0251	Xenobiotic-transporting ATPase	drugs	3.A.1.119.	55.25	9E-168
Psed_5130	ABC-type glycine betaine transport, periplasmic subunit	quaternary amine	3.A.1.12.1	26.38	5E-28
Psed_5132	ABC-type transporter, integral membrane subunit	choline	3.A.1.12.3	33.01	1E-22
Psed_5131	ABC-type transporter, integral membrane subunit	quaternary amine	3.A.1.12.4	33.17	5E-27
Psed_5133	glycine betaine/L-proline ABC transporter, ATPase subunit	quaternary amine	3.A.1.12.4	47.42	1E-78
Psed_0840	ABC transporter related	drugs	3.A.1.120.	29.69	3E-62
Psed_4807	ATP-binding cassette protein, ChvD family	drugs	3.A.1.120.	37.38	2E-93

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Psed_5589	ABC transporter related	drugs	3.A.1.120.	35.04	5E-61
Psed_3388	ABC transporter related	drugs	3.A.1.121.	29.15	2E-59
Psed_1540	Phosphonate-transporting ATPase	drugs	3.A.1.122.	45.16	7E-49
Psed_4501	secretion protein HlyD family protein	drugs	3.A.1.122.	30.19	8E-11
Psed_4502	protein of unknown function DUF214	drugs	3.A.1.122.	34.06	2E-46
Psed_4503	Phosphonate-transporting ATPase	drugs	3.A.1.122.	54.59	3E-68
Psed_4504	efflux transporter, RND family, MFP subunit	drugs	3.A.1.122.	23.95	7E-10
Psed_0883	ABC transporter related	drugs	3.A.1.124.	40.2	3E-44
Psed_0991	ABC transporter related	drugs	3.A.1.124.	34.03	6E-48
Psed_6400	Sulfate-transporting ATPase	drugs	3.A.1.124.	31.93	7E-46
Psed_1933	Phosphonate-transporting ATPase	lipoprotein	3.A.1.125.	39.82	7E-42
Psed_5702	protein of unknown function DUF214	lipoprotein	3.A.1.125.	35.8	7E-17
Psed_5703	Phosphonate-transporting ATPase	lipoprotein	3.A.1.125.	62.5	1E-76
Psed_2281	ABC transporter related	β-exotoxin	3.A.1.126.	31.55	4E-25
Psed_3120	ABC transporter, CydDC cysteine exporter (CydDC-E) family, permease/ATP-binding protein CydC	cysteine	3.A.1.129.	32.86	6E-75
Psed_3121	ABC transporter, CydDC cysteine exporter (CydDC-E) family, permease/ATP-binding protein CydD	cysteine	3.A.1.129.	36.45	9E-77
Psed_3855	Sulfate-transporting ATPase	drugs	3.A.1.131.	44.55	3E-41
Psed_4497	ABC transporter related	drugs	3.A.1.131.	36.72	6E-52
Psed_4816	Sulfate-transporting ATPase	drugs	3.A.1.131.	37.92	9E-49
Psed_1041	ATPase-like, ParA/MinD	putative	3.A.1.132.	36.76	4E-41
Psed_0309	Xenobiotic-transporting ATPase	drugs	3.A.1.135.	35.6	3E-104
Psed_0310	Xenobiotic-transporting ATPase	drugs	3.A.1.135.	33.94	1E-94

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Psed_2818	Conserved hypothetical protein CHP00245	putative	3.A.1.139.	31.34	9E-20
Psed_0246	ABC-type transporter, integral membrane subunit	Fe3+	3.A.1.14.1	48.25	8E-71
Psed_0247	ABC-type transporter, integral membrane subunit	Fe3+	3.A.1.14.1	52.84	3E-73
Psed_0248	ABC-type transporter, periplasmic subunit	Fe3+	3.A.1.14.1	31.79	6E-28
Psed_2912	ABC-type transporter, integral membrane subunit	Fe3+	3.A.1.14.1	55.37	3E-72
Psed_2913	ABC-type transporter, integral membrane subunit	Fe3+	3.A.1.14.1	49.59	2E-72
Psed_2914	Iron-chelate-transporting ATPase	Fe3+	3.A.1.14.1	65.2	2E-85
Psed_3995	ABC-type transporter, periplasmic subunit	Fe3+	3.A.1.14.1	35.71	4E-43
Psed_1734	ABC transporter related	Fe3+	3.A.1.14.2	34.27	1E-23
Psed_4413	ABC-type transporter, periplasmic subunit	Fe3+	3.A.1.14.2	31.84	0.000002
Psed_6095	Fe(3+)-transporting ATPase	Fe3+	3.A.1.14.3	36.48	3E-30
Psed_2033	ABC-type transporter, periplasmic subunit	Fe3+	3.A.1.14.4	30.34	5E-22
Psed_1187	protein of unknown function DUF214	cell division	3.A.1.140.	25.33	0.00000001
Psed_0198	ABC-2 type transporter	glycolipid	3.A.1.142.	24.66	0.0000002
Psed_4464	ABC-2 type transporter	glycolipid	3.A.1.142.	27.35	7E-12
Psed_3037	ABC-type metal ion transporter, periplasmic subunit	Mn2+/Zn2+/Fe2+	3.A.1.15.1	56.36	7E-91
Psed_3038	Sulfate-transporting ATPase	Mn2+/Zn2+/Fe2+	3.A.1.15.1	49.79	1E-63
Psed_3039	ABC-type transporter, integral membrane subunit	Mn2+/Zn2+/Fe2+	3.A.1.15.1	49.11	3E-73
Psed_6096	ABC-type transporter, integral membrane subunit	Mn2+/Zn2+/Fe2+	3.A.1.15.2	30.85	2E-19
Psed_0654	ABC transporter related	Zn2+	3.A.1.15.5	35.68	6E-16

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Psed_6094	ABC-type metal ion transporter, periplasmic subunit	heavy metal	3.A.1.15.6	27.3	3E-16
Psed_1073	hypothetical protein	Mn2+/Zn2+/Fe2+	3.A.1.15.8	30	0.000000001
Psed_3014	Taurine-transporting ATPase	nitrate/nitrite	3.A.1.16.1	42.44	4E-43
Psed_5045	Taurine-transporting ATPase	nitrate/nitrite	3.A.1.16.1	45.27	3E-53
Psed_5964	Taurine-transporting ATPase	nitrate/nitrite	3.A.1.16.1	45.85	3E-47
Psed_4191	Taurine-transporting ATPase	cyanate/nitrite	3.A.1.16.2	45.85	7E-56
Psed_6266	ABC-type nitrate/sulfonate/bicarbonate transport systems periplasmic components	cyanate/nitrite	3.A.1.16.2	27.3	1E-30
Psed_4511	Taurine-transporting ATPase	bicarbonate	3.A.1.16.3	40.17	4E-46
Psed_5965	ABC-type transporter, integral membrane subunit	bicarbonate	3.A.1.16.3	30.39	1E-29
Psed_6265	ABC-type transporter, integral membrane subunit	bicarbonate	3.A.1.16.3	33.07	3E-29
Psed_4510	aliphatic sulfonates family ABC transporter, periplsmic ligand-binding protein	taurine	3.A.1.17.1	29.83	7E-13
Psed_5046	ABC-type transporter, integral membrane subunit	taurine	3.A.1.17.2	32.77	6E-30
Psed_5047	hypothetical protein	taurine	3.A.1.17.2	26.12	0.000005
Psed_5966	ABC transporter substrate-binding protein	taurine	3.A.1.17.2	27.7	6E-20
Psed_3013	ABC-type transporter, integral membrane subunit	taurine	3.A.1.17.3	34.31	4E-32
Psed_4192	ABC-type transporter, integral membrane subunit	taurine	3.A.1.17.3	36.32	2E-31
Psed_4512	ABC-type transporter, integral membrane subunit	phthalate	3.A.1.17.5	27.27	3E-18
Psed_3012	hypothetical protein	taurine	3.A.1.17.6	25.85	3E-12

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Psed_4190	nmt1/thi5 like domain protein	taurine	3.A.1.17.6	29.49	2E-10
Psed_2321	ABC-type sugar transport system periplasmic component-like	ribose	3.A.1.2.1	23.83	0.000000002
Psed_2958	hypothetical protein	ribose	3.A.1.2.1	28.19	1E-12
Psed_3027	Monosaccharide-transporting ATPase	ribose	3.A.1.2.1	33.07	2E-76
Psed_3189	hypothetical protein	ribose	3.A.1.2.1	28.29	1E-13
Psed_4163	ABC-type sugar transport system periplasmic component-like	ribose	3.A.1.2.1	24.55	0.000000002
Psed_6928	periplasmic binding protein/LacI transcriptional regulator	ribose	3.A.1.2.1	29.23	5E-15
Psed_0683	ABC-type sugar transport system periplasmic component-like	carbohydrate	3.A.1.2.11	26.87	0.000005
Psed_3026	ABC-type transporter, integral membrane subunit	carbohydrate	3.A.1.2.11	32.16	4E-18
Psed_2164	periplasmic binding protein/LacI transcriptional regulator	carbohydrate	3.A.1.2.14	28.71	0.00000002
Psed_3191	Monosaccharide-transporting ATPase	carbohydrate	3.A.1.2.14	38.25	1E-86
Psed_4171	Monosaccharide-transporting ATPase	carbohydrate	3.A.1.2.14	33.99	3E-79
Psed_0682	ABC-type transporter, integral membrane subunit	carbohydrate	3.A.1.2.15	34.26	2E-32
Psed_2957	ABC-type transporter, integral membrane subunit	carbohydrate	3.A.1.2.16	31.46	2E-39
Psed_3190	ABC-type transporter, integral membrane subunit	carbohydrate	3.A.1.2.16	35.37	9E-40
Psed_6260	Monosaccharide-transporting ATPase	carbohydrate	3.A.1.2.16	31.38	4E-44
Psed_2956	Monosaccharide-transporting ATPase	glucose/galactose	3.A.1.2.3	34.75	5E-84
Psed_0685	Monosaccharide-transporting ATPase	xylose	3.A.1.2.4	33.01	5E-73

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Psed_2259	Monosaccharide-transporting ATPase	xylose	3.A.1.2.4	36.52	2E-84
Psed_2995	monosaccharide-transporting ATPase	xylose	3.A.1.2.4	39.56	3E-49
Psed_2997	ABC-type transporter, integral membrane subunit	xylose	3.A.1.2.4	31.44	5E-34
Psed_4161	ABC-type transporter, integral membrane subunit	carbohydrate	3.A.1.2.6	28.22	2E-24
Psed_4162	Monosaccharide-transporting ATPase	carbohydrate	3.A.1.2.6	32.88	1E-78
Psed_4170	ABC-type transporter, integral membrane subunit	carbohydrate	3.A.1.2.6	27.87	5E-21
Psed_2260	ABC-type transporter, integral membrane subunit	ribose/mannose/fructose	3.A.1.2.7	27.35	2E-22
Psed_2996	Monosaccharide-transporting ATPase	ribose/mannose/fructose	3.A.1.2.7	46.37	3E-57
Psed_3204	extracellular solute-binding protein family 1	Iron	3.A.1.20.1	27.1	0.00000003
Psed_3205	ABC-type transporter, integral membrane subunit	Iron	3.A.1.20.1	26.7	1E-16
Psed_5027	FAD-binding 9 siderophore-interacting domain protein	drugs	3.A.1.21.2	31.8	5E-16
Psed_6114	FAD-binding 9 siderophore-interacting domain protein	drugs	3.A.1.21.2	26.17	8E-10
Psed_2554	Xenobiotic-transporting ATPase	heavy metal	3.A.1.210.	35.58	2E-77
Psed_2327	cobalamin (vitamin B12) biosynthesis CbiM protein	Ni2+/Co2+	3.A.1.23.1	43.26	4E-64
Psed_3351	cobalamin (vitamin B12) biosynthesis CbiM protein	Ni2+/Co2+	3.A.1.23.1	32.21	6E-20
Psed_2325	Sulfate-transporting ATPase	Co2+	3.A.1.23.2	36.69	3E-46
Psed_2326	cobalt ABC transporter, inner membrane subunit CbiQ	Co2+	3.A.1.23.2	23.56	5E-13

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Psed_3353	cobalt ABC transporter, inner membrane subunit CbiQ	Co2+	3.A.1.23.2	29.85	0.00000001
Psed_3354	cobalt ABC transporter, ATPase subunit	Co2+	3.A.1.23.2	42.26	4E-54
Psed_5443	virulence factor Mce family protein	cholesterol	3.A.1.27.4	27.88	0.000000002
Psed_5444	virulence factor Mce family protein	cholesterol	3.A.1.27.4	27.24	0.00000005
Psed_5445	virulence factor Mce family protein	cholesterol	3.A.1.27.4	28.14	2E-19
Psed_5446	virulence factor Mce family protein	cholesterol	3.A.1.27.4	49.35	1E-69
Psed_5447	virulence factor Mce family protein	cholesterol	3.A.1.27.4	34.37	3E-49
Psed_5448	protein of unknown function DUF140	cholesterol	3.A.1.27.4	49.45	4E-66
Psed_5449	protein of unknown function DUF140	γ-HCH	3.A.1.27.5	50.42	7E-67
Psed_5450	Fe(3+)-transporting ATPase	γ-HCH	3.A.1.27.5	72.64	1E-131
Psed_1693	ectoine/hydroxyectoine ABC transporter solute-binding protein	amino acid	3.A.1.3.1	26.5	1E-12
Psed_1694	ectoine/hydroxyectoine ABC transporter, permease protein EhuC	amino acid	3.A.1.3.10	38.73	2E-31
Psed_1696	ectoine/hydroxyectoine ABC transporter, ATP-binding protein	amino acid	3.A.1.3.10	50	5E-59
Psed_4931	ABC-type transporter, periplasmic subunit family 3	amino acid	3.A.1.3.10	30.22	6E-19
Psed_6613	polar amino acid ABC transporter, inner membrane subunit	amino acid	3.A.1.3.12	39.15	7E-34
Psed_6614	Fe(3+)-transporting ATPase	cysteine	3.A.1.3.14	54.8	9E-78
Psed_4929	polar amino acid ABC transporter, inner membrane subunit	arginine	3.A.1.3.15	35.11	5E-29
Psed_1695	ectoine/hydroxyectoine ABC transporter, permease protein EhuD	glutamate/aspartate	3.A.1.3.16	30.41	9E-29

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Psed_6612	ABC-type transporter, periplasmic subunit family 3	glutamine	3.A.1.3.2	32.78	2E-29
Psed_1697	ABC-type transporter, periplasmic subunit family 3	nopaline	3.A.1.3.6	28.23	5E-16
Psed_3960	Phosphonate-transporting ATPase	glutamate	3.A.1.3.9	78.93	2E-111
Psed_3961	ABC-type transporter, periplasmic subunit family 3	glutamate	3.A.1.3.9	40.61	7E-43
Psed_3962	polar amino acid ABC transporter, inner membrane subunit	glutamate	3.A.1.3.9	47.42	7E-41
Psed_3963	polar amino acid ABC transporter, inner membrane subunit	glutamate	3.A.1.3.9	35.04	8E-31
Psed_4930	Sulfate-transporting ATPase	glutamate	3.A.1.3.9	51	1E-71
Psed_3266	ABC transporter related	Co2+	3.A.1.31.1	26.67	1E-12
Psed_3845	UvrABC system protein A	cobalamin precursor	3.A.1.32.1	33.52	0.00000004
Psed_2016	ABC transporter related	methylthioadenosine	3.A.1.33.1	28.09	4E-21
Psed_0514	ABC transporter related	leucine/isoleucine/valine	3.A.1.4.1	41.98	7E-38
Psed_1377	Polyamine-transporting ATPase	leucine/isoleucine/valine	3.A.1.4.1	39.17	1E-41
Psed_1378	ABC transporter related	leucine/isoleucine/valine	3.A.1.4.1	37.95	2E-36
Psed_2064	Extracellular ligand-binding receptor	leucine/isoleucine/valine	3.A.1.4.1	25.99	2E-15
Psed_2065	ABC-type transporter, integral membrane subunit	leucine/isoleucine/valine	3.A.1.4.1	31.25	3E-23
Psed_2066	Monosaccharide-transporting ATPase	leucine/isoleucine/valine	3.A.1.4.1	40.78	3E-51
Psed_2067	ABC transporter related	leucine/isoleucine/valine	3.A.1.4.1	41.13	1E-45
Psed_2068	ABC-type transporter, integral membrane subunit	leucine/isoleucine/valine	3.A.1.4.1	31.13	1E-25
Psed_2481	ABC transporter related	leucine/isoleucine/valine	3.A.1.4.1	28.35	1E-33
Psed_2568	Extracellular ligand-binding receptor	leucine/isoleucine/valine	3.A.1.4.1	27.34	1E-10

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Psed_2569	ABC-type transporter, integral membrane subunit	leucine/isoleucine/valine	3.A.1.4.1	29.28	1E-25
Psed_2570	Phosphonate-transporting ATPase	leucine/isoleucine/valine	3.A.1.4.1	38.71	6E-47
Psed_2571	ABC transporter related	leucine/isoleucine/valine	3.A.1.4.1	40.26	3E-41
Psed_2600	ABC-type transporter, integral membrane subunit	leucine/isoleucine/valine	3.A.1.4.1	30.82	2E-17
Psed_2603	Fe(3+)-transporting ATPase	leucine/isoleucine/valine	3.A.1.4.1	45.34	1E-48
Psed_3725	Extracellular ligand-binding receptor	leucine/isoleucine/valine	3.A.1.4.1	26.39	0.000000001
Psed_3731	Phosphonate-transporting ATPase	leucine/isoleucine/valine	3.A.1.4.1	46.35	5E-53
Psed_3732	Monosaccharide-transporting ATPase	leucine/isoleucine/valine	3.A.1.4.1	39.76	4E-50
Psed_3875	Extracellular ligand-binding receptor	leucine/isoleucine/valine	3.A.1.4.1	32.81	2E-32
Psed_3876	ABC-type transporter, integral membrane subunit	leucine/isoleucine/valine	3.A.1.4.1	39.94	4E-45
Psed_3877	ABC-type transporter, integral membrane subunit	leucine/isoleucine/valine	3.A.1.4.1	40.26	1E-39
Psed_3878	Monosaccharide-transporting ATPase	leucine/isoleucine/valine	3.A.1.4.1	51.41	2E-68
Psed_3879	Fe(3+)-transporting ATPase	leucine/isoleucine/valine	3.A.1.4.1	53.65	1E-65
Psed_2602	Monosaccharide-transporting ATPase	amino acid	3.A.1.4.2	36.18	3E-42
Psed_0511	ABC-type transporter, integral membrane subunit	amino acid	3.A.1.4.3	31.9	4E-22
Psed_0513	Monosaccharide-transporting ATPase	amino acid	3.A.1.4.3	34.4	3E-34
Psed_2601	ABC-type transporter, integral membrane subunit	amino acid	3.A.1.4.3	29.74	3E-27
Psed_3733	ABC-type transporter, integral membrane subunit	amino acid	3.A.1.4.3	29.51	9E-28

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Psed_3734	ABC-type transporter, integral membrane subunit	amino acid	3.A.1.4.3	30.38	4E-33
Psed_6261	ABC-type transporter, integral membrane subunit	amino acid	3.A.1.4.3	29.52	2E-23
Psed_6263	Extracellular ligand-binding receptor	amino acid	3.A.1.4.3	30.19	5E-17
Psed_0512	ABC-type transporter, integral membrane subunit	urea	3.A.1.4.4	26.4	4E-18
Psed_0509	Extracellular ligand-binding receptor	urea	3.A.1.4.5	26.62	0.00000006
Psed_2483	Extracellular ligand-binding receptor	urea	3.A.1.4.5	23.26	0.00000004
Psed_2484	ABC transporter related	urea	3.A.1.4.5	34.98	5E-23
Psed_2599	Extracellular ligand-binding receptor	urea	3.A.1.4.5	22.85	3E-14
Psed_3046	regulatory protein	urea	3.A.1.4.5	31.53	5E-42
Psed_3240	Extracellular ligand-binding receptor	urea	3.A.1.4.5	26.54	4E-29
Psed_3801	regulatory protein	urea	3.A.1.4.5	30.52	1E-39
Psed_5955	Extracellular ligand-binding receptor	urea	3.A.1.4.5	28.19	2E-25
Psed_6002	regulatory protein	urea	3.A.1.4.5	29.18	9E-39
Psed_2482	ABC-type transporter, integral membrane subunit	amino acid	3.A.1.4.6	26.61	3E-11
Psed_6262	ABC-type transporter, integral membrane subunit	amino acid	3.A.1.4.6	30.77	3E-20
Psed_1001	ABC-type transporter, periplasmic subunit	oligopeptide	3.A.1.5.1	28.08	1E-37
Psed_1749	Nickel-transporting ATPase	oligopeptide	3.A.1.5.1	38.77	9E-51
Psed_6018	oligopeptide/dipeptide ABC transporter, ATPase subunit	oligopeptide	3.A.1.5.1	48.13	3E-89
Psed_6923	oligopeptide/dipeptide ABC transporter, ATPase subunit	oligopeptide	3.A.1.5.1	47.34	3E-83

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Psed_0080	ABC-type transporter, integral membrane subunit	glutathione	3.A.1.5.11	35.88	4E-42
Psed_0258	ABC-type transporter, periplasmic subunit	glutathione	3.A.1.5.11	30.04	1E-45
Psed_1353	ABC-type transporter, integral membrane subunit	glutathione	3.A.1.5.11	42.92	1E-41
Psed_1354	ABC-type transporter, integral membrane subunit	glutathione	3.A.1.5.11	32.07	3E-40
Psed_1355	ABC-type transporter, periplasmic subunit	glutathione	3.A.1.5.11	28.22	2E-35
Psed_1412	ABC-type transporter, periplasmic subunit	glutathione	3.A.1.5.11	30.15	1E-50
Psed_1416	ABC-type transporter, periplasmic subunit	glutathione	3.A.1.5.11	30	5E-47
Psed_1745	ABC-type transporter, periplasmic subunit	glutathione	3.A.1.5.11	29.82	1E-30
Psed_1747	ABC-type transporter, integral membrane subunit	glutathione	3.A.1.5.11	35.23	3E-38
Psed_2187	ABC-type transporter, integral membrane subunit	glutathione	3.A.1.5.11	36.59	1E-47
Psed_2188	ABC-type transporter, integral membrane subunit	glutathione	3.A.1.5.11	34.21	6E-41
Psed_2371	ABC-type transporter, periplasmic subunit	glutathione	3.A.1.5.11	26.86	3E-30
Psed_2372	ABC-type transporter, integral membrane subunit	glutathione	3.A.1.5.11	33.66	2E-50
Psed_2373	ABC-type transporter, integral membrane subunit	glutathione	3.A.1.5.11	40.08	2E-49
Psed_2685	ABC-type transporter, periplasmic subunit	glutathione	3.A.1.5.11	29.36	3E-43
Psed_2927	ABC-type transporter, periplasmic subunit	glutathione	3.A.1.5.11	28.6	3E-44
Psed_2983	ABC-type transporter, periplasmic subunit	glutathione	3.A.1.5.11	28.95	2E-47
Psed_3694	ABC-type transporter, integral membrane subunit	glutathione	3.A.1.5.11	39.93	8E-57

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Psed_3697	ABC-type transporter, periplasmic subunit	glutathione	3.A.1.5.11	30.36	4E-47
Psed_3782	ABC-type transporter, periplasmic subunit	glutathione	3.A.1.5.11	28.66	5E-45
Psed_3920	ABC-type transporter, periplasmic subunit	glutathione	3.A.1.5.11	31.17	1E-44
Psed_4090	ABC-type transporter, integral membrane subunit	glutathione	3.A.1.5.11	35.22	5E-51
Psed_4092	ABC-type transporter, integral membrane subunit	glutathione	3.A.1.5.11	42.67	7E-59
Psed_4648	ABC-type transporter, integral membrane subunit	glutathione	3.A.1.5.11	42.07	7E-54
Psed_6013	ABC-type transporter, periplasmic subunit	glutathione	3.A.1.5.11	30.11	5E-43
Psed_6014	ABC-type transporter, integral membrane subunit	glutathione	3.A.1.5.11	39.74	7E-62
Psed_6015	ABC-type transporter, integral membrane subunit	glutathione	3.A.1.5.11	37.4	2E-43
Psed_6924	ABC-type transporter, periplasmic subunit	glutathione	3.A.1.5.11	28.19	1E-28
Psed_6926	ABC-type transporter, integral membrane subunit	glutathione	3.A.1.5.11	36.94	5E-56
Psed_4089	oligopeptide/dipeptide ABC transporter, ATPase subunit	rhaminose	3.A.1.5.12	53.87	4E-97
Psed_3693	oligopeptide/dipeptide ABC transporter, ATPase subunit	mannose	3.A.1.5.15	47.42	2E-82
Psed_0079	ABC-type transporter, periplasmic subunit	proline	3.A.1.5.17	27.52	3E-40
Psed_0081	ABC-type transporter, integral membrane subunit	proline	3.A.1.5.17	30.98	2E-21
Psed_1002	ABC-type transporter, integral membrane subunit	oligopeptide	3.A.1.5.20	36.88	2E-51

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Psed_1003	ABC-type transporter, integral membrane subunit	oligopeptide	3.A.1.5.20	41.7	1E-60
Psed_1746	ABC-type transporter, integral membrane subunit	oligopeptide	3.A.1.5.20	30.82	9E-37
Psed_2190	ABC-type transporter, periplasmic subunit	oligopeptide	3.A.1.5.20	27.31	2E-31
Psed_3692	oligopeptide/dipeptide ABC transporter, ATPase subunit	oligopeptide	3.A.1.5.20	47.84	4E-88
Psed_3695	ABC-type transporter, integral membrane subunit	oligopeptide	3.A.1.5.20	35.81	3E-50
Psed_4088	oligopeptide/dipeptide ABC transporter, ATPase subunit	oligopeptide	3.A.1.5.20	55.14	2E-106
Psed_4646	oligopeptide/dipeptide ABC transporter, ATPase subunit	oligopeptide	3.A.1.5.20	50.17	9E-85
Psed_4649	ABC-type transporter, integral membrane subunit	oligopeptide	3.A.1.5.20	36.83	2E-57
Psed_6019	oligopeptide/dipeptide ABC transporter, ATPase subunit	oligopeptide	3.A.1.5.20	46.93	3E-78
Psed_0082	Monosaccharide-transporting ATPase	drugs	3.A.1.5.21	31.59	2E-55
Psed_6922	oligopeptide/dipeptide ABC transporter, ATPase subunit	oligopeptide	3.A.1.5.22	51.56	2E-89
Psed_1000	ABC-type transporter, periplasmic subunit	EDTA	3.A.1.5.23	29.34	9E-29
Psed_2189	ABC transporter related	EDTA	3.A.1.5.23	44.73	2E-109
Psed_4936	Phosphate-transporting ATPase., Nickel-transporting ATPase	EDTA	3.A.1.5.23	49.28	3E-127
Psed_4647	oligopeptide/dipeptide ABC transporter, ATPase subunit	oligopeptide	3.A.1.5.25	45.28	2E-78

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Psed_1004	oligopeptide/dipeptide ABC transporter, ATPase subunit	oligopeptide	3.A.1.5.26	50.24	4E-161
Psed_1352	oligopeptide/dipeptide ABC transporter, ATPase subunit	oligopeptide	3.A.1.5.26	43.34	6E-113
Psed_1748	Nickel-transporting ATPase	glutathione	3.A.1.5.27	43.54	2E-59
Psed_4091	ABC-type transporter, periplasmic subunit	glutathione	3.A.1.5.27	29.41	6E-25
Psed_4645	ABC-type transporter, periplasmic subunit	glutathione	3.A.1.5.27	30.69	2E-55
Psed_6925	ABC-type transporter, integral membrane subunit	glutathione	3.A.1.5.27	36	1E-33
Psed_5656	NifC-like ABC-type porter	thiamin	3.A.1.6.1	33.49	2E-28
Psed_6267	Sulfate-transporting ATPase	thiamin	3.A.1.6.3	40.74	2E-36
Psed_5655	Molybdate-transporting ATPase	molybdate/tungsten	3.A.1.6.5	33.43	9E-45
Psed_0461	phosphate ABC transporter, periplasmic phosphate-binding protein	phosphate	3.A.1.7.2	56.86	2E-109
Psed_0462	phosphate ABC transporter, inner membrane subunit PstC	phosphate	3.A.1.7.2	63.66	1E-105
Psed_0463	phosphate ABC transporter, inner membrane subunit PstA	phosphate	3.A.1.7.2	59.74	3E-94
Psed_0464	phosphate ABC transporter, ATPase subunit	phosphate	3.A.1.7.2	88.37	1E-127
Psed_5657	molybdenum ABC transporter, periplasmic molybdate-binding protein	molybdate/tungsten	3.A.1.8.1	31.78	7E-15
Psed_0397	Pyrophosphate-energized proton pump	H+-PPase	3.A.10.2.2	68.65	0
Psed_2009	competence protein ComEA helix-hairpin-helix repeat protein	DNA	3.A.11.1.1	30.05	5E-22
Psed_5524	hydrolase	DNA	3.A.11.1.1	32.08	0.00000007
Psed_0885	transcription-repair coupling factor	DNA	3.A.11.3.1	30.51	0.000006

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Psed_3435	primosomal protein N' (replication factor Y) - superfamily II helicase-like protein	DNA	3.A.11.3.1	21.21	2E-10
Psed_4771	ATP-dependent DNA helicase RecG	DNA	3.A.11.3.1	20	8E-12
Psed_4628	cell divisionFtsK/SpoIIIE	DNA	3.A.12.1.1	40.98	3E-127
Psed_7022	cell divisionFtsK/SpoIIIE	DNA	3.A.12.1.2	26.06	0.0000002
Psed_0856	cell divisionFtsK/SpoIIIE	DNA	3.A.12.3.1	29.07	2E-26
Psed_1127	cell divisionFtsK/SpoIIIE	DNA	3.A.12.3.2	31.9	3E-32
Psed_5227	cell divisionFtsK/SpoIIIE	DNA	3.A.12.3.2	38.06	6E-37
Psed_5531	cell divisionFtsK/SpoIIIE	DNA	3.A.12.3.2	26.77	2E-12
Psed_5778	cell divisionFtsK/SpoIIIE	DNA	3.A.12.3.2	30.36	4E-32
Psed_6548	Microtubule-severing ATPase	ER-RT	3.A.16.1.1	42.54	3E-49
Psed_3319	proteasome ATPase	ER-RT	3.A.16.1.2	45.62	6E-44
Psed_3563	AAA ATPase central domain protein	ER-RT	3.A.16.1.2	35.11	0.000005
Psed_5711	Microtubule-severing ATPase	ER-RT	3.A.16.1.2	28.01	1E-82
Psed_6127	ATP-dependent metalloprotease FtsH	ER-RT	3.A.16.1.2	43.7	3E-53
Psed_4066	type III restriction protein res subunit	T7 Injectisome	3.A.17.1.1	24.87	0.000000004
Psed_1069	DEAD/DEAH box helicase domain protein	mRNA	3.A.18.1.1	33.68	4E-58
Psed_3068	DEAD/DEAH box helicase domain protein	mRNA	3.A.18.1.1	38.32	2E-72
Psed_4690	DEAD/DEAH box helicase domain protein	mRNA	3.A.18.1.1	30.14	4E-46
Psed_2770	Arsenite-transporting ATPase	arsenite	3.A.19.1.1	27.51	3E-21
Psed_1678	ATP synthase subunit a	F-type	3.A.2.1.1	29.11	6E-21
Psed_1680	ATP synthase subunit b	F-type	3.A.2.1.1	26.8	6E-12
Psed_1681	ATP synthase subunit b	F-type	3.A.2.1.1	26.54	0.000002
Psed_1682	ATP synthase subunit delta	F-type	3.A.2.1.1	26.09	0.000003
Psed_1686	ATP synthase epsilon chain	F-type	3.A.2.1.1	33.33	8E-12
Psed_1683	ATP synthase subunit alpha	F-type	3.A.2.1.2	55.36	7E-163
Psed_1684	ATP synthase gamma chain	F-type	3.A.2.1.2	34.48	2E-48

Psed_1685	ATP synthase subunit beta	F-type	3.A.2.1.3	61.15	3E-162
Psed_0937	WD40 repeat-containing protein	protein	3.A.20.1.2	30.96	3E-13
Psed_6538	AAA ATPase central domain protein	protein	3.A.20.1.2	35.09	2E-11
Psed_3062	TrkA-N domain protein	P-type	3.a.3.1.8	37.9	8E-37
Psed_3063	TrkA-N domain protein	P-type	3.a.3.1.8	32	1E-27
Psed_4265	ATPase, P-type (transporting), HAD superfamily, subfamily IC	P-type	3.A.3.2.21	35.64	1E-146
Psed_0349	ATPase, P-type (transporting), HAD superfamily, subfamily IC	P-type	3.A.3.23.1	65.51	0
Psed_4849	heavy metal translocating P-type ATPase	P-type	3.A.3.25.1	64.83	0
Psed_1288	heavy metal translocating P-type ATPase	P-type	3.A.3.5.15	39.64	1E-124
Psed_1304	heavy metal translocating P-type ATPase	P-type	3.A.3.5.18	49.04	0
Psed_1080	heavy metal translocating P-type ATPase	P-type	3.A.3.5.19	42.78	3E-170
Psed_2220	heavy metal translocating P-type ATPase	P-type	3.A.3.5.19	39.9	4E-155
Psed_4360	heavy metal translocating P-type ATPase	P-type	3.A.3.5.19	37.96	2E-143
Psed_6876	heavy metal translocating P-type ATPase	P-type	3.A.3.5.19	44.04	2E-172
Psed_6401	heavy metal translocating P-type ATPase	P-type	3.A.3.5.20	48.67	6E-168
Psed_6403	Heavy metal transport/detoxification protein	P-type	3.A.3.5.24	41.94	0.0000002
Psed_2659	XshC-Cox1-family protein	P-type	3.A.3.5.27	46.51	0.0000002
Psed_6874	copper ion binding protein	P-type	3.A.3.5.3	38.98	0.0000002
Psed_1303	Heavy metal transport/detoxification protein	P-type	3.A.3.5.6	32.86	0.000003
Psed_0565	Heavy metal transport/detoxification protein	P-type	3.A.3.5.7	34.78	0.000005
Psed_4376	heavy metal translocating P-type ATPase	P-type	3.A.3.6.1	38.05	1E-120
Psed_0039	heavy metal translocating P-type ATPase	P-type	3.A.3.6.9	35.53	1E-99
Psed_6160	heavy metal translocating P-type ATPase	P-type	3.A.3.6.9	36.14	5E-104
Psed_4145	Potassium-transporting ATPase C chain	P-type	3.A.3.7.1	37.37	4E-21

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Psed_4146	Potassium-transporting ATPase B chain	P-type	3.A.3.7.1	61.62	0
Psed_4147	Potassium-transporting ATPase A chain	P-type	3.A.3.7.1	47.21	6E-126
Psed_6497	oxyanion-translocating ATPase	arsenite	3.A.4.1.1	27.33	0.0000002
Psed_6498	oxyanion-translocating ATPase	arsenite	3.A.4.1.1	25.1	0.00000002
Psed_3416	preprotein translocase, SecG subunit	Sec	3.A.5.2.2	72	7E-22
Psed_3580	protein-export membrane protein SecF	Sec	3.A.5.2.2	53.52	6E-107
Psed_3581	protein-export membrane protein SecD	Sec	3.A.5.2.2	60.65	7E-109
Psed_3582	preprotein translocase, YajC subunit	Sec	3.A.5.2.2	48.89	2E-16
Psed_4749	signal recognition particle protein	Sec	3.A.5.2.2	72.92	0
Psed_4756	signal recognition particle-docking protein FtsY	Sec	3.A.5.2.2	75.39	2E-115
Psed_5179	Protein translocase subunit secA	Sec	3.A.5.2.2	68.98	0
Psed_5409	preprotein translocase, SecY subunit	Sec	3.A.5.2.2	74.15	0
Psed_5464	preprotein translocase, SecE subunit	Sec	3.A.5.2.2	60	3E-15
Psed_3483	chaperone DnaJ domain protein	Sec	3.A.5.8.1	31.51	0.000004
Psed_4493	Chaperone protein dnaJ	Sec	3.A.5.9.1	36.36	0.000002
Psed_6553	Chaperone protein dnaJ	Sec	3.A.5.9.1	38.89	0.0000006
Psed_1667	transcription termination factor Rho	IIISP	3.A.6.1.1	30.07	6E-16
Psed_3160	Forkhead-associated protein	IIISP	3.A.6.3.1	33.33	0.0000002
Psed_3810	Cobyrinic acid ac-diamide synthase	IVSP	3.A.7.11.1	32.91	8E-12
Psed_6686	Cobyrinic acid ac-diamide synthase	IVSP	3.A.7.11.1	31.46	3E-13
Psed_6994	Cobyrinic acid ac-diamide synthase	IVSP	3.A.7.11.1	31.76	0.000000001
Psed_1216	type IV secretory pathway VirB4 components-like protein	IVSP	3.A.7.13.1	27.81	2E-63
Psed_4738	signal peptidase I	IVSP	3.A.7.13.1	28.72	6E-11
Psed_6901	hypothetical protein	IVSP	3.A.7.13.1	21.35	9E-12

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Psed_0403	helicase/secretion neighborhood ATPase	IVSP	3.A.7.15.1	38.29	9E-64
Psed_6900	TRAG family protein	IVSP	3.A.7.2.1	25.29	0.000001
Psed_4384	type IV secretory pathway VirD4 protein-like protein	IVSP	3.A.7.5.1	23.2	0.000003
Psed_0393	DNA topoisomerase I	DNA	3.A.7.7.1	25.46	5E-25
Psed_2210	Protein grpE	MPT	3.A.8.1.1	23.29	0.000001
Psed_3484	Protein grpE	MPT	3.A.8.1.1	28.08	1E-10
Psed_6554	Protein grpE	MPT	3.A.8.1.1	28.75	0.00000001
Psed_0135	NAD-dependent epimerase/dehydratase	CEPT	3.A.9.1.1	30.77	0.000007
Psed_0237	NmrA family protein	CEPT	3.A.9.1.1	28.41	0.000001
Psed_0332	Amidase	CEPT	3.A.9.1.1	39.04	5E-14
Psed_1033	Amidase	CEPT	3.A.9.1.1	40.63	0.0000008
Psed_1194	Allophanate hydrolase	CEPT	3.A.9.1.1	26.04	2E-12
Psed_1414	Phthalate 4,5-dioxygenase	CEPT	3.A.9.1.1	32.43	2E-15
Psed_1419	Amidase	CEPT	3.A.9.1.1	41.67	9E-13
Psed_1453	Amidase	CEPT	3.A.9.1.1	23.91	2E-10
Psed_1795	Glutamyl-tRNA(Gln) amidotransferase subunit A	CEPT	3.A.9.1.1	39.81	1E-11
Psed_2209	Chaperone protein dnaK	CEPT	3.A.9.1.1	58.43	0
Psed_2452	Amidase	CEPT	3.A.9.1.1	26.13	4E-11
Psed_2519	Phthalate 4,5-dioxygenase	CEPT	3.A.9.1.1	26.54	5E-16
Psed_2964	Amidase	CEPT	3.A.9.1.1	26.74	4E-15
Psed_2966	Rieske [2Fe-2S] iron-sulphur domain	CEPT	3.A.9.1.1	24.78	1E-12
Psed_3005	40-residue YVTN family beta-propeller repeat protein	CEPT	3.A.9.1.1	30.39	5E-31
Psed_3031	Amidase	CEPT	3.A.9.1.1	39.44	3E-13

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Psed_3184	allophanate hydrolase	CEPT	3.A.9.1.1	42.95	6E-16
Psed_3241	Amidase	CEPT	3.A.9.1.1	38.46	0.000000004
Psed_3485	Chaperone protein dnaK	CEPT	3.A.9.1.1	57.3	0
Psed_3606	Rieske [2Fe-2S] iron-sulphur domain	CEPT	3.A.9.1.1	27.43	0.000002
Psed_3698	Phthalate 4,5-dioxygenase	CEPT	3.A.9.1.1	32.03	5E-15
Psed_3921	Phthalate 4,5-dioxygenase	CEPT	3.A.9.1.1	27.78	2E-14
Psed_3923	Phthalate 4,5-dioxygenase	CEPT	3.A.9.1.1	28.91	1E-15
Psed_4152	Amidase	CEPT	3.A.9.1.1	26.29	1E-16
Psed_4641	Amidase	CEPT	3.A.9.1.1	39.81	0.00000003
Psed_4642	Amidase	CEPT	3.A.9.1.1	37.04	0.000000002
Psed_4644	Amidase	CEPT	3.A.9.1.1	33.98	0.0000009
Psed_5381	Amidase	CEPT	3.A.9.1.1	31.49	5E-40
Psed_5950	Amidase	CEPT	3.A.9.1.1	26.84	1E-17
Psed_6205	Amidase	CEPT	3.A.9.1.1	26.53	1E-16
Psed_6555	Chaperone protein dnaK	CEPT	3.A.9.1.1	58.72	0
Psed_6591	Heat shock protein 70	CEPT	3.A.9.1.1	29.47	9E-28
Psed_6728	Amidase	CEPT	3.A.9.1.1	26.11	8E-17
Psed_6736	Amidase	CEPT	3.A.9.1.1	33.11	0.0000005
Psed_0502	biotin/lipoyl attachment domain-containing protein	Na+	3.B.1.1.1	41.27	0.000003
Psed_1547	Pyruvate carboxylase	Na+	3.B.1.1.1	29.9	2E-50
Psed_3034	Pyruvate carboxylase	Na+	3.B.1.1.1	30.26	2E-35
Psed_4769	pyruvate carboxylase	Na+	3.B.1.1.1	35.14	7E-83
Psed_5275	Carbamoyl-phosphate synthase L chain ATP-binding	Na+	3.B.1.1.1	44.64	0.0000001

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Psed_1573	Biotin carboxylase., Propionyl-CoA carboxylase	Na+	3.B.1.1.2	36.23	0.000001
Psed_1603	Methylcrotonoyl-CoA carboxylase	Na+	3.B.1.1.2	29.72	2E-63
Psed_5261	Propionyl-CoA carboxylase	Na+	3.B.1.1.2	56.19	2E-169
Psed_6157	Methylcrotonoyl-CoA carboxylase	Na+	3.B.1.1.2	33.47	1E-75
Psed_5269	Biotin carboxylase., Pyruvate carboxylase	Na+	3.B.1.1.3	44.62	8E-10
Psed_6156	Methylcrotonoyl-CoA carboxylase	Na+	3.B.1.1.3	44.12	0.00000004
Psed_0517	Propionyl-CoA carboxylase	Na+	3.B.1.1.5	40.41	3E-96
Psed_1545	acetyl-CoA carboxylase, biotin carboxyl carrier protein	Na+	3.B.1.1.5	37.33	0.0000003
Psed_1604	Methylcrotonoyl-CoA carboxylase	Na+	3.B.1.1.5	51.47	2E-12
Psed_2963	Acetyl-CoA carboxylase	Na+	3.B.1.1.5	24.74	6E-13
Psed_4005	Carbamoyl-phosphate synthase L chain ATP-binding	Na+	3.B.1.1.5	32.28	3E-60
Psed_5277	Propionyl-CoA carboxylase	Na+	3.B.1.1.5	41.96	5E-94
Psed_3296	molybdopterin oxidoreductase Fe4S4 region	NDH	3.D.1.1.1	27.41	7E-20
Psed_5556	NAD(P)H-quinone oxidoreductase subunit 2	NDH	3.D.1.1.1	37.5	5E-62
Psed_5557	proton-translocating NADH-quinone oxidoreductase, chain M	NDH	3.D.1.2.1	34.13	2E-84
Psed_6863	proton-translocating NADH-quinone oxidoreductase, chain M	NDH	3.D.1.2.1	36.02	2E-77
Psed_6869	NADH-ubiquinone/plastoquinone oxidoreductase chain 3	NDH	3.D.1.2.1	31.62	2E-14
Psed_0743	NADH dehydrogenase (quinone)	NDH	3.D.1.3.1	34.33	4E-48
Psed_5559	NAD(P)H-quinone oxidoreductase subunit 4L	NDH	3.D.1.3.1	62.37	5E-20
Psed_5561	NAD(P)H-quinone oxidoreductase subunit I	NDH	3.D.1.3.1	47.1	1E-35

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Psed_5564	NADH-quinone oxidoreductase, F subunit	NDH	3.D.1.3.1	51.33	7E-112
Psed_5566	NAD(P)H-quinone oxidoreductase subunit H	NDH	3.D.1.3.1	48.51	1E-106
Psed_5568	NAD(P)H-quinone oxidoreductase subunit K	NDH	3.D.1.3.1	68.24	2E-60
Psed_0755	NADH ubiquinone oxidoreductase 20 kDa subunit	H+	3.D.1.4.1	49.58	3E-29
Psed_0756	NADH dehydrogenase (quinone)	H+	3.D.1.4.3	32.39	1E-38
Psed_0757	respiratory-chain NADH dehydrogenase subunit 1	H+	3.D.1.4.3	31.69	2E-20
Psed_0760	NADH dehydrogenase (ubiquinone) 30 kDa subunit	H+	3.D.1.4.3	30.06	1E-29
Psed_3041	NADH dehydrogenase (ubiquinone) 24 kDa subunit	H+	3.D.1.5.1	34.51	6E-25
Psed_3042	NADH dehydrogenase (quinone)	H+	3.D.1.5.1	40.12	2E-104
Psed_3297	Respiratory-chain NADH dehydrogenase domain 51 kDa subunit	H+	3.D.1.5.1	37.99	2E-90
Psed_5562	NADH dehydrogenase (quinone)	H+	3.D.1.5.1	50.78	1E-87
Psed_5567	NAD(P)H-quinone oxidoreductase subunit J	H+	3.D.1.5.1	42.77	2E-27
Psed_6867	NADH dehydrogenase (quinone)	H+	3.D.1.5.1	37.37	6E-42
Psed_5565	NADH dehydrogenase (quinone)	H+	3.D.1.6.1	35.15	7E-24
Psed_5563	NADH-quinone oxidoreductase, chain G	H+	3.D.1.6.2	35.45	4E-77
Psed_2135	Acyl carrier protein	H+	3.D.1.6.4	40.3	8E-10
Psed_5558	proton-translocating NADH-quinone oxido-reductase, chain L	NDH	3.D.1.7.1	45.63	3E-97
Psed_5569	NAD(P)H-quinone oxidoreductase subunit 3	NDH	3.D.1.7.1	37.4	2E-16
Psed_6864	NADH dehydrogenase (quinone)	NDH	3.D.1.7.1	29.41	1E-53
Psed_5405	nicotinamide nucleotide transhydrogenase alpha subunit 2 PntAB	H+	3.D.2.1.1	43.33	7E-14

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Psed_5195	alanine dehydrogenase	H+	3.D.2.2.1	31.36	2E-32
Psed_5404	NAD(P)(+) transhydrogenase (AB-specific)	H+	3.D.2.2.1	47.92	2E-109
Psed_5406	NAD(P)(+) transhydrogenase (AB-specific)	H+	3.D.2.2.1	47.16	2E-79
Psed_3122	cytochrome d ubiquinol oxidase, subunit II	COX	3.D.4.3.1	35.32	1E-23
Psed_6416	cytochrome bd ubiquinol oxidase subunit II	COX	3.D.4.3.1	29.75	0.000000005
Psed_3405	Protoheme IX farnesyltransferase	COX	3.D.4.4.1	38.31	3E-30
Psed_1495	cytochrome c oxidase, subunit I	COX	3.D.4.4.2	71.1	0
Psed_2354	cytochrome c oxidase, subunit I	COX	3.D.4.4.2	68.44	0
Psed_2757	Cytochrome-c oxidase	COX	3.D.4.4.2	63.32	9E-71
Psed_4545	Cytochrome-c oxidase	COX	3.D.4.4.2	29.37	4E-16
Psed_4546	cytochrome c oxidase, subunit II	COX	3.D.4.4.2	39.32	9E-69
Psed_5003	cytochrome c oxidase, subunit I	COX	3.D.4.4.2	69.96	0
Psed_5869	cytochrome c oxidase, subunit I	COX	3.D.4.4.2	67.51	0
Psed_0073	Ferredoxin--NAD(+) reductase	Na-NDH	3.D.5.1.1	24.71	5E-15
Psed_0630	Ferredoxin--NAD(+) reductase	Na-NDH	3.D.5.1.1	28.8	2E-27
Psed_0766	Phenol 2-monooxygenase	Na-NDH	3.D.5.1.1	22.22	2E-15
Psed_5584	Nitric oxide dioxygenase	Na-NDH	3.D.5.1.1	24.85	0.000001
Psed_5921	Oxidoreductase FAD-binding domain protein	Na-NDH	3.D.5.1.1	23.81	0.000004
Psed_6551	Nitric oxide dioxygenase	Na-NDH	3.D.5.1.1	24	9E-10
Psed_6977	Ferredoxin--NAD(+) reductase	Na-NDH	3.D.5.1.1	29.27	2E-13
Psed_4030	protein of unknown function DUF224 cysteine-rich region domain protein	H+	3.D.7.1.1	24.56	6E-12
Psed_4785	protein of unknown function DUF224 cysteine-rich region domain protein	H+	3.D.7.1.1	24.16	7E-22
Psed_4790	protein of unknown function DUF224 cysteine-rich region domain protein	H+	3.D.7.1.1	23.09	3E-16

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Psed_6361	Cytochrome-c3 hydrogenase	H+	3.D.7.1.1	32.08	3E-36
Psed_6362	Cytochrome-c3 hydrogenase	H+	3.D.7.1.1	29.61	2E-53
Psed_6565	protein of unknown function DUF224 cysteine-rich region domain protein	H+	3.D.7.1.1	25.89	5E-33
Psed_4915	glutamate synthase alpha subunit domain protein	FMF-DH	3.D.8.1.2	28.17	0.000008
Psed_0759	NADH dehydrogenase (quinone)	F420H2DH	3.D.9.1.1	28.75	7E-29
Psed_5560	NADH-ubiquinone/plastoquinone oxidoreductase chain 6	F420H2DH	3.D.9.1.1	33.71	2E-15
Psed_6862	NAD(P)H-quinone oxidoreductase subunit 2	F420H2DH	3.D.9.1.1	31.26	1E-55
Psed_6865	NAD(P)H-quinone oxidoreductase subunit 4L	F420H2DH	3.D.9.1.1	39.33	0.00000003
Psed_2754	Cytochrome b/b6 domain	PRC	3.E.2.2.2	31.47	7E-25
Psed_2922	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.3	28.04	3E-17
Psed_0133	Long-chain-fatty-acid--CoA ligase	fatty acid	4.C.1.1.4	27.2	5E-26
Psed_0252	amino acid adenylation domain protein	fatty acid	4.C.1.1.4	25.27	3E-25
Psed_0293	Long-chain-fatty-acid--CoA ligase	fatty acid	4.C.1.1.4	29.35	1E-33
Psed_0499	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.4	30.4	1E-53
Psed_0510	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.4	29.06	2E-39
Psed_0542	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.4	27.88	3E-25
Psed_0623	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.4	30.36	2E-54
Psed_0848	Long-chain-fatty-acid--[acyl-carrier-protein] ligase	fatty acid	4.C.1.1.4	26.46	9E-22
Psed_1343	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.4	30.39	2E-49
Psed_1491	benzoate-CoA ligase family	fatty acid	4.C.1.1.4	27.17	5E-34
Psed_1550	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.4	31.25	4E-68
Psed_1587	Long-chain-fatty-acid--CoA ligase	fatty acid	4.C.1.1.4	34.71	2E-75

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Psed_1596	Long-chain-fatty-acid--CoA ligase	fatty acid	4.C.1.1.4	28.37	2E-38
Psed_1605	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.4	29.21	8E-48
Psed_1607	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.4	29	2E-45
Psed_1779	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.4	27.06	7E-39
Psed_2619	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.4	27.59	5E-39
Psed_2766	Long-chain-fatty-acid--CoA ligase	fatty acid	4.C.1.1.4	26.88	2E-34
Psed_3001	non-ribosomal peptide synthetase domain protein	fatty acid	4.C.1.1.4	22.2	5E-18
Psed_3740	Long-chain-fatty-acid--CoA ligase	fatty acid	4.C.1.1.4	30.19	3E-42
Psed_3899	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.4	28.57	8E-51
Psed_3988	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.4	27.39	2E-36
Psed_4095	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.4	27.98	3E-43
Psed_4246	Benzoate--CoA ligase	fatty acid	4.C.1.1.4	29.66	8E-48
Psed_4908	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.4	28.33	1E-39
Psed_5064	(2,3-dihydroxybenzoyl)adenylate synthase	fatty acid	4.C.1.1.4	28.2	2E-35
Psed_5111	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.4	32.63	2E-59
Psed_5189	Long-chain-fatty-acid--CoA ligase	fatty acid	4.C.1.1.4	30.49	1E-41
Psed_5437	Long-chain-fatty-acid--CoA ligase	fatty acid	4.C.1.1.4	34.69	3E-78
Psed_5474	4-coumarate--CoA ligase	fatty acid	4.C.1.1.4	34.66	2E-78
Psed_5499	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.4	30.28	1E-57
Psed_5600	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.4	25.99	1E-14
Psed_5624	Long-chain-fatty-acid--CoA ligase	fatty acid	4.C.1.1.4	30.92	2E-63
Psed_5652	Long-chain-fatty-acid--CoA ligase	fatty acid	4.C.1.1.4	32.18	3E-65
Psed_5917	Long-chain-fatty-acid--CoA ligase	fatty acid	4.C.1.1.4	26.73	7E-17
Psed_5934	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.4	28.62	1E-39

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Psed_6221	Long-chain-fatty-acid--CoA ligase	fatty acid	4.C.1.1.4	31.93	3E-61
Psed_6339	(2,3-dihydroxybenzoyl)adenylate synthase	fatty acid	4.C.1.1.4	24.23	3E-25
Psed_6448	(2,3-dihydroxybenzoyl)adenylate synthase	fatty acid	4.C.1.1.4	24.62	5E-23
Psed_6776	Long-chain-fatty-acid--CoA ligase	fatty acid	4.C.1.1.4	30.73	6E-39
Psed_6920	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.4	25.81	3E-29
Psed_0055	non-ribosomal peptide synthetase domain protein	fatty acid	4.C.1.1.6	23.52	9E-11
Psed_0559	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.6	29.23	4E-55
Psed_0562	Acetate--CoA ligase	fatty acid	4.C.1.1.6	24.08	1E-28
Psed_1328	Long-chain-fatty-acid--CoA ligase	fatty acid	4.C.1.1.6	27.96	3E-48
Psed_1599	Long-chain-fatty-acid--CoA ligase	fatty acid	4.C.1.1.6	29.59	1E-49
Psed_2061	Long-chain-fatty-acid--CoA ligase	fatty acid	4.C.1.1.6	31.54	1E-59
Psed_2077	Long-chain-fatty-acid--CoA ligase	fatty acid	4.C.1.1.6	38.22	5E-105
Psed_2463	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.6	30.1	4E-55
Psed_2540	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.6	28.46	4E-46
Psed_2566	Long-chain-fatty-acid--CoA ligase	fatty acid	4.C.1.1.6	27.82	4E-46
Psed_2583	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.6	28.34	2E-44
Psed_2672	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.6	30.96	7E-58
Psed_2717	Butyrate--CoA ligase	fatty acid	4.C.1.1.6	24.95	1E-31
Psed_4221	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.6	24.9	8E-37
Psed_5037	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.6	25.99	5E-31
Psed_5280	Long-chain-fatty-acid--CoA ligase	fatty acid	4.C.1.1.6	31.84	1E-62
Psed_5507	Long-chain-fatty-acid--CoA ligase	fatty acid	4.C.1.1.6	30.04	2E-55
Psed_0236	acetoacetyl-CoA synthase	fatty acid	4.C.1.1.7	23.37	0.00000002
Psed_0407	Acetyl-coenzyme A synthetase	fatty acid	4.C.1.1.7	25.84	7E-28
Psed_3363	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.7	29.07	2E-40

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Psed_4103	4-coumarate--CoA ligase	fatty acid	4.C.1.1.7	25.41	1E-28
Psed_4605	acetoacetyl-CoA synthase	fatty acid	4.C.1.1.7	21.98	7E-11
Psed_4795	Acetate--CoA ligase	fatty acid	4.C.1.1.7	23.16	5E-26
Psed_5304	o-succinylbenzoate--CoA ligase	fatty acid	4.C.1.1.7	27.15	5E-38
Psed_1916	Carnitine O-acetyltransferase	fatty acid	4.C.2.1.2	31.88	2E-69
Psed_5486	BAAT/Acyl-CoA thioester hydrolase	fatty acid	4.C.3.1.1	31.63	0.000001
Psed_5609	cytochrome c biogenesis protein transmembrane region	DsbD	5.A.1.2.1	32.34	1E-26
Psed_1300	cytochrome c biogenesis protein transmembrane region	mercuric	5.A.1.4.1	37.73	9E-16
Psed_6718	cytochrome c biogenesis protein transmembrane region	mercuric	5.A.1.4.1	94.04	1E-116
Psed_1290	Heavy metal transport/detoxification protein	heavy metal	5.A.1.6.1	35.29	0.0000002
Psed_4300	nitrate reductase, alpha subunit	H+	5.A.3.1.1	50.08	0
Psed_4301	nitrate reductase, beta subunit	H+	5.A.3.1.1	53.56	1E-160
Psed_4303	respiratory nitrate reductase, gamma subunit	PMO	5.A.3.1.2	32.88	8E-30
Psed_2417	4Fe-4S ferredoxin iron-sulfur binding domain-containing protein	H+	5.A.3.2.1	38.4	8E-44
Psed_2418	formate dehydrogenase, alpha subunit	H+	5.A.3.2.1	36.9	0
Psed_3043	formate dehydrogenase, alpha subunit	H+	5.A.3.2.1	25.6	3E-42
Psed_3298	Nitrate reductase	H+	5.A.3.2.1	24.95	8E-32
Psed_1658	Trimethylamine-N-oxide reductase (cytochrome c)	PMO	5.A.3.3.2	29.76	2E-67
Psed_2196	Trimethylamine-N-oxide reductase (cytochrome c)	PMO	5.A.3.4.2	37.81	3E-140
Psed_1374	Nitrate reductase	PMO	5.A.3.5.1	24.66	5E-26
Psed_1088	Nitrate reductase	PMO	5.A.3.6.1	23.82	4E-23

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Psed_2755	Rieske [2Fe-2S] iron-sulphur domain	PMO	5.A.3.6.1	39.47	0.00000004
Psed_4229	Nitrate reductase	PMO	5.A.3.6.1	25.1	6E-30
Psed_2846	L-aspartate oxidase	SDH	5.A.4.1.1	27.66	3E-42
Psed_4589	succinate dehydrogenase and fumarate reductase iron-sulfur protein	SDH	5.A.4.1.1	30.73	3E-20
Psed_4590	Succinate dehydrogenase (ubiquinone)	SDH	5.A.4.1.1	30.78	6E-62
Psed_4592	succinate dehydrogenase and fumarate reductase iron-sulfur protein	SDH	5.A.4.1.1	29.84	1E-24
Psed_4593	succinate dehydrogenase or fumarate reductase, flavoprotein subunit	SDH	5.A.4.1.1	37.31	2E-95
Psed_5969	L-aspartate oxidase	SDH	5.A.4.1.1	23.64	4E-13
Psed_4997	Oxidoreductase FAD-binding domain protein	Phox	5.B.1.6.1	28.4	0.000001
Psed_3260	band 7 protein	Stomatin	8.A.21.2.1	46.86	5E-53
Psed_3355	band 7 protein	Stomatin	8.A.21.2.1	45.51	3E-34
Psed_0300	Pyridoxine 4-dehydrogenase	K+	8.A.5.1.1	27.3	1E-22
Psed_4159	NADP-dependent oxidoreductase domain	K+	8.A.5.1.1	30.89	2E-34
Psed_5362	Aryl-alcohol dehydrogenase (NADP(+))	K+	8.A.5.1.1	26.49	3E-20
Psed_5591	2,5-didehydrogluconate reductase	K+	8.A.5.1.2	24.63	0.0000002
Psed_2147	2,5-didehydrogluconate reductase	K+	8.A.5.1.3	24.65	6E-12
Psed_2431	Pyridoxine 4-dehydrogenase	K+	8.A.5.1.3	29.45	1E-23
Psed_4079	Aryl-alcohol dehydrogenase (NADP(+))	K+	8.A.5.1.3	29.94	4E-30
Psed_4432	Aryl-alcohol dehydrogenase (NADP(+))	K+	8.A.5.1.3	33.74	1E-40
Psed_4986	NADP-dependent oxidoreductase domain	K+	8.A.5.1.3	32.35	5E-41
Psed_5092	NADP-dependent oxidoreductase domain	K+	8.A.5.1.3	29.67	1E-28
Psed_5978	Aryl-alcohol dehydrogenase (NADP(+))	K+	8.A.5.1.3	34.63	2E-36

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Psed_2974	phosphoenolpyruvate-protein phosphotrans-ferase	EI	8.A.7.1.1	36.78	4E-91
Psed_4581	pyruvate, phosphate dikinase	EI	8.A.7.1.1	25.99	6E-21
Psed_1729	trehalose synthase	amino acid	8.A.9.1.1	28.67	2E-54
Psed_4802	alpha amylase catalytic region	amino acid	8.A.9.1.1	30.54	2E-59
Psed_6477	peptidase M10A and M12B matrixin and adamalysin	putative	8.B.14.2.1	28.48	0.000000007
Psed_1273	multicopper oxidase type 3	heavy metal	9.A.10.1.4	22.34	0.0000009
Psed_6851	multicopper oxidase type 3	heavy metal	9.A.10.1.7	29.78	1E-14
Psed_0889	Tat-translocated protein	Fe2+	9.A.10.2.3	37.89	4E-63
Psed_0890	Peptidase M75, Imelysin	Fe2+	9.A.10.2.3	37.93	1E-59
Psed_0891	iron permease FTR1	Fe2+	9.A.10.2.3	38.78	6E-42
Psed_0892	iron permease FTR1	Fe2+	9.A.10.2.3	42.26	1E-42
Psed_0893	Peptidase M75, Imelysin	Fe2+	9.A.10.2.3	41.11	1E-76
Psed_0894	Tat-translocated protein	Fe2+	9.A.10.2.3	37.5	5E-66
Psed_4831	Tat-translocated protein	Fe2+	9.A.10.2.3	38.3	5E-70
Psed_4832	Peptidase M75, Imelysin	Fe2+	9.A.10.2.3	34.74	2E-51
Psed_4833	iron permease FTR1	Fe2+	9.A.10.2.3	35.19	1E-35
Psed_0040	MgtE intracellular region	Mg2+	9.A.19.1.2	30.21	8E-14
Psed_0708	CBS domain containing protein	Mg2+	9.A.19.1.2	52.83	0.0000002
Psed_1044	MgtE intracellular region	Mg2+	9.A.19.1.2	33.19	2E-23
Psed_3786	CBS domain containing protein	Mg2+	9.A.19.1.2	33.64	0.000001
Psed_5606	hypothetical protein	RD1	9.A.25.1.1	38.57	1E-50
Psed_6072	chromosome partitioning ATPase	RD1	9.A.25.1.1	34.97	1E-35
Psed_6251	CbbX protein	RD1	9.A.25.1.1	38.49	3E-40
Psed_6588	chromosome partitioning ATPase	RD1	9.A.25.1.1	34.78	9E-37

Appendix 4. Transport proteins in *P. dioxanivorans* CB1190

Psed_6754	hypothetical protein	RD1	9.A.25.1.1	32.53	6E-30
Psed_6954	chromosome partitioning ATPase	RD1	9.A.25.1.1	32.3	6E-30
Psed_5851	integral membrane protein, TerC family	tellerium	9.A.30.1.1	34.97	5E-42
Psed_2442	sigma-54 factor interaction domain-containing protein	VISP	9.A.34.1.1	22.55	0.000004
Psed_4116	ATPase AAA-2 domain protein	VISP	9.A.34.1.1	34.15	5E-69
Psed_2213	ATP-dependent chaperone ClpB	VISP	9.A.34.2.1	40.21	1E-152
Psed_3481	ATP-dependent chaperone ClpB	VISP	9.A.34.2.1	39.58	8E-150
Psed_6113	ATPase AAA-2 domain protein	VISP	9.A.34.2.1	34.87	7E-126
Psed_6547	ATP-dependent chaperone ClpB	VISP	9.A.34.2.1	39.3	4E-148
Psed_3166	protein of unknown function DUF21	HCC	9.A.40.2.1	26.02	1E-36
Psed_3167	protein of unknown function DUF21	HCC	9.A.40.2.1	28.18	3E-52
Psed_4466	protein of unknown function DUF21	HCC	9.A.40.2.1	26.25	2E-42
Psed_4467	protein of unknown function DUF21	HCC	9.A.40.2.1	25.88	5E-31
Psed_4485	CBS domain containing protein	HCC	9.A.40.2.1	26.71	8E-44
Psed_5388	cell divisionFtsK/SpoIIIE	MPSS	9.A.42.1.1	32.91	1E-115
Psed_5390	peptidase S8 and S53 subtilisin kexin sedolisin	MPSS	9.A.42.1.1	35.38	2E-28
Psed_5391	protein of unknown function DUF690	MPSS	9.A.42.1.1	28.84	7E-21
Psed_5118	cell divisionFtsK/SpoIIIE	EPSP	9.A.44.1.1	28.99	2E-15
Psed_1220	NLP/P60 protein	conjugation	9.A.49.1.1	43.18	4E-10
Psed_2764	NLP/P60 protein	conjugation	9.A.49.1.1	33.91	0.00000001
Psed_3375	NLP/P60 protein	conjugation	9.A.49.1.1	32.76	0.000000009
Psed_4534	NLP/P60 protein	conjugation	9.A.49.1.1	39.05	9E-12
Psed_6210	NLP/P60 protein	conjugation	9.A.49.1.1	34.56	4E-11
Psed_6211	NLP/P60 protein	conjugation	9.A.49.1.1	35.25	6E-11

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Psed_6559	NLP/P60 protein	conjugation	9.A.49.1.1	35.51	0.0000002
Psed_6562	hypothetical protein	conjugation	9.A.49.1.1	23.01	2E-12
Psed_6579	NLP/P60 protein	conjugation	9.A.49.1.1	31.43	0.000000009
Psed_6665	NLP/P60 protein	conjugation	9.A.49.1.1	33.93	0.000000006
Psed_6742	NLP/P60 protein	conjugation	9.A.49.1.1	36.7	0.000005
Psed_6959	NLP/P60 protein	conjugation	9.A.49.1.1	33.86	6E-13
Psed_3803	GTP-binding protein engA	Fe2+	9.A.8.1.3	37.29	0.000004
Psed_2203	cobalamin synthesis CobW domain protein	Zn2+	9.B.10.1.1	26.84	2E-29
Psed_5015	cobalamin synthesis protein P47K	Zn2+	9.B.10.1.1	30.64	3E-24
Psed_1642	protein of unknown function DUF395 YeeE/ YedE	putative	9.B.102.2.	37.89	1E-44
Psed_0455	Thiosulfate sulfurtransferase	sulfate	9.B.102.4.	29.33	1E-21
Psed_2199	Ketopantoate reductase ApbA/PanE domain protein	sulfate	9.B.102.4.	33.88	0.000000001
Psed_4901	3-mercaptopyruvate sulfurtransferase	sulfate	9.B.102.4.	26.51	0.00000009
Psed_5264	Thiosulfate sulfurtransferase	sulfate	9.B.102.4.	33.22	3E-26
Psed_5607	cytochrome c-type biogenesis protein CcsB	HHP	9.B.14.1.3	28.42	0.000008
Psed_4741	MgtC/SapB transporter	Mg2+	9.B.20.1.1	37.72	3E-18
Psed_1594	protein of unknown function UPF0016	putative	9.B.26.1.1	32.28	7E-12
Psed_1522	SNARE associated Golgi protein	putative	9.B.27.1.1	26.74	9E-11
Psed_2817	SNARE associated Golgi protein	selenite	9.B.27.2.3	34.25	4E-23
Psed_4824	SNARE associated Golgi protein	selenite	9.B.27.2.3	35.52	1E-21
Psed_4866	phosphoesterase PA-phosphatase related	selenite	9.B.27.2.3	33.59	1E-15
Psed_6526	SNARE associated Golgi protein	selenite	9.B.27.2.3	38.59	4E-37
Psed_6527	SNARE associated Golgi protein	selenite	9.B.27.2.3	31.98	2E-23
Psed_5070	SNARE associated Golgi protein	putative	9.B.27.2.5	26.34	0.000000004

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Psed_0657	Protein of unknown function DUF318, transmembrane	putative	9.B.28.1.3	25.84	0.000003
Psed_0741	protein of unknown function DUF204	putative	9.B.29.1.1	28.57	1E-12
Psed_4870	penicillin-binding protein transpeptidase	drugs	9.B.3.1.2	34.38	3E-32
Psed_0056	cell division protein FtsW	putative	9.B.3.1.3	38.58	8E-48
Psed_0117	cell cycle protein	putative	9.B.3.1.3	37.24	1E-39
Psed_2805	cell division protein FtsW	putative	9.B.3.1.3	42.35	2E-64
Psed_0948	channel protein, hemolysin III family	putative	9.B.30.1.1	32.28	4E-14
Psed_5694	glycosyl transferase family 2	VGP	9.B.32.1.2	25.57	2E-13
Psed_5941	glycosyl transferase family 2	VGP	9.B.32.1.2	28.44	0.0000002
Psed_0571	polysaccharide deacetylase	VGP	9.B.32.1.3	40.36	1E-39
Psed_0574	polysaccharide deacetylase	VGP	9.B.32.1.3	39.91	8E-41
Psed_1884	glycosyl transferase family 2	VGP	9.B.32.1.3	25.52	0.00000003
Psed_5325	glycosyl transferase family 2	VGP	9.B.32.1.3	25.77	3E-10
Psed_0151	histidine kinase internal region	SHK	9.B.33.1.1	31.46	1E-43
Psed_3144	histidine kinase internal region	SHK	9.B.33.1.1	31.86	3E-10
Psed_0440	diguanylate cyclase	KPSH	9.B.34.1.1	38.33	0.0000002
Psed_0469	diguanylate cyclase	KPSH	9.B.34.1.1	35.84	3E-14
Psed_0674	diguanylate cyclase	KPSH	9.B.34.1.1	32.24	0.00000007
Psed_2886	diguanylate cyclase	KPSH	9.B.34.1.1	41.09	1E-13
Psed_2887	diguanylate cyclase	KPSH	9.B.34.1.1	40.91	3E-13
Psed_4081	diguanylate cyclase	KPSH	9.B.34.1.1	34.76	0.00000001
Psed_5134	diguanylate cyclase	KPSH	9.B.34.1.1	34.5	0.000002
Psed_1767	hydroxyisourate hydrolase	Transthyretin	9.B.35.2.1	37.93	2E-15
Psed_0114	Calcium/calmodulin-dependent protein kinase	RDD	9.B.45.1.3	31	4E-16

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Psed_0115	Calcium/calmodulin-dependent protein kinase	RDD	9.B.45.1.3	25.44	1E-12
Psed_1900	Mitogen-activated protein kinase kinase kinase	RDD	9.B.45.1.3	27.14	4E-15
Psed_2637	Calcium/calmodulin-dependent protein kinase	RDD	9.B.45.1.3	29.41	7E-11
Psed_3172	Serine/threonine protein kinase-related	RDD	9.B.45.1.3	30	6E-19
Psed_4235	Mitogen-activated protein kinase kinase kinase	RDD	9.B.45.1.3	29.6	6E-17
Psed_5309	Calcium/calmodulin-dependent protein kinase	RDD	9.B.45.1.3	29.07	6E-18
Psed_3088	NADP oxidoreductase coenzyme F420-dependent	NPS	9.B.66.1.1	27.46	7E-10
Psed_4049	NADP oxidoreductase coenzyme F420-dependent	NPS	9.B.66.1.1	26.32	0.000002
Psed_0586	CrcB-like protein	camphor	9.B.71.1.1	33.9	0.00000002
Psed_0587	CrcB-like protein	camphor	9.B.71.1.1	32.26	0.000000001
Psed_1072	YhgE/Pip C-terminal domain protein	PIP	9.B.74.1.2	32.86	3E-77
Psed_5908	Cupin 2 conserved barrel domain protein	Eut	9.B.75.3.1	37.25	0.00000001
Psed_3193	Cupin 2 conserved barrel domain protein	Eut	9.B.75.4.1	30.86	0.0000001
Psed_0190	acyltransferase 3	ATAT	9.B.97.3.1	27.86	3E-10

Abbreviations: PPD – Plant Plasmodesmata, Hsp70 – Heatshock Protein-70, GJ-CC – Gap Junction-like Channel-forming Complex, TRIC – Homotrimeric Cation Channel, MIP – Major Intrinsic Protein, Bam Complex – Outer Memberane Protein Insertion Porin, ESAT-6 – Pore-forming 6kDa early secretory antigenic target protein, SVF-Pore – Synaptosomal Vesical Fusion Pore, SSS – Solute:Sodium Symporter, APC – Amino Acid-Polyamine-Organocation, TRAP-T – Tripartite ATP-independent Periplasmic Transporter, RND – Resistance-Nodulation-Cell Division, TAT – Twin Arginine Targeting, γ-HCH – γ-Hexachlorocyclohexane, H+-PPase – H+-translocating Pyrophosphatase, T7 Injectisome – Phage T7 Injectisome, F-type – H+- or Na+-translocating F-type, V-type and

A-type ATPase, P-type – P-type ATPase, Sec – General Secretory Pathway, IIISP – Type III (Virulence-related) Secretory Pathway, IVSP – Type IV (Conjugal DNA-Protein Transfer or VirB) Secretory Pathway, MPT – Mitochondrial Protein Translocase, CEPT – Chloroplast Envelope Protein Translocase, NDH – H+ or Na+-translocating NADH Dehydrogenase, COX – Proton-translocating Cytochrome Oxidase, Na-NDH – Na+-translocating NADH:Quinone Dehydrogenase, FMF-DH – Na+ or H+ Pumping Formyl Methanofuran Dehydrogenase, F420H2DH – H+-translocating F420H2 Dehydrogenase, DsbD – Disulfide Bond Oxidoreductase D, PMO – Prokaryotic Molybdopterin-containing Oxidoreductase, SDH – Prokaryotic Succinate Dehydrogenase, Phox – gp91phox Phagocyte NADPH Oxidase-associated Cytochrome b558, Stomatin – Stomatin/Podocin/Band 7/Nephrosis.2/SPFH, EI – Phosphotransferase System Enzyme I, RD1 – RD1 or ESX-1/Snm Protein Secretion System, VISP – Putative Type VI Symbiosis/Virulence Secretory Pathway, HCC – HlyC/CorC, MPSS – Mycobacterial PPE41 Protein Secretion System, EPSP – EsxA/EsxB Protein Secretion Pathway, HHP – Putative Heme Handling Protein, VGP – Putative Vectorial Glycosyl Polymerization, SHK – Sensor Histidine Kinase, KPSH – Kinase/Phosphotase/Cyclic-GMP Synthase/Cyclic di-GMP Hydrolase, Transthyretin – Putative Thyroxine –Transporting Transthyretin, RDD – Arg/Asp/Asp Family Protein, NPS – Animal Nonclassical Protein Secretion, PIP – Phage Infection Protein, Eut – Ethanol Utilization/Transport Protein, ATAT – Acyltransferase-3/Putative Acetyl-CoA Transporter

Appendix 5

Genes differentially expressed on 1,4-dioxane relative to pyruvate.

Appendix 5. Genes differentially expressed on 1,4-dioxane relative to pyruvate. Differentially expressed genes have an adjusted p-value < 0.01 and have a $log_2FC > 1$ or log2FC < -1 relative to pyruvate and are sorted by log2FC.

Genes	Protein	log_2FC	p-value	adjusted p-value
Psed_3889	2-hydroxy-3-oxopropionate reductase	7.06	7.57E-16	2.04E-12
Psed_3888	Hydroxypyruvate isomerase	6.99	9.41E-16	2.04E-12
Psed_3890	glyoxylate carboligase	6.75	2.45E-16	1.60E-12
Psed_3891	glycerate kinase	5.64	6.01E-15	7.39E-12
Psed_4788	D-lactate dehydrogenase (cytochrome)	5.50	5.20E-15	7.39E-12
Psed_4790	protein of unknown function DUF224 cysteine-rich region domain protein	4.84	9.75E-11	4.51E-08
Psed_4789	FAD linked oxidase domain protein	4.74	6.81E-15	7.39E-12
Psed_6787	Formyl-CoA transferase	3.89	1.04E-10	4.51E-08
Psed_6982	Mn2+/Fe2+ transporter, NRAMP family	3.87	6.78E-09	1.00E-06
Psed_4782	Malate synthase	3.38	5.61E-11	2.81E-08
Psed_6981	Aldehyde Dehydrogenase	3.38	2.70E-10	1.10E-07
Psed_5371	hypothetical protein	3.34	1.23E-05	3.01E-04
Psed_6780	hypothetical protein	3.18	1.33E-11	9.61E-09
Psed_6749	hypothetical protein	3.05	1.72E-08	2.16E-06
Psed_6779	Integrase catalytic region	2.90	8.80E-12	7.16E-09
Psed_6970	D-lactate dehydrogenase (cytochrome)	2.80	8.83E-07	4.67E-05
Psed_6980	hypothetical protein	2.60	9.72E-10	2.88E-07
Psed_6746	hypothetical protein	2.60	1.32E-09	3.59E-07
Psed_6743	hypothetical protein	2.52	4.06E-09	7.14E-07
Psed_6971	Hydroxyacid-oxoacid transhydrogenase	2.47	1.45E-07	1.26E-05
Psed_1658	Trimethylamine-N-oxide reductase (cytochrome c)	2.43	2.63E-05	5.38E-04
Psed_5025	4-hydroxyacetophenone monooxygenase	2.42	3.55E-05	6.37E-04
Psed_6782	Hydroxyacid-oxoacid transhydrogenase	2.33	3.65E-08	4.17E-06
Psed_6751	Transglycosylase-like domain protein	2.33	4.32E-08	4.69E-06
Psed_6748	hypothetical protein	2.28	9.60E-09	1.33E-06
Psed_6977	Ferredoxin--NAD(+) reductase	2.26	1.10E-09	3.11E-07
Psed_6752	hypothetical protein	2.15	4.11E-08	4.62E-06
Psed_6750	hypothetical protein	2.09	6.66E-09	1.00E-06

Psed_6600	acyl-CoA dehydrogenase domain-containing protein	2.00	9.53E-05	1.32E-03
Psed_6784	Alkylglycerone-phosphate synthase	1.98	7.20E-09	1.04E-06
Psed_6979	monooxygenase component MmoB/DmpM	1.98	2.14E-09	4.76E-07
Psed_6744	hypothetical protein	1.97	3.27E-09	6.26E-07
Psed_6972	GntR domain protein	1.93	9.62E-06	2.49E-04
Psed_6747	hypothetical protein	1.92	7.92E-09	1.12E-06
Psed_6745	ATP-binding protein	1.91	1.71E-12	1.59E-09
Psed_6729	hypothetical protein	1.91	8.91E-08	8.40E-06
Psed_6754	hypothetical protein	1.86	5.33E-08	5.51E-06
Psed_6974	Ethyl tert-butyl ether degradation EthD	1.85	1.47E-09	3.82E-07
Psed_1302	protein of unknown function DUF156	1.85	1.32E-04	1.65E-03
Psed_6755	hypothetical protein	1.83	1.31E-07	1.18E-05
Psed_4148	K+-transporting ATPase, F subunit	1.81	2.45E-04	2.66E-03
Psed_6791	IstB domain protein ATP-binding protein	1.79	4.49E-11	2.44E-08
Psed_6753	hypothetical protein	1.77	8.67E-05	1.23E-03
Psed_1584	6-phosphofructokinase	1.76	5.13E-09	8.57E-07
Psed_3522	response regulator receiver	1.74	6.75E-06	1.99E-04
Psed_6175	regulatory protein TetR	1.73	1.57E-05	3.57E-04
Psed_6730	Luciferase-like, subgroup	1.63	6.73E-05	1.02E-03
Psed_6978	methane/phenol/toluene hydroxylase	1.60	6.49E-10	2.35E-07
Psed_0350	Bile acid:sodium symporter	1.60	3.24E-05	6.07E-04
Psed_4146	Potassium-transporting ATPase B chain	1.58	3.43E-05	6.27E-04
Psed_3564	Aromatic-amino-acid transaminase	1.55	1.48E-05	3.45E-04
Psed_6973	hypothetical protein	1.53	1.47E-05	3.44E-04
Psed_3653	hypothetical protein	1.51	4.22E-05	7.13E-04
Psed_7002	transcription factor WhiB	1.51	1.10E-06	5.22E-05
Psed_6259	FMN-dependent oxidoreductase, nitrilotriacetate monooxygenase family	1.51	8.21E-06	2.22E-04
Psed_6742	NLP/P60 protein	1.50	4.20E-10	1.61E-07
Psed_7003	hypothetical protein	1.48	3.71E-09	6.71E-07
Psed_6975	Betaine-aldehyde dehydrogenase	1.47	7.79E-10	2.41E-07
Psed_3935	GntR domain protein	1.46	2.64E-05	5.38E-04
Psed_1303	Heavy metal transport/detoxification protein	1.43	1.30E-05	3.16E-04
Psed_4424	hypothetical protein	1.42	1.28E-08	1.63E-06
Psed_5736	Transglycosylase-like domain protein	1.41	3.11E-04	3.18E-03
Psed_7007	hypothetical protein	1.40	2.03E-08	2.40E-06

Appendix 5. Genes differentially expressed on 1,4-dioxane relative to pyruvate.

Psed_5135	malic protein NAD-binding	1.40	4.81E-05	7.86E-04
Psed_1304	heavy metal translocating P-type ATPase	1.38	7.22E-04	6.02E-03
Psed_6728	Amidase	1.37	3.58E-06	1.26E-04
Psed_1076	short-chain dehydrogenase/reductase SDR	1.35	5.83E-05	9.19E-04
Psed_6261	ABC-type transporter, integral membrane subunit	1.31	7.02E-06	2.02E-04
Psed_4147	Potassium-transporting ATPase A chain	1.27	6.47E-05	9.86E-04
Psed_6913	hypothetical protein	1.27	1.22E-06	5.60E-05
Psed_4512	ABC-type transporter, integral membrane subunit	1.25	1.37E-03	9.72E-03
Psed_4513	cobalamin (vitamin B12) biosynthesis CbiX protein	1.24	2.13E-05	4.55E-04
Psed_0038	regulatory protein ArsR	1.22	1.72E-06	7.22E-05
Psed_3934	major facilitator superfamily MFS_1	1.22	4.87E-06	1.57E-04
Psed_6263	Extracellular ligand-binding receptor	1.21	5.09E-06	1.61E-04
Psed_5938	glycoside hydrolase family 13 domain-containing protein	1.21	8.35E-06	2.24E-04
Psed_6262	ABC-type transporter, integral membrane subunit	1.20	8.99E-06	2.39E-04
Psed_7008	hypothetical protein	1.20	1.02E-08	1.38E-06
Psed_1594	protein of unknown function UPF0016	1.19	7.30E-10	2.41E-07
Psed_6732	MaoC domain protein dehydratase	1.19	1.00E-04	1.37E-03
Psed_5026	regulatory protein TetR	1.19	3.21E-05	6.06E-04
Psed_6758	hypothetical protein	1.17	9.93E-07	4.91E-05
Psed_6888	C-5 cytosine-specific DNA methylase	1.17	2.13E-06	8.45E-05
Psed_4149	hypothetical protein	1.14	8.87E-05	1.25E-03
Psed_4755	ammonium transporter	1.14	7.89E-04	6.42E-03
Psed_2752	hypothetical protein	1.12	3.60E-04	3.57E-03
Psed_6740	hypothetical protein	1.12	1.23E-05	3.01E-04
Psed_2030	Linalool 8-monooxygenase	1.09	1.56E-04	1.88E-03
Psed_5524	hydrolase	1.08	1.03E-05	2.59E-04
Psed_3041	NADH dehydrogenase (ubiquinone) 24 kDa subunit	1.07	6.65E-04	5.68E-03
Psed_5406	NAD(P)(+) transhydrogenase (AB-specific)	1.06	1.18E-03	8.69E-03
Psed_6241	hypothetical protein	1.05	3.32E-05	6.15E-04
Psed_2371	ABC-type transporter, periplasmic subunit	1.05	7.42E-04	6.14E-03

Appendix 5. Genes differentially expressed on 1,4-dioxane relative to pyruvate.

Psed_4306	hypothetical protein	1.05	3.95E-05	6.83E-04
Psed_5405	nicotinamide nucleotide transhydrogenase alpha subunit 2 PntAB	1.04	7.83E-04	6.40E-03
Psed_6757	hypothetical protein	1.03	7.80E-06	2.15E-04
Psed_5404	NAD(P)(+) transhydrogenase (AB-specific)	1.02	1.87E-05	4.13E-04
Psed_6609	Pyruvate dehydrogenase (acetyl-transferring)	-1.00	1.41E-04	1.72E-03
Psed_4514	hypothetical protein	-1.00	7.06E-05	1.05E-03
Psed_5273	Dihydrolipoyl dehydrogenase	-1.00	6.80E-06	1.99E-04
Psed_2132	hypothetical protein	-1.01	4.38E-04	4.13E-03
Psed_6322	Peptide methionine sulfoxide reductase msrA	-1.01	1.16E-04	1.51E-03
Psed_2123	Peptidoglycan-binding lysin domain	-1.01	4.11E-06	1.37E-04
Psed_7016	hypothetical protein	-1.01	7.23E-05	1.07E-03
Psed_4449	DNA primase	-1.01	6.20E-05	9.52E-04
Psed_0881	Protein of unknown function DUF2277	-1.01	7.12E-07	4.05E-05
Psed_6645	hypothetical protein	-1.01	6.92E-05	1.04E-03
Psed_2789	hypothetical protein	-1.02	2.80E-05	5.50E-04
Psed_0565	Heavy metal transport/detoxification protein	-1.02	1.09E-03	8.21E-03
Psed_2430	3-oxoacyl-[acyl-carrier-protein] reductase	-1.02	1.00E-06	4.91E-05
Psed_5316	hypothetical protein	-1.03	1.50E-04	1.81E-03
Psed_1420	hypothetical protein	-1.03	3.64E-05	6.44E-04
Psed_5567	NAD(P)H-quinone oxidoreductase subunit J	-1.03	3.84E-04	3.76E-03
Psed_1457	alpha/beta hydrolase fold	-1.03	6.64E-07	3.86E-05
Psed_2809	cell division protein FtsZ	-1.03	3.31E-04	3.33E-03
Psed_1675	glycosyl transferase family 4	-1.03	2.77E-07	2.12E-05
Psed_1684	ATP synthase gamma chain	-1.03	7.27E-04	6.05E-03
Psed_5793	hypothetical protein	-1.04	7.24E-04	6.03E-03
Psed_5110	Xenobiotic-transporting ATPase., Peptide-transporting ATPase	-1.04	1.78E-05	3.97E-04
Psed_2888	1-deoxy-D-xylulose-5-phosphate synthase	-1.04	7.45E-05	1.09E-03
Psed_0390	hypothetical protein	-1.04	9.59E-04	7.43E-03
Psed_3161	protein of unknown function DUF151	-1.04	4.91E-04	4.50E-03
Psed_0080	ABC-type transporter, integral membrane subunit	-1.04	4.52E-07	2.99E-05

Appendix 5. Genes differentially expressed on 1,4-dioxane relative to pyruvate.

Psed_5565	NADH dehydrogenase (quinone)	-1.04	1.09E-04	1.45E-03
Psed_0958	Fumarate hydratase class II	-1.05	9.82E-06	2.52E-04
Psed_6508	hypothetical protein	-1.05	1.36E-05	3.23E-04
Psed_5616	Porphobilinogen synthase	-1.05	1.24E-06	5.61E-05
Psed_5080	putative transcriptional regulator	-1.05	2.88E-05	5.61E-04
Psed_2978	RNA polymerase sigma factor, sigma-70 family	-1.05	9.65E-05	1.34E-03
Psed_4870	penicillin-binding protein transpeptidase	-1.05	5.98E-04	5.26E-03
Psed_1712	DivIVA domain	-1.06	9.32E-07	4.78E-05
Psed_4409	methylmalonyl-CoA mutase, large subunit	-1.06	1.32E-04	1.65E-03
Psed_2294	hypothetical protein	-1.06	6.05E-04	5.29E-03
Psed_4570	putative F420-dependent oxidoreductase	-1.07	2.75E-05	5.48E-04
Psed_5312	isocitrate dehydrogenase, NADP-dependent	-1.07	1.58E-04	1.89E-03
Psed_5920	oxidoreductase molybdopterin binding	-1.07	6.50E-04	5.61E-03
Psed_6509	endonuclease III	-1.07	1.11E-03	8.33E-03
Psed_5913	5-oxoprolinase (ATP-hydrolyzing)	-1.08	9.86E-07	4.91E-05
Psed_6523	fructose-bisphosphate aldolase, class II	-1.08	7.13E-04	5.96E-03
Psed_2725	Cobyrinic acid A,C-diamide synthase	-1.08	3.87E-07	2.72E-05
Psed_1024	protein of unknown function DUF1470	-1.08	1.37E-05	3.24E-04
Psed_5194	hypothetical protein	-1.08	8.39E-05	1.20E-03
Psed_2831	DNA polymerase III, alpha subunit	-1.08	3.67E-05	6.47E-04
Psed_2351	helix-turn-helix domain protein	-1.08	9.78E-06	2.52E-04
Psed_5640	2,3-bisphosphoglycerate-dependent phosphoglycerate mutase	-1.08	1.72E-04	2.01E-03
Psed_3667	SSS sodium solute transporter superfamily	-1.08	3.71E-04	3.66E-03
Psed_5215	cell envelope-related function transcriptional attenuator, LytR/CpsA family	-1.08	4.45E-05	7.40E-04
Psed_3614	hypothetical protein	-1.10	9.20E-06	2.41E-04
Psed_4845	gluconate kinase	-1.10	3.54E-04	3.52E-03
Psed_2690	DSBA oxidoreductase	-1.10	2.12E-05	4.55E-04
Psed_0325	Glyoxalase/bleomycin resistance protein/dioxygenase	-1.11	6.20E-04	5.38E-03
Psed_2654	Carbon-monoxide dehydrogenase (acceptor)	-1.12	6.90E-08	6.71E-06

Appendix 5. Genes differentially expressed on 1,4-dioxane relative to pyruvate.

Psed_2352	methionine aminopeptidase, type I	-1.12	1.06E-03	8.04E-03
Psed_1320	hypothetical protein	-1.12	1.58E-09	3.95E-07
Psed_1685	ATP synthase subunit beta	-1.12	9.12E-04	7.12E-03
Psed_2282	protein of unknown function UPF0089	-1.13	3.14E-04	3.21E-03
Psed_2655	hypothetical protein	-1.13	1.17E-08	1.52E-06
Psed_1851	Heparinase II/III family protein	-1.13	1.17E-06	5.45E-05
Psed_6289	hypothetical protein	-1.13	5.11E-07	3.20E-05
Psed_2143	alkyl hydroperoxide reductase/ Thiol specific antioxidant/ Mal allergen	-1.14	6.87E-04	5.80E-03
Psed_5563	NADH-quinone oxidoreductase, chain G	-1.14	1.49E-04	1.80E-03
Psed_0967	RmuC-domain protein	-1.15	1.53E-06	6.58E-05
Psed_2502	hypothetical protein	-1.15	3.23E-05	6.07E-04
Psed_5019	protein of unknown function DUF1707	-1.15	6.88E-05	1.03E-03
Psed_3038	Sulfate-transporting ATPase	-1.15	3.94E-06	1.35E-04
Psed_2122	LexA repressor	-1.16	4.57E-04	4.25E-03
Psed_1447	Phosphoribosylaminoimidazolecarboxamide formyltransferase	-1.16	3.05E-07	2.26E-05
Psed_3308	dihydrolipoamide dehydrogenase	-1.16	3.59E-04	3.57E-03
Psed_3615	hypothetical protein	-1.16	5.13E-04	4.66E-03
Psed_5781	hypothetical protein	-1.17	7.72E-04	6.35E-03
Psed_4801	globin	-1.17	1.17E-04	1.51E-03
Psed_3658	RNA polymerase sigma-70 factor	-1.18	3.10E-09	6.12E-07
Psed_2641	ATPase associated with various cellular activities AAA_5	-1.18	3.95E-06	1.35E-04
Psed_2763	hypothetical protein	-1.18	1.02E-05	2.57E-04
Psed_5826	L-2,4-diaminobutyric acid acetyltransferase	-1.19	3.28E-06	1.19E-04
Psed_1654	hypothetical protein	-1.19	5.15E-06	1.61E-04
Psed_3926	protein of unknown function DUF1707	-1.19	4.26E-04	4.05E-03
Psed_1844	exopolysaccharide biosynthesis polyprenyl glycosylphosphotransferase	-1.20	8.88E-06	2.37E-04
Psed_3668	hypothetical protein	-1.20	5.43E-05	8.60E-04
Psed_1762	Carbon-monoxide dehydrogenase (acceptor)	-1.20	1.63E-04	1.93E-03
Psed_1769	xanthine permease	-1.20	1.09E-04	1.45E-03
Psed_5264	Thiosulfate sulfurtransferase	-1.20	1.87E-05	4.13E-04
Psed_2875	Malate dehydrogenase	-1.20	2.74E-05	5.48E-04
Psed_5796	hypothetical protein	-1.20	9.10E-04	7.11E-03
Psed_1686	ATP synthase epsilon chain	-1.22	7.16E-06	2.04E-04

Appendix 5. Genes differentially expressed on 1,4-dioxane relative to pyruvate.

Psed_2499	Carbon-monoxide dehydrogenase (acceptor)	-1.22	1.39E-06	6.09E-05
Psed_2650	hypothetical protein	-1.22	1.52E-07	1.30E-05
Psed_1761	S-adenosylhomocysteine deaminase	-1.23	3.10E-05	5.93E-04
Psed_1828	monooxygenase FAD-binding	-1.23	3.27E-07	2.39E-05
Psed_1899	Aldehyde Dehydrogenase	-1.23	5.83E-07	3.50E-05
Psed_2995	monosaccharide-transporting ATPase	-1.23	2.08E-05	4.47E-04
Psed_1166	hypothetical protein	-1.24	1.01E-06	4.91E-05
Psed_6599	N-acetylmuramoyl-L-alanine amidase family 2	-1.24	4.95E-06	1.58E-04
Psed_0459	transcriptional regulator domain-containing protein	-1.24	2.25E-05	4.73E-04
Psed_4709	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase	-1.26	1.17E-06	5.45E-05
Psed_1633	hypothetical protein	-1.26	4.15E-05	7.08E-04
Psed_4983	Aminocarboxymuconate-semialdehyde decarboxylase	-1.26	3.96E-06	1.35E-04
Psed_0886	hypothetical protein	-1.27	1.21E-04	1.54E-03
Psed_5562	NADH dehydrogenase (quinone)	-1.27	2.55E-04	2.74E-03
Psed_6640	extracellular solute-binding protein family 1	-1.27	5.55E-04	4.97E-03
Psed_5185	Thymidylate kinase	-1.28	4.47E-04	4.20E-03
Psed_1656	hypothetical protein	-1.28	8.92E-07	4.69E-05
Psed_4110	MmgE/PrpD family protein	-1.29	4.81E-05	7.86E-04
Psed_1657	Cobalt transporter subunit CbtB putative	-1.29	7.24E-06	2.05E-04
Psed_3713	helix-turn-helix domain-containing protein AraC type	-1.29	7.44E-10	2.41E-07
Psed_2432	helix-turn-helix domain protein	-1.29	6.05E-04	5.29E-03
Psed_1858	polysaccharide biosynthesis protein	-1.30	3.03E-05	5.84E-04
Psed_5465	Aspartate transaminase	-1.31	3.45E-05	6.29E-04
Psed_1674	Glycine hydroxymethyltransferase	-1.33	1.00E-03	7.72E-03
Psed_0953	PhoH family protein	-1.33	3.61E-05	6.43E-04
Psed_0560	hypothetical protein	-1.34	3.85E-04	3.76E-03
Psed_3377	aconitate hydratase 1	-1.35	2.10E-04	2.36E-03
Psed_2649	hypothetical protein	-1.35	4.56E-05	7.49E-04
Psed_2812	Cell division protein sepF	-1.35	6.24E-05	9.56E-04
Psed_5484	peptidase S15	-1.35	4.32E-07	2.93E-05
Psed_4008	hypothetical protein	-1.36	4.18E-05	7.11E-04

Appendix 5. Genes differentially expressed on 1,4-dioxane relative to pyruvate.

Psed_5557	proton-translocating NADH-quinone oxidoreductase, chain M	-1.36	6.54E-04	5.61E-03
Psed_2973	dihydroxyacetone kinase, L subunit	-1.36	1.13E-08	1.50E-06
Psed_1116	transcription factor WhiB	-1.38	2.37E-07	1.94E-05
Psed_0382	hypothetical protein	-1.39	2.38E-04	2.60E-03
Psed_6565	protein of unknown function DUF224 cysteine-rich region domain protein	-1.39	9.96E-06	2.54E-04
Psed_3863	hypothetical protein	-1.39	9.06E-06	2.39E-04
Psed_1492	Alcohol dehydrogenase zinc-binding domain protein	-1.40	1.48E-04	1.79E-03
Psed_0389	hypothetical protein	-1.41	1.18E-03	8.72E-03
Psed_4532	band 7 protein	-1.41	8.46E-05	1.21E-03
Psed_6619	Phosphoenolpyruvate carboxykinase [GTP]	-1.41	5.59E-08	5.69E-06
Psed_4302	nitrate reductase molybdenum cofactor assembly chaperone	-1.42	1.08E-04	1.45E-03
Psed_6641	ABC-type transporter, integral membrane subunit	-1.42	3.31E-04	3.34E-03
Psed_3621	hypothetical protein	-1.42	5.31E-09	8.64E-07
Psed_1730	alpha amylase catalytic region	-1.42	2.67E-06	1.03E-04
Psed_4021	Bifunctional protein folD	-1.43	6.20E-05	9.52E-04
Psed_3995	ABC-type transporter, periplasmic subunit	-1.43	9.07E-07	4.69E-05
Psed_1771	Electron transfer flavoprotein alpha/beta-subunit	-1.46	4.50E-05	7.46E-04
Psed_1849	asparagine synthase (glutamine-hydrolyzing)	-1.46	5.27E-08	5.51E-06
Psed_0914	Luciferase-like, subgroup	-1.46	2.74E-05	5.48E-04
Psed_1060	magnesium and cobalt transport protein CorA	-1.47	1.03E-07	9.61E-06
Psed_5180	ribosomal subunit interface protein	-1.49	6.00E-05	9.35E-04
Psed_4872	hypothetical protein	-1.50	3.62E-05	6.43E-04
Psed_3727	Pyruvate dehydrogenase (acetyl-transferring)	-1.50	5.01E-06	1.59E-04
Psed_4299	major facilitator superfamily MFS_1	-1.51	5.54E-04	4.97E-03
Psed_1880	glycosyl transferase family 2	-1.51	1.35E-04	1.67E-03
Psed_3871	hypothetical protein	-1.51	2.08E-05	4.47E-04
Psed_0137	copper resistance D domain protein	-1.52	3.09E-06	1.14E-04
Psed_1857	glycosyl transferase family 2	-1.53	6.78E-06	1.99E-04
Psed_6304	FAD-dependent pyridine nucleotide-disulphide oxidoreductase	-1.53	2.79E-05	5.50E-04

Appendix 5. Genes differentially expressed on 1,4-dioxane relative to pyruvate.

Psed_1052	Glycerol-3-phosphate-transporting ATPase	-1.54	4.15E-05	7.08E-04
Psed_1847	lipopolysaccharide biosynthesis protein	-1.54	7.21E-07	4.05E-05
Psed_1054	1-aminocyclopropane-1-carboxylate deaminase	-1.56	5.32E-05	8.47E-04
Psed_4080	protein of unknown function DUF574	-1.56	4.47E-06	1.47E-04
Psed_1860	Glucose-1-phosphate cytidylyltransferase	-1.56	6.93E-06	2.00E-04
Psed_4860	hypothetical protein	-1.57	1.04E-03	7.88E-03
Psed_5186	NUDIX hydrolase	-1.57	3.41E-05	6.25E-04
Psed_1050	ABC-type transporter, integral membrane subunit	-1.58	5.96E-05	9.31E-04
Psed_3941	Glyoxalase/bleomycin resistance protein/dioxygenase	-1.59	4.52E-05	7.46E-04
Psed_5823	ectoine hydroxylase	-1.59	1.34E-03	9.62E-03
Psed_1468	Cupin 2 conserved barrel domain protein	-1.61	2.28E-09	4.79E-07
Psed_4176	GntR domain protein	-1.61	5.28E-06	1.63E-04
Psed_3121	ABC transporter, CydDC cysteine exporter (CydDC-E) family, permease/ATP-binding protein CydD	-1.62	1.13E-03	8.43E-03
Psed_5187	Adenosylhomocysteinase	-1.63	1.72E-05	3.85E-04
Psed_2941	regulatory protein MarR	-1.63	1.80E-06	7.43E-05
Psed_1562	hypothetical protein	-1.65	4.95E-05	8.03E-04
Psed_1117	hypothetical protein	-1.66	4.65E-07	3.00E-05
Psed_1848	nucleotide sugar dehydrogenase	-1.66	5.93E-08	5.94E-06
Psed_2498	Carbon-monoxide dehydrogenase (acceptor)	-1.66	1.58E-06	6.73E-05
Psed_1049	extracellular solute-binding protein family 1	-1.66	9.43E-06	2.46E-04
Psed_2291	pyridoxamine 5'-phosphate oxidase-related FMN-binding protein	-1.67	8.33E-07	4.48E-05
Psed_6628	hypothetical protein	-1.68	5.08E-05	8.19E-04
Psed_1770	hypothetical protein	-1.68	4.11E-07	2.84E-05
Psed_3037	ABC-type metal ion transporter, periplasmic subunit	-1.69	4.60E-07	3.00E-05
Psed_1051	ABC-type transporter, integral membrane subunit	-1.70	1.13E-04	1.49E-03
Psed_1422	Ferritin Dps family protein	-1.71	2.59E-07	2.08E-05
Psed_5825	diaminobutyrate/2-oxoglutarate aminotransferase	-1.71	1.36E-04	1.68E-03

Appendix 5. Genes differentially expressed on 1,4-dioxane relative to pyruvate.

Psed_4655	protein of unknown function DUF1778	-1.71	3.00E-07	2.25E-05
Psed_3724	3-oxoacyl-[acyl-carrier-protein] reductase	-1.72	4.13E-05	7.07E-04
Psed_5789	hypothetical protein	-1.73	1.06E-04	1.43E-03
Psed_3278	LmbE family protein	-1.73	7.40E-06	2.07E-04
Psed_3591	hypothetical protein	-1.74	1.38E-07	1.22E-05
Psed_3720	amidohydrolase 2	-1.75	6.55E-07	3.86E-05
Psed_3717	Transcriptional regulator IclR	-1.76	8.76E-07	4.67E-05
Psed_2972	dihydroxyacetone kinase, DhaK subunit	-1.78	1.20E-07	1.10E-05
Psed_2283	UspA domain-containing protein	-1.81	3.06E-04	3.15E-03
Psed_2434	GAF domain protein	-1.82	1.58E-06	6.73E-05
Psed_2640	hypothetical protein	-1.83	2.92E-08	3.39E-06
Psed_5111	o-succinylbenzoate--CoA ligase	-1.85	7.04E-06	2.02E-04
Psed_0140	hypothetical protein	-1.85	6.49E-06	1.94E-04
Psed_1772	Electron transfer flavoprotein alpha subunit	-1.85	3.62E-06	1.27E-04
Psed_4688	polysaccharide deacetylase	-1.86	5.79E-09	9.20E-07
Psed_3730	Carboxymuconolactone decarboxylase	-1.87	3.70E-05	6.50E-04
Psed_1763	molybdopterin dehydrogenase FAD-binding	-1.89	3.17E-06	1.17E-04
Psed_5788	peptidase S14 ClpP	-1.89	8.16E-07	4.43E-05
Psed_4300	nitrate reductase, alpha subunit	-1.90	7.97E-06	2.17E-04
Psed_4976	NADP oxidoreductase coenzyme F420-dependent	-1.92	1.98E-07	1.65E-05
Psed_4849	heavy metal translocating P-type ATPase	-1.93	5.59E-04	4.99E-03
Psed_1421	ferric-uptake regulator	-1.93	1.30E-06	5.79E-05
Psed_3725	Extracellular ligand-binding receptor	-1.94	2.87E-06	1.08E-04
Psed_5922	Carboxylesterase	-1.95	3.97E-05	6.84E-04
Psed_3723	oxidoreductase domain protein	-1.95	1.18E-06	5.45E-05
Psed_4847	CBS domain containing protein	-1.97	1.19E-04	1.53E-03
Psed_4842	UspA domain-containing protein	-1.97	2.54E-04	2.74E-03
Psed_3120	ABC transporter, CydDC cysteine exporter (CydDC-E) family, permease/ ATP-binding protein CydC	-1.98	3.15E-04	3.22E-03
Psed_6301	dienelactone hydrolase	-2.00	5.26E-07	3.26E-05
Psed_2318	regulatory protein MerR	-2.00	9.65E-07	4.90E-05
Psed_2299	hypothetical protein	-2.01	4.64E-06	1.51E-04
Psed_3721	alpha/beta hydrolase fold	-2.02	5.86E-06	1.76E-04

Appendix 5. Genes differentially expressed on 1,4-dioxane relative to pyruvate.

Psed_5795	hypothetical protein	-2.03	2.48E-06	9.62E-05
Psed_3729	3-oxoacyl-[acyl-carrier-protein] reductase	-2.03	3.51E-06	1.25E-04
Psed_3602	small GTP-binding protein	-2.04	1.17E-05	2.90E-04
Psed_4984	isochorismatase hydrolase	-2.04	4.22E-08	4.66E-06
Psed_3589	stress protein	-2.04	1.99E-08	2.40E-06
Psed_4861	cell shape determining protein MreB/Mrl	-2.05	3.35E-06	1.21E-04
Psed_4303	respiratory nitrate reductase, gamma subunit	-2.07	2.41E-05	5.00E-04
Psed_5872	hypothetical protein	-2.08	2.68E-07	2.10E-05
Psed_5794	hypothetical protein	-2.08	2.38E-07	1.94E-05
Psed_4874	Glyoxalase/bleomycin resistance protein/dioxygenase	-2.08	1.14E-04	1.49E-03
Psed_5791	hypothetical protein	-2.10	6.24E-09	9.67E-07
Psed_3594	Tellurite resistance TerB	-2.11	9.09E-06	2.39E-04
Psed_3731	Phosphonate-transporting ATPase	-2.12	1.75E-06	7.25E-05
Psed_3728	Pyruvate dehydrogenase (acetyl-transferring)	-2.12	1.03E-06	4.97E-05
Psed_2297	hypothetical protein	-2.12	3.65E-04	3.61E-03
Psed_2298	UspA domain-containing protein	-2.12	3.00E-04	3.11E-03
Psed_3718	3-oxoacyl-[acyl-carrier-protein] reductase	-2.16	4.81E-08	5.13E-06
Psed_3726	Xylose isomerase domain-containing protein TIM barrel	-2.16	2.38E-05	4.95E-04
Psed_3047	hypothetical protein	-2.20	5.64E-04	5.01E-03
Psed_2290	hypothetical protein	-2.20	1.30E-04	1.64E-03
Psed_4656	hypothetical protein	-2.21	6.52E-08	6.43E-06
Psed_2292	response regulator receiver	-2.21	5.29E-05	8.44E-04
Psed_1846	lipopolysaccharide biosynthesis protein	-2.25	1.81E-08	2.22E-06
Psed_2281	ABC transporter related	-2.25	1.32E-07	1.18E-05
Psed_0902	NADH dehydrogenase (ubiquinone)	-2.26	4.65E-09	7.96E-07
Psed_3722	hypothetical protein	-2.26	3.54E-07	2.56E-05
Psed_1852	lipoprotein	-2.32	8.02E-08	7.67E-06
Psed_3590	Tellurium resistance	-2.37	3.88E-07	2.72E-05
Psed_3719	cyclase family protein	-2.37	5.83E-07	3.50E-05
Psed_2289	hypothetical protein	-2.42	1.39E-06	6.09E-05
Psed_2286	hypothetical protein	-2.44	1.33E-04	1.66E-03
Psed_3733	ABC-type transporter, integral membrane subunit	-2.48	1.23E-05	3.01E-04

Appendix 5. Genes differentially expressed on 1,4-dioxane relative to pyruvate.

Psed_1023	hypothetical protein	-2.54	2.14E-09	4.76E-07
Psed_5790	hypothetical protein	-2.57	9.70E-07	4.90E-05
Psed_4843	UspA domain-containing protein	-2.58	9.63E-06	2.49E-04
Psed_4301	nitrate reductase, beta subunit	-2.62	4.80E-05	7.86E-04
Psed_4840	CBS domain containing protein	-2.63	1.02E-04	1.39E-03
Psed_0078	Methyltransferase type 12	-2.64	2.29E-11	1.49E-08
Psed_2436	nitroreductase	-2.64	1.36E-05	3.23E-04
Psed_0138	copper resistance protein CopC	-2.67	5.60E-07	3.44E-05
Psed_4839	phosphoketolase	-2.70	2.86E-04	3.01E-03
Psed_2296	hypothetical protein	-2.71	7.49E-04	6.18E-03
Psed_0079	ABC-type transporter, periplasmic subunit	-2.74	1.71E-09	4.12E-07
Psed_0139	nuclear export factor GLE1	-2.76	2.00E-06	8.09E-05
Psed_3732	Monosaccharide-transporting ATPase	-2.80	4.93E-07	3.11E-05
Psed_0312	Sulfate transporter/antisigma-factor antagonist STAS	-2.81	3.53E-09	6.57E-07
Psed_2295	Domain of unknown function DUF1918	-2.83	1.23E-06	5.61E-05
Psed_2437	CBS domain containing protein	-2.83	3.61E-05	6.43E-04
Psed_0276	major facilitator superfamily MFS_1	-2.83	3.15E-11	1.86E-08
Psed_4841	UspA domain-containing protein	-2.86	3.30E-05	6.14E-04
Psed_4837	hypothetical protein	-2.89	3.04E-04	3.13E-03
Psed_4844	glyceraldehyde-3-phosphate dehydrogenase, type I	-3.05	2.96E-05	5.73E-04
Psed_4873	Domain of unknown function DUF1876	-3.05	5.34E-06	1.64E-04
Psed_3734	ABC-type transporter, integral membrane subunit	-3.18	2.13E-06	8.45E-05
Psed_3670	Butyryl-CoA dehydrogenase	-3.27	3.40E-05	6.25E-04
Psed_4848	hypothetical protein	-3.53	5.81E-06	1.75E-04
Psed_5195	alanine dehydrogenase	-3.89	2.99E-09	6.09E-07
Psed_2433	DoxX family protein	-4.16	1.66E-05	3.73E-04

Appendix 5. Genes differentially expressed on 1,4-dioxane relative to pyruvate.

Appendix 6

Genes differentially expressed on glycolate relative to pyruvate.

Appendix 6. Genes differentially expressed on glycolate relative to pyruvate. Differentially expressed genes have an adjusted p-value < 0.01 and have a $log_2FC > 1$ or $log_2FC < -1$ relative to pyruvate and are sorted by log_2FC.

Genes	Protein	log_2FC	p-value	adjusted p-value
Psed_3889	2-hydroxy-3-oxopropionate reductase	7.02	2.05E-15	6.23E-12
Psed_3888	Hydroxypyruvate isomerase	6.89	2.87E-15	6.23E-12
Psed_0089	SSS sodium solute transporter superfamily	6.87	8.28E-15	1.08E-11
Psed_3890	glyoxylate carboligase	6.83	5.24E-16	3.41E-12
Psed_0088	protein of unknown function DUF485	6.41	4.55E-15	7.40E-12
Psed_3891	glycerate kinase	5.81	1.01E-14	1.09E-11
Psed_4788	D-lactate dehydrogenase (cytochrome)	5.46	1.44E-14	1.34E-11
Psed_4790	protein of unknown function DUF224 cysteine-rich region domain protein	4.88	2.15E-10	5.83E-08
Psed_4789	FAD linked oxidase domain protein	4.61	2.51E-14	2.04E-11
Psed_0090	hypothetical protein	3.93	6.12E-13	4.19E-10
Psed_2680	Cysteine desulfurase	3.92	8.01E-12	4.35E-09
Psed_2554	Xenobiotic-transporting ATPase	3.78	1.33E-10	3.78E-08
Psed_0350	Bile acid:sodium symporter	3.74	2.85E-09	4.32E-07
Psed_6787	Formyl-CoA transferase	3.68	5.41E-10	1.22E-07
Psed_5371	hypothetical protein	3.66	9.62E-06	1.70E-04
Psed_1302	protein of unknown function DUF156	3.53	2.25E-07	8.82E-06
Psed_4782	Malate synthase	3.51	8.11E-11	2.40E-08
Psed_6665	NLP/P60 protein	3.45	7.39E-08	4.11E-06
Psed_4755	ammonium transporter	3.29	1.56E-08	1.27E-06
Psed_1304	heavy metal translocating P-type ATPase	3.23	1.89E-07	7.92E-06
Psed_6780	hypothetical protein	3.12	4.07E-11	1.46E-08
Psed_5782	Collagen triple helix repeat-containing protein	3.09	1.53E-07	6.94E-06
Psed_5736	Transglycosylase-like domain protein	2.98	1.95E-07	7.99E-06
Psed_1303	Heavy metal transport/detoxification protein	2.95	4.94E-09	5.97E-07
Psed_0554	hypothetical protein	2.93	1.76E-05	2.80E-04
Psed_5795	hypothetical protein	2.92	7.10E-08	4.08E-06
Psed_5801	hypothetical protein	2.84	8.43E-08	4.42E-06
Psed_3486	hypothetical protein	2.79	1.50E-07	6.92E-06
Psed_5804	hypothetical protein	2.77	3.09E-07	1.12E-05
Psed_5799	hypothetical protein	2.76	3.30E-09	4.77E-07

Psed_5796	hypothetical protein	2.72	4.20E-07	1.37E-05
Psed_5798	hypothetical protein	2.70	1.87E-07	7.90E-06
Psed_5787	hypothetical protein	2.64	6.60E-09	7.16E-07
Psed_3669	hypothetical protein	2.61	2.15E-08	1.59E-06
Psed_5815	hypothetical protein	2.52	9.67E-07	2.66E-05
Psed_5794	hypothetical protein	2.50	5.48E-08	3.29E-06
Psed_3487	heat shock protein Hsp20	2.47	1.91E-07	7.94E-06
Psed_3108	hypothetical protein	2.47	8.68E-06	1.56E-04
Psed_2011	threonine synthase	2.47	1.13E-11	5.67E-09
Psed_5783	hypothetical protein	2.45	3.46E-06	7.28E-05
Psed_3109	hypothetical protein	2.44	1.12E-06	2.95E-05
Psed_6779	Integrase catalytic region	2.44	2.26E-10	5.89E-08
Psed_5784	hypothetical protein	2.42	3.49E-08	2.34E-06
Psed_3485	Chaperone protein dnaK	2.42	1.31E-07	6.22E-06
Psed_5811	hypothetical protein	2.38	3.47E-07	1.23E-05
Psed_5788	peptidase S14 ClpP	2.34	1.40E-07	6.57E-06
Psed_5192	Uncharacterised protein family UPF0324	2.34	2.87E-07	1.07E-05
Psed_5792	hypothetical protein	2.33	9.93E-06	1.74E-04
Psed_3451	Domain of unknown function DUF1931	2.29	1.21E-07	5.90E-06
Psed_3497	transport-associated	2.29	2.69E-09	4.27E-07
Psed_5791	hypothetical protein	2.29	4.72E-09	5.97E-07
Psed_5797	hypothetical protein	2.28	6.99E-07	2.07E-05
Psed_3496	hypothetical protein	2.27	6.83E-08	3.97E-06
Psed_5793	hypothetical protein	2.27	4.46E-07	1.42E-05
Psed_2679	NADH dehydrogenase (ubiquinone)	2.25	1.37E-08	1.18E-06
Psed_3460	formaldehyde dehydrogenase, glutathi-one-independent	2.24	4.51E-08	2.85E-06
Psed_6782	Hydroxyacid-oxoacid transhydroge-nase	2.24	1.39E-07	6.54E-06
Psed_5786	phage terminase	2.20	2.94E-06	6.42E-05
Psed_5789	hypothetical protein	2.19	1.94E-05	3.02E-04
Psed_2386	cyclase/dehydrase	2.18	1.74E-07	7.55E-06
Psed_4754	nitrogen regulatory protein P-II	2.17	1.83E-07	7.80E-06
Psed_3508	intracellular protease, PfpI family	2.13	1.63E-07	7.30E-06
Psed_5358	transcription factor WhiB	2.13	2.17E-07	8.71E-06
Psed_5814	hypothetical protein	2.11	3.37E-05	4.77E-04
Psed_3484	Protein grpE	2.10	2.69E-07	1.01E-05
Psed_5790	hypothetical protein	2.10	1.98E-05	3.06E-04

Appendix 6. Genes differentially expressed on glycolate relative to pyruvate.

Psed_5808	hypothetical protein	2.04	1.47E-05	2.41E-04
Psed_3446	Ferritin Dps family protein	2.03	5.51E-08	3.29E-06
Psed_3453	hypothetical protein	2.03	5.89E-07	1.78E-05
Psed_5800	hypothetical protein	1.99	1.90E-07	7.93E-06
Psed_6175	regulatory protein TetR	1.98	7.64E-06	1.41E-04
Psed_6547	ATP-dependent chaperone ClpB	1.97	3.72E-08	2.45E-06
Psed_2385	hypothetical protein	1.97	9.82E-07	2.68E-05
Psed_3858	protein of unknown function DUF6 transmembrane	1.96	1.25E-07	5.98E-06
Psed_3463	hypothetical protein	1.96	8.94E-07	2.50E-05
Psed_5458	hypothetical protein	1.96	1.70E-07	7.49E-06
Psed_6784	Alkylglycerone-phosphate synthase	1.96	2.04E-08	1.53E-06
Psed_1113	glutaredoxin-like protein	1.95	2.77E-08	1.96E-06
Psed_3522	response regulator receiver	1.93	4.50E-06	9.02E-05
Psed_0091	regulatory protein MerR	1.93	3.05E-09	4.51E-07
Psed_4577	Cold-shock protein DNA-binding	1.93	1.70E-04	1.82E-03
Psed_3483	chaperone DnaJ domain protein	1.91	1.14E-07	5.58E-06
Psed_1927	hypothetical protein	1.88	2.26E-07	8.82E-06
Psed_2271	GCN5-related N-acetyltransferase	1.86	3.27E-07	1.16E-05
Psed_1584	6-phosphofructokinase	1.85	6.30E-09	6.95E-07
Psed_5025	4-hydroxyacetophenone monooxygenase	1.82	8.89E-04	6.73E-03
Psed_6855	beta-lactamase domain protein	1.79	1.70E-07	7.49E-06
Psed_4578	Stearoyl-CoA 9-desaturase	1.78	4.56E-05	6.09E-04
Psed_5809	hypothetical protein	1.77	6.58E-06	1.24E-04
Psed_2370	hypothetical protein	1.77	2.25E-05	3.37E-04
Psed_6857	protein of unknown function DUF156	1.76	1.25E-07	5.98E-06
Psed_0338	hypothetical protein	1.75	1.59E-06	3.85E-05
Psed_5813	hypothetical protein	1.74	3.31E-05	4.69E-04
Psed_0903	hypothetical protein	1.73	3.56E-05	5.00E-04
Psed_5803	hypothetical protein	1.71	3.12E-06	6.80E-05
Psed_6600	acyl-CoA dehydrogenase domain-containing protein	1.70	7.55E-04	5.90E-03
Psed_2869	pyruvate kinase	1.69	1.18E-05	2.00E-04
Psed_5810	hypothetical protein	1.68	4.79E-05	6.34E-04
Psed_2372	ABC-type transporter, integral membrane subunit	1.66	7.93E-05	9.69E-04
Psed_3481	ATP-dependent chaperone ClpB	1.63	4.93E-05	6.46E-04
Psed_3500	glucose-6-phosphate 1-dehydrogenase	1.62	1.22E-05	2.06E-04

Appendix 6. Genes differentially expressed on glycolate relative to pyruvate.

Psed_4576	regulatory protein TetR	1.61	5.57E-06	1.07E-04
Psed_5812	hypothetical protein	1.60	1.47E-04	1.62E-03
Psed_5785	hypothetical protein	1.60	2.86E-06	6.28E-05
Psed_2660	regulatory protein TetR	1.59	1.96E-08	1.50E-06
Psed_6552	regulatory protein MerR	1.58	2.65E-05	3.92E-04
Psed_2630	Xenobiotic-transporting ATPase	1.58	1.83E-07	7.80E-06
Psed_2191	FMN-dependent oxidoreductase, nitrilotriacetate monooxygenase family	1.57	1.31E-05	2.17E-04
Psed_0388	Acetylcholinesterase	1.56	6.69E-06	1.26E-04
Psed_4548	GCN5-related N-acetyltransferase	1.54	7.97E-05	9.70E-04
Psed_2080	GCN5-related N-acetyltransferase	1.54	1.03E-05	1.79E-04
Psed_4106	Catalase	1.54	1.48E-05	2.41E-04
Psed_3840	tRNA/rRNA methyltransferase (SpoU)	1.50	2.82E-05	4.12E-04
Psed_6812	hypothetical protein	1.49	2.86E-07	1.07E-05
Psed_5802	hypothetical protein	1.49	3.63E-05	5.08E-04
Psed_4512	ABC-type transporter, integral membrane subunit	1.47	6.37E-04	5.19E-03
Psed_4333	UBA/THIF-type NAD/FAD binding protein	1.47	4.29E-04	3.81E-03
Psed_4753	nitrogen regulatory protein P-II	1.47	1.54E-06	3.77E-05
Psed_2371	ABC-type transporter, periplasmic subunit	1.47	6.68E-05	8.38E-04
Psed_2553	Glycine dehydrogenase [decarboxylating]	1.46	1.16E-09	2.23E-07
Psed_6506	major facilitator superfamily MFS_1	1.44	6.54E-08	3.84E-06
Psed_3964	Lysine exporter protein (LYSE/YGGA)	1.43	5.86E-06	1.12E-04
Psed_3456	Thioredoxin-disulfide reductase	1.41	1.89E-06	4.41E-05
Psed_5026	regulatory protein TetR	1.41	1.05E-05	1.82E-04
Psed_2631	DNA topoisomerase IB	1.40	1.58E-04	1.71E-03
Psed_0311	regulatory protein TetR	1.40	9.41E-06	1.67E-04
Psed_2550	Glycine hydroxymethyltransferase	1.39	8.94E-09	8.95E-07
Psed_4031	iron-sulfur cluster binding protein	1.39	5.41E-06	1.05E-04
Psed_5747	hypothetical protein	1.36	3.80E-07	1.28E-05
Psed_4689	RIO-like kinase	1.35	4.64E-08	2.91E-06
Psed_5805	hypothetical protein	1.35	1.13E-03	8.10E-03
Psed_4332	hypothetical protein	1.31	4.95E-04	4.26E-03
Psed_6811	type IV secretory pathway VirD4 protein-like protein	1.31	1.76E-04	1.86E-03
Psed_5748	hypothetical protein	1.31	6.31E-08	3.73E-06

Appendix 6. Genes differentially expressed on glycolate relative to pyruvate.

Psed_2978	RNA polymerase sigma factor, sigma-70 family	1.31	2.11E-05	3.20E-04
Psed_3513	Helix-turn-helix, AraC domain	1.30	2.46E-04	2.47E-03
Psed_6853	protein of unknown function DUF302	1.30	4.14E-09	5.61E-07
Psed_2030	Linalool 8-monooxygenase	1.29	5.52E-05	7.13E-04
Psed_5758	hypothetical protein	1.29	1.02E-04	1.20E-03
Psed_6553	Chaperone protein dnaJ	1.26	6.73E-05	8.43E-04
Psed_5520	hypothetical protein	1.26	1.11E-03	8.01E-03
Psed_3482	hypothetical protein	1.26	3.85E-04	3.50E-03
Psed_4513	cobalamin (vitamin B12) biosynthesis CbiX protein	1.25	4.03E-05	5.55E-04
Psed_3501	hypothetical protein	1.24	1.77E-05	2.80E-04
Psed_2661	drug resistance transporter, EmrB/QacA subfamily	1.24	9.54E-04	7.10E-03
Psed_6791	IstB domain protein ATP-binding protein	1.24	1.28E-08	1.13E-06
Psed_2388	Gas vesicle synthesis GvpLGvpF	1.24	1.23E-04	1.41E-03
Psed_0473	hypothetical protein	1.24	4.38E-09	5.76E-07
Psed_3472	protein of unknown function (DUF461)	1.23	3.47E-06	7.29E-05
Psed_5044	isochorismatase hydrolase	1.23	7.96E-04	6.14E-03
Psed_6854	protein of unknown function DUF81	1.22	1.49E-06	3.65E-05
Psed_3473	Pyruvate dehydrogenase (cytochrome)	1.22	1.87E-04	1.96E-03
Psed_2552	Aminomethyltransferase	1.21	7.18E-08	4.08E-06
Psed_6672	tRNA adenylyltransferase	1.20	4.91E-06	9.67E-05
Psed_3448	hypothetical protein	1.19	1.18E-06	3.02E-05
Psed_5763	hypothetical protein	1.19	8.25E-05	9.93E-04
Psed_3388	ABC transporter related	1.18	1.62E-05	2.62E-04
Psed_5404	NAD(P)(+) transhydrogenase (AB-specific)	1.18	8.19E-06	1.50E-04
Psed_5374	hypothetical protein	1.18	5.10E-04	4.37E-03
Psed_6852	Protein of unknown function DUF2078, membrane	1.18	1.24E-06	3.16E-05
Psed_2549	L-serine dehydratase 1	1.17	1.10E-05	1.88E-04
Psed_3461	transport-associated	1.17	2.68E-04	2.64E-03
Psed_4051	hypothetical protein	1.17	3.63E-07	1.25E-05
Psed_4549	Stearoyl-CoA 9-desaturase	1.17	7.27E-08	4.08E-06
Psed_2373	ABC-type transporter, integral membrane subunit	1.16	4.07E-05	5.57E-04
Psed_4662	processing peptidase	1.14	3.17E-05	4.53E-04

Appendix 6. Genes differentially expressed on glycolate relative to pyruvate.

Psed_6813	hypothetical protein	1.14	2.64E-06	5.91E-05
Psed_4461	hypothetical protein	1.14	2.87E-05	4.16E-04
Psed_1644	6-phosphofructokinase	1.13	9.95E-05	1.17E-03
Psed_5756	hypothetical protein	1.13	2.76E-04	2.70E-03
Psed_5632	pyrroline-5-carboxylate reductase	1.12	7.44E-07	2.17E-05
Psed_4030	protein of unknown function DUF224 cysteine-rich region domain protein	1.12	2.42E-05	3.61E-04
Psed_2714	Catalase-peroxidase	1.11	7.62E-04	5.94E-03
Psed_2321	ABC-type sugar transport system periplasmic component-like	1.10	2.36E-04	2.39E-03
Psed_5405	nicotinamide nucleotide transhydrogenase alpha subunit 2 PntAB	1.10	8.85E-04	6.71E-03
Psed_0355	hypothetical protein	1.08	1.98E-06	4.60E-05
Psed_6856	Rhodanese-like protein	1.08	4.24E-05	5.73E-04
Psed_2190	ABC-type transporter, periplasmic subunit	1.08	1.43E-04	1.59E-03
Psed_5395	tRNA pseudouridine synthase A	1.07	2.25E-05	3.37E-04
Psed_3780	conserved hypothetical protein	1.07	1.88E-04	1.96E-03
Psed_3015	hypothetical protein	1.07	6.99E-05	8.70E-04
Psed_5749	hypothetical protein	1.06	6.88E-04	5.52E-03
Psed_5375	phosphoglucosamine mutase	1.05	7.49E-04	5.88E-03
Psed_3967	GCN5-related N-acetyltransferase	1.05	7.42E-05	9.15E-04
Psed_4401	DoxX family protein	1.05	3.76E-05	5.21E-04
Psed_5762	hypothetical protein	1.05	1.75E-05	2.78E-04
Psed_5320	thioredoxin	1.03	4.81E-05	6.35E-04
Psed_2823	Abortive infection protein	1.03	2.18E-07	8.73E-06
Psed_4601	regulatory protein TetR	1.00	5.94E-07	1.78E-05
Psed_3971	glutamate--cysteine ligase GCS2	-1.00	3.70E-04	3.40E-03
Psed_4593	succinate dehydrogenase or fumarate reductase, flavoprotein subunit	-1.00	3.46E-04	3.21E-03
Psed_4014	exodeoxyribonuclease V, gamma subunit	-1.00	4.15E-06	8.44E-05
Psed_1899	Aldehyde Dehydrogenase	-1.00	1.31E-05	2.17E-04
Psed_2640	hypothetical protein	-1.00	6.26E-05	7.92E-04
Psed_5911	Hydantoinase/oxoprolinase	-1.02	2.56E-05	3.79E-04
Psed_3972	nitroreductase	-1.02	1.45E-05	2.37E-04
Psed_1761	S-adenosylhomocysteine deaminase	-1.02	3.48E-04	3.23E-03
Psed_0459	transcriptional regulator domain-containing protein	-1.02	2.89E-04	2.80E-03
Psed_6999	hypothetical protein	-1.02	3.08E-05	4.42E-04

Appendix 6. Genes differentially expressed on glycolate relative to pyruvate.

Psed_6203	Luciferase-like, subgroup	-1.02	2.98E-05	4.31E-04
Psed_4594	succinate dehydrogenase (or fumarate reductase) cytochrome b subunit, b558 family	-1.03	2.84E-04	2.76E-03
Psed_5110	Xenobiotic-transporting ATPase., Peptide-transporting ATPase	-1.03	3.92E-05	5.40E-04
Psed_1320	hypothetical protein	-1.03	1.12E-08	1.00E-06
Psed_3951	hypothetical protein	-1.04	1.56E-04	1.70E-03
Psed_2649	hypothetical protein	-1.04	9.13E-04	6.86E-03
Psed_5223	putative sporulation-associated protein	-1.04	4.09E-06	8.35E-05
Psed_5541	helix-turn-helix domain-containing protein AraC type	-1.04	2.06E-06	4.74E-05
Psed_5912	Acetone carboxylase gamma subunit	-1.04	1.99E-07	8.10E-06
Psed_3308	dihydrolipoamide dehydrogenase	-1.05	1.44E-03	9.83E-03
Psed_3136	hydrolase	-1.06	1.44E-06	3.59E-05
Psed_3116	YbhB YbcL family protein	-1.06	1.23E-03	8.64E-03
Psed_0081	ABC-type transporter, integral membrane subunit	-1.06	1.02E-04	1.19E-03
Psed_4067	protein of unknown function DUF1470	-1.07	1.84E-05	2.88E-04
Psed_3906	Luciferase-like, subgroup	-1.07	4.37E-05	5.85E-04
Psed_4085	Glyoxalase/bleomycin resistance protein/dioxygenase	-1.08	3.53E-07	1.23E-05
Psed_1769	xanthine permease	-1.08	5.14E-04	4.38E-03
Psed_3962	polar amino acid ABC transporter, inner membrane subunit	-1.08	4.18E-05	5.68E-04
Psed_0546	alpha/beta hydrolase fold	-1.09	7.89E-07	2.26E-05
Psed_0022	hypothetical protein	-1.09	1.99E-09	3.33E-07
Psed_2871	OsmC family protein	-1.09	5.52E-07	1.69E-05
Psed_3961	ABC-type transporter, periplasmic subunit family 3	-1.10	7.91E-05	9.68E-04
Psed_7017	hypothetical protein	-1.10	5.31E-07	1.65E-05
Psed_1632	regulatory protein MarR	-1.11	4.26E-04	3.80E-03
Psed_1762	Carbon-monoxide dehydrogenase (acceptor)	-1.11	6.01E-04	4.94E-03
Psed_2789	hypothetical protein	-1.11	2.20E-05	3.30E-04
Psed_2101	S-adenosylmethionine synthase	-1.11	2.26E-06	5.15E-05
Psed_1951	hypothetical protein	-1.11	4.83E-08	2.99E-06
Psed_5856	alkylhydroperoxidase like protein, AhpD family	-1.12	4.06E-07	1.36E-05
Psed_0953	PhoH family protein	-1.12	3.69E-04	3.39E-03

Appendix 6. Genes differentially expressed on glycolate relative to pyruvate.

Psed_0325	Glyoxalase/bleomycin resistance protein/dioxygenase	-1.12	9.91E-04	7.31E-03
Psed_3102	3-oxoacyl-[acyl-carrier-protein] reductase	-1.12	1.02E-03	7.50E-03
Psed_7015	hypothetical protein	-1.12	2.93E-07	1.08E-05
Psed_2498	Carbon-monoxide dehydrogenase (acceptor)	-1.12	1.93E-04	2.01E-03
Psed_1086	Conserved hypothetical protein CHP02569	-1.13	3.13E-07	1.13E-05
Psed_4397	Polyketide cyclase/dehydrase	-1.13	2.05E-05	3.12E-04
Psed_6994	Cobyrinic acid ac-diamide synthase	-1.13	1.74E-04	1.85E-03
Psed_0082	Monosaccharide-transporting ATPase	-1.13	5.80E-07	1.76E-05
Psed_5267	protein of unknown function DUF692	-1.13	2.42E-04	2.44E-03
Psed_0545	Cna B domain protein	-1.13	1.64E-07	7.30E-06
Psed_2798	Protein mraZ	-1.14	4.84E-06	9.54E-05
Psed_6302	hypothetical protein	-1.14	3.47E-05	4.89E-04
Psed_0233	putative transglycosylase associated protein	-1.14	1.53E-05	2.49E-04
Psed_6578	hypothetical protein	-1.14	7.41E-07	2.17E-05
Psed_6565	protein of unknown function DUF224 cysteine-rich region domain protein	-1.15	1.40E-04	1.57E-03
Psed_5460	Alpha-methylacyl-CoA racemase	-1.15	2.00E-04	2.07E-03
Psed_4021	Bifunctional protein folD	-1.15	8.12E-04	6.23E-03
Psed_3162	regulatory protein MerR	-1.15	1.13E-03	8.09E-03
Psed_2907	hypothetical protein	-1.16	4.72E-04	4.10E-03
Psed_1447	Phosphoribosylaminoimidazolecarboxamide formyltransferase	-1.16	7.22E-07	2.13E-05
Psed_5874	ErfK/YbiS/YcfS/YnhG family protein	-1.16	4.42E-07	1.42E-05
Psed_1710	3-hydroxyacyl-CoA dehydrogenase., 3-hydroxybutyryl-CoA dehydrogenase	-1.16	7.25E-04	5.74E-03
Psed_0246	ABC-type transporter, integral membrane subunit	-1.16	7.24E-05	8.96E-04
Psed_1259	hypothetical protein	-1.16	1.03E-04	1.20E-03
Psed_6693	hypothetical protein	-1.16	4.48E-06	9.01E-05
Psed_2910	protein of unknown function DUF322	-1.16	4.31E-04	3.83E-03
Psed_2125	ribonucleoside-diphosphate reductase, adenosylcobalamin-dependent	-1.16	1.46E-04	1.62E-03
Psed_0550	Rhodanese-like protein	-1.16	1.82E-07	7.80E-06
Psed_1721	hypothetical protein	-1.17	1.48E-06	3.65E-05
Psed_3387	transcriptional regulator	-1.17	1.06E-06	2.82E-05

Appendix 6. Genes differentially expressed on glycolate relative to pyruvate.

Psed_3635	GTP cyclohydrolase 1	-1.17	2.01E-04	2.07E-03
Psed_1858	polysaccharide biosynthesis protein	-1.18	1.52E-04	1.67E-03
Psed_5925	cation diffusion facilitator family transporter	-1.18	9.69E-06	1.71E-04
Psed_3590	Tellurium resistance	-1.18	9.55E-04	7.10E-03
Psed_3356	protein of unknown function DUF107	-1.19	2.03E-05	3.10E-04
Psed_2845	Quinolinate synthase A	-1.19	3.35E-04	3.15E-03
Psed_0958	Fumarate hydratase class II	-1.19	4.73E-06	9.40E-05
Psed_2906	hypothetical protein	-1.20	3.89E-06	7.96E-05
Psed_5202	hypothetical protein	-1.20	7.13E-04	5.67E-03
Psed_5111	o-succinylbenzoate--CoA ligase	-1.21	8.31E-04	6.34E-03
Psed_3621	hypothetical protein	-1.21	8.86E-08	4.54E-06
Psed_1828	monooxygenase FAD-binding	-1.22	8.45E-07	2.39E-05
Psed_3589	stress protein	-1.22	1.94E-05	3.02E-04
Psed_7013	hypothetical protein	-1.22	8.96E-05	1.07E-03
Psed_6817	hypothetical protein	-1.23	1.48E-04	1.63E-03
Psed_4597	Protein recA	-1.23	5.02E-07	1.58E-05
Psed_5601	SH3 type 3 domain protein	-1.24	6.82E-07	2.03E-05
Psed_4603	DEAD/H associated domain protein	-1.24	3.12E-08	2.12E-06
Psed_2893	hypothetical protein	-1.24	2.60E-07	9.89E-06
Psed_4109	HpcH/HpaI aldolase	-1.24	4.91E-05	6.45E-04
Psed_3926	protein of unknown function DUF1707	-1.25	5.20E-04	4.43E-03
Psed_7000	hypothetical protein	-1.25	3.02E-04	2.91E-03
Psed_1054	1-aminocyclopropane-1-carboxylate deaminase	-1.25	7.29E-04	5.76E-03
Psed_3023	cobaltochelatase, CobN subunit	-1.25	5.01E-08	3.08E-06
Psed_5080	putative transcriptional regulator	-1.26	8.54E-06	1.55E-04
Psed_2902	hypothetical protein	-1.26	1.02E-06	2.75E-05
Psed_0375	regulatory protein TetR	-1.27	1.79E-06	4.23E-05
Psed_6542	hypothetical protein	-1.28	3.34E-06	7.16E-05
Psed_1166	hypothetical protein	-1.28	1.48E-06	3.65E-05
Psed_3812	Tyrosine recombinase xerC	-1.29	9.24E-09	9.11E-07
Psed_1261	hypothetical protein	-1.29	2.18E-06	4.99E-05
Psed_2717	Butyrate--CoA ligase	-1.29	1.63E-06	3.96E-05
Psed_5709	hypothetical protein	-1.30	1.63E-04	1.75E-03
Psed_6290	dienelactone hydrolase	-1.30	1.81E-08	1.41E-06
Psed_5204	transcription factor WhiB	-1.31	1.94E-07	7.99E-06
Psed_6929	oxidoreductase domain protein	-1.31	1.06E-06	2.82E-05
Psed_0038	regulatory protein ArsR	-1.31	1.72E-06	4.12E-05

Appendix 6. Genes differentially expressed on glycolate relative to pyruvate.

Psed_2914	Iron-chelate-transporting ATPase	-1.31	9.51E-06	1.68E-04
Psed_3101	Acetamidase/Formamidase	-1.32	5.66E-05	7.26E-04
Psed_1847	lipopolysaccharide biosynthesis protein	-1.32	8.79E-06	1.58E-04
Psed_0549	cysteine dioxygenase type I	-1.33	1.60E-08	1.28E-06
Psed_1857	glycosyl transferase family 2	-1.34	5.68E-05	7.27E-04
Psed_3677	squalene synthase HpnD	-1.34	1.10E-05	1.88E-04
Psed_1993	ATP-dependent DNA helicase, RecQ family	-1.34	1.01E-06	2.72E-05
Psed_7016	hypothetical protein	-1.34	7.86E-06	1.45E-04
Psed_7014	hypothetical protein	-1.34	1.70E-05	2.73E-04
Psed_1849	asparagine synthase (glutamine-hydrolyzing)	-1.35	3.08E-07	1.12E-05
Psed_0080	ABC-type transporter, integral membrane subunit	-1.36	4.06E-08	2.62E-06
Psed_2700	phenylacetate-CoA oxygenase, PaaG subunit	-1.36	5.41E-06	1.05E-04
Psed_7019	hypothetical protein	-1.36	3.75E-05	5.21E-04
Psed_5209	Peroxiredoxin	-1.37	1.12E-08	1.00E-06
Psed_3678	squalene-associated FAD-dependent desaturase	-1.37	1.26E-06	3.20E-05
Psed_3928	major facilitator superfamily MFS_1	-1.38	2.65E-07	1.00E-05
Psed_5913	5-oxoprolinase (ATP-hydrolyzing)	-1.38	1.13E-07	5.57E-06
Psed_3898	3-oxoacyl-[acyl-carrier-protein] reductase	-1.38	1.47E-07	6.84E-06
Psed_2908	hypothetical protein	-1.38	1.54E-04	1.69E-03
Psed_6486	Conserved hypothetical protein CHP02680	-1.39	3.73E-08	2.45E-06
Psed_1024	protein of unknown function DUF1470	-1.39	1.68E-06	4.05E-05
Psed_3680	squalene-hopene cyclase	-1.39	4.41E-07	1.42E-05
Psed_1653	hypothetical protein	-1.39	1.18E-06	3.02E-05
Psed_3602	small GTP-binding protein	-1.39	8.31E-04	6.34E-03
Psed_1908	hypothetical protein	-1.40	4.24E-04	3.78E-03
Psed_0537	short-chain dehydrogenase/reductase SDR	-1.41	5.46E-05	7.08E-04
Psed_6289	hypothetical protein	-1.42	7.52E-08	4.15E-06
Psed_1457	alpha/beta hydrolase fold	-1.42	2.94E-08	2.02E-06
Psed_5134	diguanylate cyclase	-1.43	2.03E-08	1.53E-06
Psed_5881	Heme oxygenase	-1.43	3.41E-06	7.23E-05
Psed_1848	nucleotide sugar dehydrogenase	-1.44	7.59E-07	2.21E-05

Appendix 6. Genes differentially expressed on glycolate relative to pyruvate.

Psed_0023	regulatory protein GntR HTH	-1.45	7.27E-11	2.34E-08
Psed_1771	Electron transfer flavoprotein alpha/ beta-subunit	-1.47	8.11E-05	9.82E-04
Psed_3357	hypothetical protein	-1.47	3.24E-07	1.16E-05
Psed_1852	lipoprotein	-1.47	3.31E-05	4.69E-04
Psed_2038	sporulation and cell division protein SsgA	-1.47	1.23E-05	2.07E-04
Psed_1060	magnesium and cobalt transport protein CorA	-1.47	2.25E-07	8.82E-06
Psed_0012	hypothetical protein	-1.48	1.07E-05	1.85E-04
Psed_3713	helix-turn-helix domain-containing protein AraC type	-1.48	2.96E-10	6.97E-08
Psed_7018	putative partitioning protein ParA	-1.48	1.78E-06	4.21E-05
Psed_0902	NADH dehydrogenase (ubiquinone)	-1.48	1.84E-06	4.32E-05
Psed_2913	ABC-type transporter, integral membrane subunit	-1.49	3.36E-06	7.17E-05
Psed_0282	esterase	-1.49	7.25E-08	4.08E-06
Psed_1691	methylmalonyl-CoA mutase, large subunit	-1.50	1.20E-04	1.38E-03
Psed_2352	methionine aminopeptidase, type I	-1.50	1.43E-04	1.59E-03
Psed_6961	hypothetical protein	-1.51	9.53E-09	9.17E-07
Psed_3594	Tellurite resistance TerB	-1.52	4.49E-04	3.96E-03
Psed_1633	hypothetical protein	-1.53	1.15E-05	1.97E-04
Psed_0967	RmuC-domain protein	-1.53	1.07E-07	5.40E-06
Psed_2831	DNA polymerase III, alpha subunit	-1.54	1.57E-06	3.83E-05
Psed_5710	hypothetical protein	-1.54	3.71E-06	7.67E-05
Psed_0580	highly repetitive protein	-1.54	8.14E-05	9.83E-04
Psed_4657	protein of unknown function DUF1006	-1.54	1.66E-06	4.01E-05
Psed_1260	DEAD/DEAH box helicase domain protein	-1.55	3.07E-07	1.12E-05
Psed_0137	copper resistance D domain protein	-1.55	5.10E-06	9.98E-05
Psed_5466	hypothetical protein	-1.57	1.55E-07	6.99E-06
Psed_1770	hypothetical protein	-1.57	1.98E-06	4.59E-05
Psed_3817	hypothetical protein	-1.57	5.04E-06	9.88E-05
Psed_6114	FAD-binding 9 siderophore-interacting domain protein	-1.57	1.10E-06	2.89E-05
Psed_5081	DoxX family protein	-1.57	3.75E-05	5.21E-04
Psed_3681	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	-1.58	9.39E-07	2.59E-05
Psed_6890	hypothetical protein	-1.59	7.68E-07	2.22E-05

Appendix 6. Genes differentially expressed on glycolate relative to pyruvate.

Psed_6645	hypothetical protein	-1.60	1.03E-06	2.75E-05
Psed_3937	hypothetical protein	-1.61	4.15E-07	1.37E-05
Psed_3038	Sulfate-transporting ATPase	-1.61	1.72E-07	7.51E-06
Psed_1713	crotonyl-CoA reductase	-1.62	8.06E-05	9.77E-04
Psed_4003	hypothetical protein	-1.62	8.56E-08	4.44E-06
Psed_1468	Cupin 2 conserved barrel domain protein	-1.62	4.95E-09	5.97E-07
Psed_5488	FAD-dependent pyridine nucleotide-disulphide oxidoreductase	-1.63	4.62E-06	9.23E-05
Psed_6889	hypothetical protein	-1.65	1.12E-07	5.57E-06
Psed_3666	regulatory protein DeoR	-1.66	5.40E-07	1.66E-05
Psed_6893	hypothetical protein	-1.67	3.59E-07	1.25E-05
Psed_5355	regulatory protein LuxR	-1.68	1.30E-06	3.27E-05
Psed_1772	Electron transfer flavoprotein alpha subunit	-1.69	2.02E-05	3.10E-04
Psed_3871	hypothetical protein	-1.70	1.22E-05	2.06E-04
Psed_1049	extracellular solute-binding protein family 1	-1.70	1.45E-05	2.38E-04
Psed_3375	NLP/P60 protein	-1.72	4.17E-07	1.37E-05
Psed_6892	hypothetical protein	-1.72	5.76E-09	6.47E-07
Psed_0951	hypothetical protein	-1.74	1.46E-06	3.63E-05
Psed_1763	molybdopterin dehydrogenase FAD-binding	-1.74	1.63E-05	2.63E-04
Psed_4655	protein of unknown function DUF1778	-1.75	5.28E-07	1.65E-05
Psed_4110	MmgE/PrpD family protein	-1.75	3.67E-06	7.61E-05
Psed_0021	isochorismatase hydrolase	-1.75	4.51E-08	2.85E-06
Psed_3894	ErfK/YbiS/YcfS/YnhG family protein	-1.75	1.44E-08	1.21E-06
Psed_1730	alpha amylase catalytic region	-1.75	5.14E-07	1.61E-05
Psed_2123	Peptidoglycan-binding lysin domain	-1.76	1.08E-08	1.00E-06
Psed_0247	ABC-type transporter, integral membrane subunit	-1.79	9.83E-06	1.73E-04
Psed_4176	GntR domain protein	-1.82	2.81E-06	6.19E-05
Psed_2887	diguanylate cyclase	-1.83	8.12E-08	4.33E-06
Psed_3658	RNA polymerase sigma-70 factor	-1.83	2.17E-11	9.42E-09
Psed_6202	pyrimidine utilization protein A	-1.84	2.42E-06	5.46E-05
Psed_0950	hypothetical protein	-1.84	1.13E-04	1.31E-03
Psed_2799	Ribosomal RNA small subunit methyltransferase H	-1.85	8.24E-07	2.35E-05
Psed_0140	hypothetical protein	-1.87	1.17E-05	1.99E-04
Psed_3278	LmbE family protein	-1.88	6.32E-06	1.20E-04

Appendix 6. Genes differentially expressed on glycolate relative to pyruvate.

Psed_6619	Phosphoenolpyruvate carboxykinase [GTP]	-1.88	3.49E-09	4.93E-07
Psed_1846	lipopolysaccharide biosynthesis protein	-1.88	3.79E-07	1.28E-05
Psed_3996	Methionyl-tRNA formyltransferase	-1.88	1.34E-04	1.51E-03
Psed_1655	hypothetical protein	-1.89	1.22E-09	2.25E-07
Psed_1052	Glycerol-3-phosphate-transporting ATPase	-1.90	9.12E-06	1.63E-04
Psed_1050	ABC-type transporter, integral membrane subunit	-1.90	1.78E-05	2.81E-04
Psed_4080	protein of unknown function DUF574	-1.90	9.77E-07	2.67E-05
Psed_3682	hopanoid biosynthesis associated radical SAM protein HpnH	-1.91	2.32E-07	8.99E-06
Psed_2318	regulatory protein MerR	-1.92	3.40E-06	7.22E-05
Psed_6968	transposase mutator type	-1.95	9.63E-10	2.01E-07
Psed_2800	penicillin-binding protein transpeptidase	-1.95	1.37E-06	3.43E-05
Psed_5922	Carboxylesterase	-1.97	7.00E-05	8.70E-04
Psed_2033	ABC-type transporter, periplasmic subunit	-1.97	5.60E-05	7.22E-04
Psed_3679	Dimethylallyltranstransferase	-2.00	8.11E-06	1.49E-04
Psed_3668	hypothetical protein	-2.01	3.71E-07	1.26E-05
Psed_4656	hypothetical protein	-2.03	4.17E-07	1.37E-05
Psed_1051	ABC-type transporter, integral membrane subunit	-2.03	3.84E-05	5.33E-04
Psed_4976	NADP oxidoreductase coenzyme F420-dependent	-2.04	2.07E-07	8.39E-06
Psed_1023	hypothetical protein	-2.05	7.59E-08	4.15E-06
Psed_0248	ABC-type transporter, periplasmic subunit	-2.06	1.72E-09	3.01E-07
Psed_3440	SH3 type 3 domain protein	-2.06	2.90E-06	6.37E-05
Psed_0013	hypothetical protein	-2.06	5.31E-09	6.12E-07
Psed_0252	amino acid adenylation domain protein	-2.09	1.50E-08	1.24E-06
Psed_2132	hypothetical protein	-2.12	3.50E-07	1.23E-05
Psed_6948	transposase mutator type	-2.12	5.36E-09	6.12E-07
Psed_5215	cell envelope-related function transcriptional attenuator, LytR/CpsA family	-2.14	3.83E-08	2.49E-06
Psed_2032	50S ribosomal protein L31 type B	-2.15	3.28E-05	4.69E-04
Psed_6096	ABC-type transporter, integral membrane subunit	-2.18	4.63E-07	1.46E-05

Appendix 6. Genes differentially expressed on glycolate relative to pyruvate.

Psed_3676	squalene synthase HpnC	-2.20	3.67E-09	5.09E-07
Psed_2122	LexA repressor	-2.20	1.20E-06	3.07E-05
Psed_6914	amidohydrolase	-2.21	2.11E-09	3.43E-07
Psed_3727	Pyruvate dehydrogenase (acetyl-transferring)	-2.21	1.12E-07	5.57E-06
Psed_2203	cobalamin synthesis CobW domain protein	-2.21	4.31E-07	1.40E-05
Psed_3863	hypothetical protein	-2.23	8.17E-08	4.33E-06
Psed_2763	hypothetical protein	-2.23	1.12E-08	1.00E-06
Psed_2809	cell division protein FtsZ	-2.25	1.51E-07	6.92E-06
Psed_3037	ABC-type metal ion transporter, periplasmic subunit	-2.25	2.90E-08	2.01E-06
Psed_6301	dienelactone hydrolase	-2.27	2.58E-07	9.89E-06
Psed_6162	MbtH domain protein	-2.28	8.60E-08	4.44E-06
Psed_3717	Transcriptional regulator IclR	-2.29	8.19E-08	4.33E-06
Psed_3724	3-oxoacyl-[acyl-carrier-protein] reductase	-2.33	3.32E-06	7.12E-05
Psed_3731	Phosphonate-transporting ATPase	-2.39	9.33E-07	2.59E-05
Psed_5195	alanine dehydrogenase	-2.39	2.69E-06	5.99E-05
Psed_0251	Xenobiotic-transporting ATPase	-2.48	1.25E-09	2.25E-07
Psed_6161	Siderophore-interacting protein	-2.49	1.32E-08	1.15E-06
Psed_2329	Peroxidase	-2.49	2.41E-07	9.29E-06
Psed_2995	monosaccharide-transporting ATPase	-2.51	1.04E-08	9.82E-07
Psed_6441	beta-lactamase	-2.51	3.45E-06	7.28E-05
Psed_3730	Carboxymuconolactone decarboxylase	-2.54	2.79E-06	6.18E-05
Psed_1654	hypothetical protein	-2.56	9.43E-10	2.01E-07
Psed_3721	alpha/beta hydrolase fold	-2.57	8.27E-07	2.35E-05
Psed_0138	copper resistance protein CopC	-2.59	1.76E-06	4.19E-05
Psed_3941	Glyoxalase/bleomycin resistance protein/dioxygenase	-2.61	3.64E-07	1.25E-05
Psed_0164	hypothetical protein	-2.62	5.07E-08	3.08E-06
Psed_3995	ABC-type transporter, periplasmic subunit	-2.64	1.01E-09	2.01E-07
Psed_0250	Xenobiotic-transporting ATPase	-2.64	8.01E-09	8.15E-07
Psed_3720	amidohydrolase 2	-2.67	7.65E-09	7.90E-07
Psed_3174	hypothetical protein	-2.71	1.76E-09	3.01E-07
Psed_3733	ABC-type transporter, integral membrane subunit	-2.78	7.50E-06	1.39E-04
Psed_0249	L-lysine 6-monooxygenase (NADPH)	-2.84	7.91E-08	4.29E-06
Psed_2812	Cell division protein sepF	-2.89	2.20E-08	1.61E-06

Appendix 6. Genes differentially expressed on glycolate relative to pyruvate.

Psed_3718	3-oxoacyl-[acyl-carrier-protein] reductase	-2.89	2.79E-09	4.32E-07
Psed_6599	N-acetylmuramoyl-L-alanine amidase family 2	-2.93	2.64E-10	6.62E-08
Psed_6805	hypothetical protein	-2.94	1.02E-05	1.78E-04
Psed_3723	oxidoreductase domain protein	-2.96	1.61E-08	1.28E-06
Psed_4688	polysaccharide deacetylase	-2.97	2.99E-11	1.22E-08
Psed_0276	major facilitator superfamily MFS_1	-3.00	3.65E-11	1.40E-08
Psed_3732	Monosaccharide-transporting ATPase	-3.05	3.98E-07	1.33E-05
Psed_1657	Cobalt transporter subunit CbtB putative	-3.11	3.00E-10	6.97E-08
Psed_0011	hypothetical protein	-3.15	7.56E-11	2.34E-08
Psed_0139	nuclear export factor GLE1	-3.16	8.99E-07	2.50E-05
Psed_1656	hypothetical protein	-3.18	1.83E-11	8.51E-09
Psed_3728	Pyruvate dehydrogenase (acetyl-transferring)	-3.19	1.51E-08	1.24E-06
Psed_3904	CO dehydrogenase maturation factor-like protein	-3.20	2.53E-08	1.81E-06
Psed_5471	hypothetical protein	-3.22	4.42E-09	5.76E-07
Psed_3903	hypothetical protein	-3.23	4.78E-09	5.97E-07
Psed_3729	3-oxoacyl-[acyl-carrier-protein] reductase	-3.24	2.90E-08	2.01E-06
Psed_6095	Fe(3+)-transporting ATPase	-3.26	1.17E-06	3.02E-05
Psed_5843	hypothetical protein	-3.27	2.38E-08	1.72E-06
Psed_3719	cyclase family protein	-3.37	1.76E-08	1.38E-06
Psed_3725	Extracellular ligand-binding receptor	-3.38	7.40E-09	7.77E-07
Psed_3722	hypothetical protein	-3.38	5.30E-09	6.12E-07
Psed_3667	SSS sodium solute transporter superfamily	-3.54	1.02E-09	2.01E-07
Psed_0078	Methyltransferase type 12	-3.67	6.43E-13	4.19E-10
Psed_6094	ABC-type metal ion transporter, periplasmic subunit	-3.67	9.58E-09	9.17E-07
Psed_3734	ABC-type transporter, integral membrane subunit	-3.71	7.73E-07	2.23E-05
Psed_3726	Xylose isomerase domain-containing protein TIM barrel	-3.73	9.84E-08	5.00E-06
Psed_5844	hypothetical protein	-3.85	7.12E-09	7.60E-07
Psed_0079	ABC-type transporter, periplasmic subunit	-3.87	4.26E-11	1.46E-08
Psed_3670	Butyryl-CoA dehydrogenase	-4.05	7.19E-06	1.34E-04

Appendix 6. Genes differentially expressed on glycolate relative to pyruvate.

Psed_2204	50S ribosomal protein L33	-5.44	6.47E-12	3.83E-09

Appendix 6. Genes differentially expressed on glycolate relative to pyruvate.

Appendix 7

Genes differentially expressed on succinate relative to pyruvate.

Appendix 7. Genes differentially expressed on succinate relative to pyruvate. Differentially expressed genes have an adjusted p-value < 0.01 and have a $log_2FC > 1$ or $log_2FC < -1$ relative to pyruvate and are sorted by log_2FC.

Genes	Protein	log_2FC	p-value	adjusted p-value
Psed_0089	SSS sodium solute transporter superfamily	6.25	3.02E-14	3.47E-11
Psed_1555	peptidase S1 and S6 chymotrypsin/Hap	6.08	4.66E-08	1.79E-06
Psed_5371	hypothetical protein	5.99	2.77E-08	1.17E-06
Psed_3486	hypothetical protein	5.97	7.41E-12	1.12E-09
Psed_3473	Pyruvate dehydrogenase (cytochrome)	5.91	7.28E-13	1.98E-10
Psed_3463	hypothetical protein	5.84	6.01E-13	1.86E-10
Psed_3484	Protein grpE	5.82	4.16E-13	1.50E-10
Psed_0088	protein of unknown function DUF485	5.57	3.19E-14	3.47E-11
Psed_3487	heat shock protein Hsp20	5.55	4.85E-12	7.90E-10
Psed_3443	hypothetical protein	5.53	1.80E-11	2.29E-09
Psed_3480	thioredoxin	5.51	9.12E-13	2.20E-10
Psed_3508	intracellular protease, PfpI family	5.48	6.33E-13	1.87E-10
Psed_3485	Chaperone protein dnaK	5.47	3.03E-12	5.81E-10
Psed_3497	transport-associated	5.46	2.20E-14	3.47E-11
Psed_3481	ATP-dependent chaperone ClpB	5.44	1.90E-11	2.38E-09
Psed_3460	formaldehyde dehydrogenase, glutathione-independent	5.43	3.64E-13	1.50E-10
Psed_3500	glucose-6-phosphate 1-dehydrogenase	5.43	2.82E-12	5.81E-10
Psed_3446	Ferritin Dps family protein	5.42	1.20E-13	7.10E-11
Psed_3496	hypothetical protein	5.31	8.83E-13	2.20E-10
Psed_3918	FAD linked oxidase domain protein	5.31	2.42E-07	6.71E-06
Psed_3490	major facilitator superfamily MFS_1	5.10	5.74E-13	1.86E-10
Psed_3451	Domain of unknown function DUF1931	5.07	3.74E-12	6.59E-10
Psed_1539	hypothetical protein	4.95	4.21E-09	2.43E-07
Psed_3461	transport-associated	4.94	8.34E-12	1.21E-09
Psed_3477	Erythromycin esterase	4.89	2.85E-14	3.47E-11
Psed_3493	ParB domain protein nuclease	4.89	8.76E-13	2.20E-10
Psed_3453	hypothetical protein	4.84	7.78E-12	1.15E-09
Psed_3462	1-deoxy-D-xylulose-5-phosphate synthase	4.81	4.15E-14	3.86E-11
Psed_3483	chaperone DnaJ domain protein	4.75	7.08E-13	1.98E-10
Psed_3478	RNA polymerase sigma-70 factor, sigma-B/F/G subfamily	4.69	3.00E-12	5.81E-10
Psed_3510	transcription elongation factor GreA	4.69	2.69E-10	2.54E-08

Psed_5539	hypothetical protein	4.63	3.46E-08	1.43E-06
Psed_3495	protein of unknown function DUF308 membrane	4.61	3.85E-11	4.56E-09
Psed_3513	Helix-turn-helix, AraC domain	4.61	7.74E-11	8.00E-09
Psed_3482	hypothetical protein	4.55	1.15E-10	1.17E-08
Psed_1566	protocatechuate 3,4-dioxygenase, alpha subunit	4.46	6.47E-07	1.52E-05
Psed_3472	protein of unknown function (DUF461)	4.45	2.14E-13	1.16E-10
Psed_3474	membrane protein	4.35	1.01E-11	1.42E-09
Psed_5025	4-hydroxyacetophenone monooxygenase	4.26	1.18E-07	3.86E-06
Psed_3476	dienelactone hydrolase	4.20	1.77E-11	2.29E-09
Psed_3456	Thioredoxin-disulfide reductase	4.20	1.50E-12	3.50E-10
Psed_6600	acyl-CoA dehydrogenase domain-containing protein	4.20	4.73E-08	1.81E-06
Psed_3448	hypothetical protein	4.18	9.05E-14	6.36E-11
Psed_1490	benzoyl-CoA-dihydrodiol lyase	4.18	5.19E-11	5.75E-09
Psed_3444	hypothetical protein	4.18	2.54E-13	1.27E-10
Psed_3794	Methyltransferase type 11	4.14	1.37E-10	1.36E-08
Psed_3458	hypothetical protein	4.14	3.14E-12	5.84E-10
Psed_1589	hypothetical protein	4.08	1.31E-07	4.09E-06
Psed_3514	glycosyl transferase group 1	4.07	2.98E-12	5.81E-10
Psed_3522	response regulator receiver	3.98	5.77E-10	4.70E-08
Psed_1564	4-hydroxybenzoate 3-monooxygenase	3.90	1.44E-06	2.96E-05
Psed_6066	4-hydroxyphenylacetate 3-hydroxylase	3.87	1.02E-11	1.42E-09
Psed_1489	benzoyl-CoA oxygenase, B subunit	3.84	3.25E-12	5.87E-10
Psed_6176	Acyl-CoA oxidase	3.80	3.07E-06	5.25E-05
Psed_3504	dienelactone hydrolase	3.80	4.14E-10	3.54E-08
Psed_3470	MscS Mechanosensitive ion channel	3.79	4.99E-14	4.06E-11
Psed_1491	benzoate-CoA ligase family	3.78	4.77E-10	4.03E-08
Psed_6172	MaoC domain protein dehydratase	3.72	1.90E-06	3.64E-05
Psed_1178	hypothetical protein	3.71	2.72E-09	1.65E-07
Psed_6067	flavin reductase domain protein FMN-binding	3.66	6.15E-11	6.46E-09
Psed_3475	hypothetical protein	3.64	5.63E-15	3.47E-11
Psed_1565	protocatechuate 3,4-dioxygenase, beta subunit	3.63	1.03E-06	2.21E-05
Psed_3447	hypothetical protein	3.54	1.20E-09	8.98E-08
Psed_3515	hypothetical protein	3.49	6.42E-12	9.95E-10

Appendix 7. Genes differentially expressed on succinate relative to pyruvate.

Psed_6173	3-oxoacyl-[acyl-carrier-protein] reductase	3.49	2.46E-05	2.92E-04
Psed_4478	hypothetical protein	3.41	1.80E-12	4.05E-10
Psed_3755	Xanthine dehydrogenase	3.32	4.63E-04	3.21E-03
Psed_3042	NADH dehydrogenase (quinone)	3.31	2.36E-09	1.51E-07
Psed_3041	NADH dehydrogenase (ubiquinone) 24 kDa subunit	3.30	5.24E-09	2.84E-07
Psed_3756	molybdopterin dehydrogenase FAD-binding	3.26	6.97E-04	4.46E-03
Psed_6065	catechol 1,2-dioxygenase	3.26	2.78E-12	5.81E-10
Psed_0349	ATPase, P-type (transporting), HAD superfamily, subfamily IC	3.25	3.84E-10	3.38E-08
Psed_3701	GntR domain protein	3.24	2.23E-14	3.47E-11
Psed_5782	Collagen triple helix repeat-containing protein	3.22	9.36E-08	3.19E-06
Psed_1635	regulatory protein LuxR	3.21	3.55E-13	1.50E-10
Psed_6174	acetyl-CoA acetyltransferase	3.21	2.75E-06	4.82E-05
Psed_5796	hypothetical protein	3.18	6.09E-08	2.23E-06
Psed_6175	regulatory protein TetR	3.17	2.74E-08	1.17E-06
Psed_5787	hypothetical protein	3.16	6.37E-10	5.12E-08
Psed_3457	polyphosphate:nucleotide phosphotransferase, PPK2 family	3.10	3.71E-11	4.48E-09
Psed_3489	Protein of unknown function DUF2267	3.09	3.95E-13	1.50E-10
Psed_6878	hypothetical protein	3.05	4.04E-13	1.50E-10
Psed_3502	hypothetical protein	3.00	4.75E-13	1.63E-10
Psed_3376	hypothetical protein	3.00	1.70E-06	3.39E-05
Psed_5804	hypothetical protein	2.98	1.26E-07	4.00E-06
Psed_5801	hypothetical protein	2.98	4.75E-08	1.81E-06
Psed_1559	response regulator receiver	2.97	2.40E-06	4.37E-05
Psed_3757	carbon monoxide dehydrogenase subunit G	2.97	8.02E-04	5.00E-03
Psed_5799	hypothetical protein	2.91	1.66E-09	1.17E-07
Psed_5795	hypothetical protein	2.90	7.71E-08	2.74E-06
Psed_0126	Methionine synthase vitamin-B12 independent	2.88	2.50E-07	6.86E-06
Psed_5798	hypothetical protein	2.88	8.26E-08	2.87E-06
Psed_3501	hypothetical protein	2.88	6.64E-10	5.27E-08
Psed_3752	UbiD family decarboxylase	2.88	8.42E-04	5.22E-03
Psed_1077	hypothetical protein	2.88	3.85E-06	6.33E-05
Psed_5815	hypothetical protein	2.85	2.20E-07	6.17E-06

Appendix 7. Genes differentially expressed on succinate relative to pyruvate.

Psed_0489	alpha/beta hydrolase fold-3	2.85	6.43E-09	3.35E-07
Psed_1183	acyl-CoA dehydrogenase domain-containing protein	2.84	1.76E-07	5.13E-06
Psed_6427	amino acid permease-associated region	2.81	1.83E-09	1.25E-07
Psed_6062	Phenol 2-monooxygenase	2.81	4.51E-07	1.10E-05
Psed_3449	NADH dehydrogenase (ubiquinone)	2.77	6.33E-12	9.95E-10
Psed_3043	formate dehydrogenase, alpha subunit	2.70	1.34E-08	6.40E-07
Psed_3445	hypothetical protein	2.69	4.18E-12	7.16E-10
Psed_3780	conserved hypothetical protein	2.69	5.10E-09	2.79E-07
Psed_6064	muconate and chloromuconate cycloisomerase	2.69	2.42E-09	1.53E-07
Psed_5800	hypothetical protein	2.67	4.73E-09	2.65E-07
Psed_3753	alpha/beta hydrolase fold	2.62	3.42E-04	2.50E-03
Psed_5792	hypothetical protein	2.61	2.78E-06	4.82E-05
Psed_4333	UBA/THIF-type NAD/FAD binding protein	2.60	1.23E-06	2.58E-05
Psed_5797	hypothetical protein	2.59	1.53E-07	4.58E-06
Psed_3511	hypothetical protein	2.59	6.28E-08	2.28E-06
Psed_5811	hypothetical protein	2.58	1.27E-07	4.00E-06
Psed_1416	ABC-type transporter, periplasmic subunit	2.58	1.23E-09	9.13E-08
Psed_5794	hypothetical protein	2.56	4.03E-08	1.59E-06
Psed_6063	muconolactone delta-isomerase	2.55	7.17E-07	1.66E-05
Psed_5793	hypothetical protein	2.54	1.11E-07	3.66E-06
Psed_5791	hypothetical protein	2.53	1.31E-09	9.58E-08
Psed_0090	hypothetical protein	2.53	2.36E-10	2.30E-08
Psed_4577	Cold-shock protein DNA-binding	2.48	1.34E-05	1.78E-04
Psed_4306	hypothetical protein	2.47	3.58E-09	2.10E-07
Psed_6196	protein of unknown function DUF151	2.47	5.30E-11	5.75E-09
Psed_5929	beta-lactamase	2.46	1.56E-11	2.07E-09
Psed_5934	o-succinylbenzoate--CoA ligase	2.45	2.38E-08	1.05E-06
Psed_3503	PDZ/DHR/GLGF domain-containing protein	2.43	4.37E-07	1.07E-05
Psed_6031	response regulator receiver	2.41	1.51E-06	3.08E-05
Psed_5026	regulatory protein TetR	2.41	1.85E-08	8.40E-07
Psed_3759	major facilitator superfamily MFS_1	2.38	1.10E-03	6.42E-03
Psed_5370	YjeF-related protein	2.37	4.88E-09	2.69E-07
Psed_3450	hypothetical protein	2.36	2.48E-06	4.46E-05
Psed_5788	peptidase S14 ClpP	2.36	1.25E-07	4.00E-06
Psed_4578	Stearoyl-CoA 9-desaturase	2.34	2.35E-06	4.33E-05

Appendix 7. Genes differentially expressed on succinate relative to pyruvate.

Psed_5681	Radical SAM domain protein	2.31	1.44E-08	6.78E-07
Psed_4697	hypothetical protein	2.31	1.54E-04	1.33E-03
Psed_3754	[2Fe-2S]-binding domain-containing protein	2.30	2.81E-04	2.14E-03
Psed_1182	acyl-CoA dehydrogenase domain-containing protein	2.29	2.31E-07	6.46E-06
Psed_5786	phage terminase	2.28	1.92E-06	3.66E-05
Psed_4576	regulatory protein TetR	2.27	1.01E-07	3.39E-06
Psed_5814	hypothetical protein	2.26	1.62E-05	2.03E-04
Psed_5909	GntR domain protein	2.25	2.23E-08	9.94E-07
Psed_6426	Copper amine oxidase domain-containing protein	2.25	2.65E-09	1.63E-07
Psed_0922	hypothetical protein	2.22	4.19E-09	2.43E-07
Psed_5784	hypothetical protein	2.19	1.26E-07	4.00E-06
Psed_0891	iron permease FTR1	2.17	2.83E-05	3.28E-04
Psed_5808	hypothetical protein	2.15	8.33E-06	1.22E-04
Psed_3702	Methionine synthase vitamin-B12 independent	2.12	4.84E-09	2.69E-07
Psed_5812	hypothetical protein	2.12	8.19E-06	1.21E-04
Psed_1078	alpha/beta hydrolase fold	2.11	1.32E-04	1.17E-03
Psed_5789	hypothetical protein	2.10	2.97E-05	3.39E-04
Psed_5790	hypothetical protein	2.06	2.44E-05	2.90E-04
Psed_1567	GtrA family protein	2.03	6.58E-04	4.28E-03
Psed_5682	hypothetical protein	2.03	1.61E-05	2.03E-04
Psed_4052	(S)-2-hydroxy-acid oxidase	2.00	2.75E-06	4.82E-05
Psed_6921	Xylose isomerase domain-containing protein TIM barrel	1.99	1.49E-07	4.50E-06
Psed_1076	short-chain dehydrogenase/reductase SDR	1.99	1.74E-06	3.45E-05
Psed_6591	Heat shock protein 70	1.99	2.12E-06	3.96E-05
Psed_3204	extracellular solute-binding protein family 1	1.99	3.00E-07	7.97E-06
Psed_6924	ABC-type transporter, periplasmic subunit	1.98	5.15E-07	1.24E-05
Psed_1521	Carboxymuconolactone decarboxylase	1.98	6.13E-06	9.51E-05
Psed_5135	malic protein NAD-binding	1.97	2.37E-06	4.34E-05
Psed_0889	Tat-translocated protein	1.97	2.33E-05	2.80E-04
Psed_3700	LmbE family protein	1.96	1.62E-07	4.81E-06
Psed_4332	hypothetical protein	1.95	1.02E-05	1.43E-04
Psed_5809	hypothetical protein	1.95	2.26E-06	4.19E-05

Appendix 7. Genes differentially expressed on succinate relative to pyruvate.

Psed_2496	protein of unknown function DUF849	1.94	3.12E-10	2.86E-08
Psed_2497	cyclase family protein	1.93	3.46E-10	3.09E-08
Psed_6362	Cytochrome-c3 hydrogenase	1.92	1.56E-07	4.65E-06
Psed_0531	G3E family GTPase	1.92	3.59E-06	6.00E-05
Psed_5810	hypothetical protein	1.91	1.31E-05	1.75E-04
Psed_3731	Phosphonate-transporting ATPase	1.91	1.19E-05	1.62E-04
Psed_5358	transcription factor WhiB	1.90	8.26E-07	1.85E-05
Psed_5024	Mandelate racemase/muconate lactonizing protein	1.90	1.20E-04	1.09E-03
Psed_1844	exopolysaccharide biosynthesis polyprenyl glycosylphosphotransferase	1.90	8.76E-08	3.03E-06
Psed_1791	fatty acid desaturase	1.90	8.73E-06	1.26E-04
Psed_6539	hypothetical protein	1.88	1.33E-07	4.14E-06
Psed_5473	Linalool 8-monooxygenase	1.87	2.04E-06	3.85E-05
Psed_5266	hypothetical protein	1.85	2.12E-06	3.96E-05
Psed_6006	hypothetical protein	1.85	2.76E-09	1.66E-07
Psed_5803	hypothetical protein	1.83	1.37E-06	2.84E-05
Psed_5081	DoxX family protein	1.83	7.45E-06	1.12E-04
Psed_3291	hypothetical protein	1.83	1.35E-08	6.40E-07
Psed_1790	ferredoxin	1.82	1.51E-04	1.30E-03
Psed_2777	hypothetical protein	1.82	1.32E-05	1.75E-04
Psed_3205	ABC-type transporter, integral membrane subunit	1.81	8.93E-09	4.51E-07
Psed_3488	Sulfate transporter/antisigma-factor antagonist STAS	1.79	3.85E-07	9.67E-06
Psed_2030	Linalool 8-monooxygenase	1.78	1.71E-06	3.39E-05
Psed_3795	bifunctional deaminase-reductase domain protein	1.77	3.76E-08	1.50E-06
Psed_1982	hypothetical protein	1.75	1.69E-05	2.12E-04
Psed_5802	hypothetical protein	1.74	6.85E-06	1.04E-04
Psed_1184	transcriptional regulator PadR family protein	1.73	1.17E-05	1.60E-04
Psed_4958	hypothetical protein	1.71	1.40E-05	1.83E-04
Psed_4477	transcription activator effector binding protein	1.71	5.26E-11	5.75E-09
Psed_4755	ammonium transporter	1.70	3.34E-05	3.74E-04
Psed_1708	Alcohol dehydrogenase zinc-binding domain protein	1.70	4.93E-07	1.19E-05
Psed_3512	hypothetical protein	1.69	1.52E-05	1.95E-04
Psed_5257	Acyl-CoA dehydrogenase	1.68	1.69E-06	3.39E-05

Appendix 7. Genes differentially expressed on succinate relative to pyruvate.

Psed_3044	hypothetical protein	1.68	2.02E-06	3.83E-05
Psed_3723	oxidoreductase domain protein	1.67	1.41E-05	1.84E-04
Psed_5785	hypothetical protein	1.66	1.82E-06	3.56E-05
Psed_3242	Nitrile hydratase	1.66	1.92E-09	1.29E-07
Psed_5720	PspC domain protein	1.65	4.23E-08	1.65E-06
Psed_3751	hypothetical protein	1.65	8.42E-04	5.22E-03
Psed_5783	hypothetical protein	1.65	2.03E-04	1.65E-03
Psed_0914	Luciferase-like, subgroup	1.65	1.60E-05	2.03E-04
Psed_6922	oligopeptide/dipeptide ABC transporter, ATPase subunit	1.64	8.00E-10	6.27E-08
Psed_0551	Glutaryl-CoA dehydrogenase	1.63	1.30E-04	1.16E-03
Psed_5805	hypothetical protein	1.62	2.25E-04	1.79E-03
Psed_4220	Luciferase-like, subgroup	1.62	3.00E-04	2.25E-03
Psed_3760	major facilitator superfamily MFS_1	1.62	1.43E-03	7.91E-03
Psed_1857	glycosyl transferase family 2	1.61	7.79E-06	1.16E-04
Psed_4524	Barstar (barnase inhibitor)	1.61	3.46E-09	2.07E-07
Psed_3492	transcription factor WhiB	1.61	5.21E-10	4.30E-08
Psed_5816	hypothetical protein	1.61	1.25E-06	2.60E-05
Psed_4698	amine oxidase	1.57	1.15E-03	6.68E-03
Psed_6740	hypothetical protein	1.57	5.87E-07	1.39E-05
Psed_3975	hypothetical protein	1.56	9.44E-07	2.07E-05
Psed_0253	Pentachlorophenol monooxygenase	1.56	4.98E-08	1.88E-06
Psed_1149	ThiJ/PfpI domain-containing protein	1.55	3.77E-07	9.56E-06
Psed_3182	hypothetical protein	1.54	1.34E-07	4.14E-06
Psed_1858	polysaccharide biosynthesis protein	1.53	1.08E-05	1.51E-04
Psed_3243	nitrile hydratase, alpha subunit	1.53	5.20E-11	5.75E-09
Psed_3241	Amidase	1.52	2.97E-08	1.24E-06
Psed_5813	hypothetical protein	1.51	1.30E-04	1.16E-03
Psed_6425	hypothetical protein	1.51	1.36E-05	1.79E-04
Psed_3725	Extracellular ligand-binding receptor	1.50	8.69E-05	8.46E-04
Psed_1452	Nitrile hydratase	1.50	6.82E-04	4.39E-03
Psed_2498	Carbon-monoxide dehydrogenase (acceptor)	1.49	1.09E-05	1.52E-04
Psed_5316	hypothetical protein	1.49	6.40E-06	9.87E-05
Psed_1558	protein of unknown function DUF125 transmembrane	1.48	1.01E-05	1.42E-04
Psed_1866	ATP-grasp fold domain protein, DUF201-type	1.47	1.77E-08	8.12E-07
Psed_5910	Phenylacetate--CoA ligase	1.47	2.33E-08	1.03E-06

Appendix 7. Genes differentially expressed on succinate relative to pyruvate.

Psed_6224	hypothetical protein	1.46	6.33E-05	6.55E-04
Psed_2039	RNA polymerase sigma factor, sigma-70 family	1.46	1.59E-05	2.02E-04
Psed_0227	hypothetical protein	1.45	8.86E-09	4.51E-07
Psed_3271	BFD domain protein [2Fe-2S]-binding domain protein	1.45	2.11E-06	3.96E-05
Psed_3889	2-hydroxy-3-oxopropionate reductase	1.45	2.10E-06	3.96E-05
Psed_6925	ABC-type transporter, integral membrane subunit	1.45	1.16E-06	2.45E-05
Psed_3111	Hemerythrin HHE cation binding domain protein	1.45	9.56E-05	9.16E-04
Psed_5621	beta-Ig-H3/fasciclin	1.45	9.32E-04	5.65E-03
Psed_5540	hypothetical protein	1.44	2.50E-07	6.86E-06
Psed_3724	3-oxoacyl-[acyl-carrier-protein] reductase	1.43	4.46E-04	3.12E-03
Psed_0890	Peptidase M75, Imelysin	1.43	3.45E-04	2.51E-03
Psed_6923	oligopeptide/dipeptide ABC transporter, ATPase subunit	1.41	2.92E-06	5.05E-05
Psed_3716	hypothetical protein	1.40	2.83E-07	7.68E-06
Psed_2501	GntR domain protein	1.40	7.55E-07	1.72E-05
Psed_6561	hypothetical protein	1.39	1.15E-05	1.59E-04
Psed_3727	Pyruvate dehydrogenase (acetyl-transferring)	1.39	2.39E-05	2.86E-04
Psed_6538	AAA ATPase central domain protein	1.38	9.48E-06	1.36E-04
Psed_5484	peptidase S15	1.38	7.28E-07	1.67E-05
Psed_0124	Transcriptional regulator IclR	1.38	4.52E-11	5.26E-09
Psed_6477	peptidase M10A and M12B matrixin and adamalysin	1.37	3.84E-06	6.33E-05
Psed_5343	PspC domain protein	1.37	4.12E-07	1.02E-05
Psed_4330	hypothetical protein	1.36	2.81E-05	3.27E-04
Psed_5100	hypothetical protein	1.36	2.74E-07	7.47E-06
Psed_3722	hypothetical protein	1.36	1.81E-04	1.51E-03
Psed_5911	Hydantoinase/oxoprolinase	1.36	1.08E-06	2.31E-05
Psed_4115	Protein of unknown function DUF2236	1.35	1.25E-04	1.13E-03
Psed_5683	hypothetical protein	1.35	2.48E-08	1.08E-06
Psed_5912	Acetone carboxylase gamma subunit	1.34	8.46E-09	4.34E-07
Psed_0473	hypothetical protein	1.34	1.53E-09	1.10E-07
Psed_3717	Transcriptional regulator IclR	1.33	3.90E-05	4.31E-04
Psed_1845	Glutamate-1-semialdehyde 2,1-aminomutase	1.33	1.87E-06	3.60E-05

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Psed_1855	hypothetical protein	1.32	3.50E-06	5.87E-05
Psed_5344	hypothetical protein	1.31	7.01E-06	1.06E-04
Psed_6636	Nitric-oxide synthase	1.30	1.06E-04	9.89E-04
Psed_5374	hypothetical protein	1.30	2.11E-04	1.70E-03
Psed_2026	glutamate decarboxylase	1.30	3.37E-08	1.40E-06
Psed_4569	hypothetical protein	1.30	2.37E-07	6.58E-06
Psed_1922	major facilitator superfamily MFS_1	1.29	2.97E-06	5.12E-05
Psed_1179	protein tyrosine/serine phosphatase	1.29	4.08E-08	1.60E-06
Psed_2752	hypothetical protein	1.29	1.90E-04	1.57E-03
Psed_5404	NAD(P)(+) transhydrogenase (AB-specific)	1.28	3.34E-06	5.64E-05
Psed_4329	hypothetical protein	1.28	3.16E-05	3.58E-04
Psed_1854	glycosyl transferase group 1	1.28	9.96E-08	3.38E-06
Psed_2040	putative transmembrane anti-sigma factor	1.27	1.56E-05	1.99E-04
Psed_1451	nitrile hydratase, alpha subunit	1.27	3.02E-04	2.25E-03
Psed_0488	Cyclohexanone monooxygenase	1.26	3.30E-07	8.66E-06
Psed_3280	PspC domain protein	1.26	1.67E-07	4.89E-06
Psed_1896	C-methyltransferase	1.25	5.49E-06	8.70E-05
Psed_5913	5-oxoprolinase (ATP-hydrolyzing)	1.24	3.94E-07	9.83E-06
Psed_0280	Alanine--tRNA ligase	1.24	1.20E-07	3.91E-06
Psed_1590	Formyl-CoA transferase	1.24	8.61E-05	8.42E-04
Psed_3715	Phosphoglycerate dehydrogenase	1.24	4.57E-06	7.38E-05
Psed_2008	degV family protein	1.24	3.16E-10	2.86E-08
Psed_2780	transcriptional regulatory protein	1.23	3.52E-08	1.43E-06
Psed_0334	hypothetical protein	1.23	1.05E-08	5.27E-07
Psed_4142	hypothetical protein	1.23	4.05E-10	3.52E-08
Psed_2869	pyruvate kinase	1.23	2.83E-04	2.15E-03
Psed_6861	hypothetical protein	1.22	2.27E-05	2.74E-04
Psed_1849	asparagine synthase (glutamine-hydrolyzing)	1.22	1.02E-06	2.21E-05
Psed_0327	Ku protein	1.22	4.33E-06	7.03E-05
Psed_5405	nicotinamide nucleotide transhydrogenase alpha subunit 2 PntAB	1.22	3.71E-04	2.67E-03
Psed_1709	Aldehyde Dehydrogenase	1.22	8.23E-08	2.87E-06
Psed_6004	Nitrile hydratase	1.21	1.34E-03	7.51E-03
Psed_5773	hypothetical protein	1.21	6.92E-06	1.05E-04
Psed_0246	ABC-type transporter, integral membrane subunit	1.20	4.91E-05	5.25E-04
Psed_5916	Acetyl-CoA C-acetyltransferase	1.20	3.75E-07	9.53E-06

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Psed_0527	hypothetical protein	1.20	8.22E-08	2.87E-06
Psed_4789	FAD linked oxidase domain protein	1.20	1.18E-06	2.49E-05
Psed_3128	HpcH/HpaI aldolase	1.20	1.08E-08	5.35E-07
Psed_1453	Amidase	1.19	4.70E-04	3.24E-03
Psed_5456	hypothetical protein	1.19	3.72E-05	4.13E-04
Psed_0381	hypothetical protein	1.18	2.13E-04	1.71E-03
Psed_3919	regulatory protein GntR HTH	1.18	1.05E-04	9.86E-04
Psed_6980	hypothetical protein	1.18	3.01E-05	3.43E-04
Psed_3720	amidohydrolase 2	1.18	9.95E-05	9.44E-04
Psed_6563	hypothetical protein	1.17	4.44E-05	4.84E-04
Psed_1950	SNARE associated Golgi protein	1.17	5.55E-06	8.76E-05
Psed_0350	Bile acid:sodium symporter	1.17	1.08E-03	6.34E-03
Psed_6357	hydrogenase accessory protein HypB	1.16	4.05E-04	2.88E-03
Psed_1493	ATPase associated with various cellular activities AAA_5	1.16	2.55E-04	1.97E-03
Psed_6005	nitrile hydratase, alpha subunit	1.16	2.79E-05	3.25E-04
Psed_6882	filamentation induced by cAMP protein Fic	1.16	1.02E-03	6.07E-03
Psed_3719	cyclase family protein	1.16	1.52E-03	8.34E-03
Psed_4077	hypothetical protein	1.15	2.20E-09	1.42E-07
Psed_5406	NAD(P)(+) transhydrogenase (AB-specific)	1.15	1.04E-03	6.16E-03
Psed_3193	Cupin 2 conserved barrel domain protein	1.15	2.03E-07	5.75E-06
Psed_3699	Dimethylmenaquinone methyltransferase	1.14	5.75E-06	9.00E-05
Psed_0385	Radical SAM domain protein	1.14	6.76E-05	6.90E-04
Psed_3718	3-oxoacyl-[acyl-carrier-protein] reductase	1.13	1.46E-04	1.27E-03
Psed_0986	phospho-2-dehydro-3-deoxyheptonate aldolase	1.13	6.80E-06	1.04E-04
Psed_2612	Uncharacterised conserved protein UCP007542	1.13	6.99E-07	1.63E-05
Psed_1892	C-methyltransferase	1.11	2.72E-08	1.17E-06
Psed_0930	hypothetical protein	1.11	5.85E-08	2.15E-06
Psed_4331	protein of unknown function DUF955	1.11	1.75E-03	9.30E-03
Psed_2615	regulatory protein TetR	1.11	2.38E-04	1.87E-03
Psed_2784	Geranyltranstransferase	1.10	3.84E-06	6.33E-05
Psed_6550	pyridoxamine 5'-phosphate oxidase-related FMN-binding	1.10	5.36E-06	8.52E-05

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Psed_0567	hypothetical protein	1.10	1.76E-04	1.48E-03
Psed_0247	ABC-type transporter, integral membrane subunit	1.10	1.04E-03	6.14E-03
Psed_4788	D-lactate dehydrogenase (cytochrome)	1.10	1.36E-05	1.79E-04
Psed_3696	4Fe-4S ferredoxin iron-sulfur binding domain-containing protein	1.10	4.24E-06	6.91E-05
Psed_1913	hypothetical protein	1.09	1.61E-05	2.03E-04
Psed_3882	hypothetical protein	1.09	7.59E-08	2.71E-06
Psed_5267	protein of unknown function DUF692	1.09	3.51E-04	2.56E-03
Psed_5027	FAD-binding 9 siderophore-interacting domain protein	1.09	4.98E-04	3.38E-03
Psed_0895	Tetratricopeptide TPR_1 repeat-containing protein	1.09	1.80E-07	5.19E-06
Psed_6650	hypothetical protein	1.09	9.53E-04	5.75E-03
Psed_6601	CBS domain containing protein	1.08	2.04E-04	1.66E-03
Psed_0001	Chromosomal replication initiator protein dnaA	1.08	4.46E-06	7.21E-05
Psed_5289	lipolytic protein G-D-S-L family	1.07	2.78E-06	4.82E-05
Psed_5268	hypothetical protein	1.07	3.80E-05	4.22E-04
Psed_5604	DNA binding domain protein, excisionase family	1.07	2.73E-05	3.19E-04
Psed_6572	UDP-N-acetylglucosamine 2-epimerase	1.07	3.43E-07	8.91E-06
Psed_4271	hypothetical protein	1.07	7.53E-06	1.12E-04
Psed_3840	tRNA/rRNA methyltransferase (SpoU)	1.06	6.93E-04	4.45E-03
Psed_0425	Threonine aldolase	1.06	5.34E-08	1.99E-06
Psed_4421	alpha/beta hydrolase fold	1.06	9.71E-06	1.38E-04
Psed_2527	catechol 2,3 dioxygenase	1.06	1.84E-03	9.66E-03
Psed_5101	Phosphoglycerate mutase	1.06	2.96E-07	7.91E-06
Psed_3414	hypothetical protein	1.06	2.52E-05	2.99E-04
Psed_1587	Long-chain-fatty-acid--CoA ligase	1.05	4.86E-06	7.79E-05
Psed_5273	Dihydrolipoyl dehydrogenase	1.05	8.48E-06	1.23E-04
Psed_5114	Phosphoglycerate mutase	1.05	8.03E-06	1.19E-04
Psed_4693	GCN5-related N-acetyltransferase	1.04	4.61E-04	3.20E-03
Psed_5914	Hydantoinase B/oxoprolinase	1.04	1.12E-04	1.03E-03
Psed_4949	Hydroxypyruvate isomerase	1.04	1.90E-06	3.64E-05
Psed_2505	RNA polymerase sigma-70 factor, sigma-B/F/G subfamily	1.04	8.62E-05	8.42E-04
Psed_4662	processing peptidase	1.03	8.67E-05	8.45E-04
Psed_1540	Phosphonate-transporting ATPase	1.03	2.03E-05	2.48E-04
Psed_5373	hypothetical protein	1.03	2.14E-04	1.72E-03

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Psed_4552	regulatory protein TetR	1.03	4.04E-05	4.45E-04
Psed_6811	type IV secretory pathway VirD4 protein-like protein	1.03	1.43E-03	7.91E-03
Psed_6996	hypothetical protein	1.03	2.77E-06	4.82E-05
Psed_3960	Phosphonate-transporting ATPase	1.03	5.09E-05	5.40E-04
Psed_6309	hypothetical protein	1.03	1.17E-05	1.60E-04
Psed_2843	peptidase S16 lon domain protein	1.02	6.09E-05	6.35E-04
Psed_2499	Carbon-monoxide dehydrogenase (acceptor)	1.02	2.05E-05	2.49E-04
Psed_6599	N-acetylmuramoyl-L-alanine amidase family 2	1.02	7.91E-05	7.87E-04
Psed_3114	hypothetical protein	1.02	9.58E-06	1.37E-04
Psed_5113	putative F420-dependent oxidoreductase	1.01	1.12E-04	1.03E-03
Psed_3494	hypothetical protein	1.01	7.61E-06	1.13E-04
Psed_3698	Phthalate 4,5-dioxygenase	1.01	4.99E-04	3.39E-03
Psed_5348	death-on-curing family protein	1.01	4.19E-04	2.96E-03
Psed_5652	Long-chain-fatty-acid--CoA ligase	1.00	2.62E-05	3.08E-04
Psed_1456	RNA polymerase sigma factor, sigma-70 family	1.00	7.85E-06	1.16E-04
Psed_1450	nitrile hydratase b-subunit	1.00	1.33E-05	1.77E-04
Psed_3979	hydro-lyase, Fe-S type, tartrate/fumarate subfamily, beta subunit	-1.00	5.96E-04	3.95E-03
Psed_4517	Alkanesulfonate monooxygenase	-1.00	8.31E-05	8.20E-04
Psed_2791	hypothetical protein	-1.01	1.63E-04	1.39E-03
Psed_1726	hypothetical protein	-1.02	8.57E-06	1.24E-04
Psed_1037	Asp/Glu/hydantoin racemase	-1.02	7.11E-05	7.17E-04
Psed_5330	antigen 34 kDa family protein	-1.02	5.22E-04	3.52E-03
Psed_1348	GntR domain protein	-1.02	1.63E-06	3.29E-05
Psed_1785	Serine--pyruvate transaminase	-1.03	9.60E-05	9.17E-04
Psed_4845	gluconate kinase	-1.03	1.05E-03	6.19E-03
Psed_5608	ResB family protein	-1.03	5.36E-04	3.59E-03
Psed_2778	phospho-2-dehydro-3-deoxyheptonate aldolase	-1.03	1.28E-04	1.15E-03
Psed_6951	hypothetical protein	-1.04	5.16E-05	5.46E-04
Psed_6169	Glyoxalase/bleomycin resistance protein/dioxygenase	-1.04	2.49E-06	4.46E-05
Psed_6170	regulatory protein MerR	-1.04	4.50E-05	4.87E-04
Psed_6893	hypothetical protein	-1.04	6.85E-05	6.96E-04
Psed_1585	hypothetical protein	-1.04	3.24E-04	2.39E-03
Psed_5701	hypothetical protein	-1.04	2.29E-06	4.22E-05

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Psed_6792	hypothetical protein	-1.04	2.90E-04	2.19E-03
Psed_3039	ABC-type transporter, integral membrane subunit	-1.05	8.45E-05	8.31E-04
Psed_4067	protein of unknown function DUF1470	-1.06	2.15E-05	2.60E-04
Psed_5147	Methyltransferase type 11	-1.06	1.11E-05	1.54E-04
Psed_6948	transposase mutator type	-1.06	2.00E-05	2.45E-04
Psed_1519	Ribonuclease PH	-1.07	1.12E-05	1.56E-04
Psed_1710	3-hydroxyacyl-CoA dehydrogenase., 3-hydroxybutyryl-CoA dehydrogenase	-1.07	1.40E-03	7.80E-03
Psed_5567	NAD(P)H-quinone oxidoreductase subunit J	-1.07	4.92E-04	3.36E-03
Psed_3424	hypothetical protein	-1.07	1.30E-07	4.09E-06
Psed_1727	RNA polymerase sigma factor, sigma-70 family	-1.07	6.97E-04	4.46E-03
Psed_5568	NAD(P)H-quinone oxidoreductase subunit K	-1.08	6.66E-05	6.83E-04
Psed_0565	Heavy metal transport/detoxification protein	-1.08	1.15E-03	6.66E-03
Psed_3677	squalene synthase HpnD	-1.08	9.60E-05	9.17E-04
Psed_4655	protein of unknown function DUF1778	-1.08	9.62E-05	9.17E-04
Psed_5637	Ppx/GppA phosphatase	-1.09	1.62E-07	4.81E-06
Psed_3585	Crossover junction endodeoxyribonuclease ruvC	-1.09	2.65E-06	4.69E-05
Psed_4172	ABC-type sugar transport system periplasmic component-like	-1.10	1.55E-06	3.15E-05
Psed_1993	ATP-dependent DNA helicase, RecQ family	-1.10	9.50E-06	1.36E-04
Psed_3350	Succinate-semialdehyde dehydrogenase	-1.10	4.45E-05	4.84E-04
Psed_5149	ErfK/YbiS/YcfS/YnhG family protein	-1.10	3.13E-05	3.54E-04
Psed_5186	NUDIX hydrolase	-1.10	1.58E-03	8.56E-03
Psed_6817	hypothetical protein	-1.10	4.06E-04	2.88E-03
Psed_2700	phenylacetate-CoA oxygenase, PaaG subunit	-1.11	4.83E-05	5.18E-04
Psed_1966	Formyl-CoA transferase	-1.11	5.38E-07	1.29E-05
Psed_0450	hypothetical protein	-1.12	4.99E-06	7.94E-05
Psed_4012	hypothetical protein	-1.12	4.11E-06	6.70E-05
Psed_5869	cytochrome c oxidase, subunit I	-1.12	1.04E-09	7.84E-08
Psed_1447	Phosphoribosylaminoimidazolecarboxamide formyltransferase	-1.12	1.00E-06	2.19E-05
Psed_3198	hypothetical protein	-1.13	1.52E-03	8.34E-03

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Psed_5563	NADH-quinone oxidoreductase, chain G	-1.13	2.96E-04	2.23E-03
Psed_1934	protein of unknown function DUF214	-1.13	2.22E-04	1.77E-03
Psed_1648	hypothetical protein	-1.14	5.57E-05	5.85E-04
Psed_5485	phenylacetic acid degradation-related protein	-1.14	4.33E-07	1.07E-05
Psed_3530	major facilitator superfamily MFS_1	-1.14	1.43E-04	1.25E-03
Psed_4021	Bifunctional protein folD	-1.15	8.51E-04	5.24E-03
Psed_5871	hypothetical protein	-1.16	4.71E-04	3.24E-03
Psed_6185	drug resistance transporter, Bcr/CflA subfamily	-1.16	4.05E-06	6.62E-05
Psed_5732	Transglycosylase-like domain protein	-1.16	1.28E-04	1.15E-03
Psed_0886	hypothetical protein	-1.16	4.84E-04	3.32E-03
Psed_3038	Sulfate-transporting ATPase	-1.16	7.35E-06	1.11E-04
Psed_6721	regulatory protein ArsR	-1.17	6.84E-06	1.04E-04
Psed_0459	transcriptional regulator domain-containing protein	-1.18	7.50E-05	7.50E-04
Psed_4656	hypothetical protein	-1.18	1.46E-04	1.27E-03
Psed_5187	Adenosylhomocysteinase	-1.18	6.76E-04	4.36E-03
Psed_3524	carbamoyl-phosphate synthase, large subunit	-1.18	1.48E-04	1.28E-03
Psed_6890	hypothetical protein	-1.18	2.03E-05	2.47E-04
Psed_0011	hypothetical protein	-1.19	1.23E-05	1.67E-04
Psed_2650	hypothetical protein	-1.20	4.21E-07	1.04E-05
Psed_1769	xanthine permease	-1.20	2.02E-04	1.65E-03
Psed_2125	ribonucleoside-diphosphate reductase, adenosylcobalamin-dependent	-1.21	1.05E-04	9.83E-04
Psed_1880	glycosyl transferase family 2	-1.21	1.57E-03	8.55E-03
Psed_3651	hypothetical protein	-1.22	2.87E-04	2.17E-03
Psed_3937	hypothetical protein	-1.22	9.70E-06	1.38E-04
Psed_3658	RNA polymerase sigma-70 factor	-1.22	4.61E-09	2.63E-07
Psed_3017	precorrin-6y C5,15-methyltransferase (decarboxylating), CbiE subunit	-1.22	6.22E-05	6.47E-04
Psed_2038	sporulation and cell division protein SsgA	-1.23	8.08E-05	8.02E-04
Psed_6301	dienelactone hydrolase	-1.23	2.01E-04	1.64E-03
Psed_5899	hypothetical protein	-1.23	3.18E-06	5.39E-05
Psed_1828	monooxygenase FAD-binding	-1.24	6.90E-07	1.61E-05
Psed_6105	hypothetical protein	-1.24	3.86E-07	9.67E-06

Appendix 7. Genes differentially expressed on succinate relative to pyruvate.

Psed_0390	hypothetical protein	-1.24	3.89E-04	2.78E-03
Psed_4627	YceI family protein	-1.24	1.84E-05	2.28E-04
Psed_3679	Dimethylallyltranstransferase	-1.24	7.95E-04	4.96E-03
Psed_1021	DNA-3-methyladenine glycosylase I	-1.25	6.99E-06	1.06E-04
Psed_4003	hypothetical protein	-1.26	1.78E-06	3.51E-05
Psed_2887	diguanylate cyclase	-1.27	5.94E-06	9.26E-05
Psed_6642	Fe(3+)-transporting ATPase	-1.27	1.12E-03	6.52E-03
Psed_4548	GCN5-related N-acetyltransferase	-1.27	4.70E-04	3.24E-03
Psed_2352	methionine aminopeptidase, type I	-1.27	6.28E-04	4.12E-03
Psed_2021	DoxX family protein	-1.27	2.43E-05	2.90E-04
Psed_5874	ErfK/YbiS/YcfS/YnhG family protein	-1.28	1.34E-07	4.14E-06
Psed_4819	integral membrane protein	-1.28	6.21E-04	4.09E-03
Psed_3278	LmbE family protein	-1.29	2.84E-04	2.15E-03
Psed_7017	hypothetical protein	-1.29	8.03E-08	2.84E-06
Psed_2303	Glutamate dehydrogenase (NADP(+))	-1.30	1.66E-06	3.33E-05
Psed_1024	protein of unknown function DUF1470	-1.30	3.61E-06	6.02E-05
Psed_3871	hypothetical protein	-1.30	1.82E-04	1.51E-03
Psed_1054	1-aminocyclopropane-1-carboxylate deaminase	-1.30	5.21E-04	3.52E-03
Psed_5562	NADH dehydrogenase (quinone)	-1.30	3.59E-04	2.60E-03
Psed_0082	Monosaccharide-transporting ATPase	-1.31	1.01E-07	3.39E-06
Psed_0282	esterase	-1.31	3.53E-07	9.11E-06
Psed_4111	Formyl-CoA transferase	-1.34	2.38E-06	4.35E-05
Psed_3926	protein of unknown function DUF1707	-1.34	2.76E-04	2.11E-03
Psed_0013	hypothetical protein	-1.35	1.02E-06	2.21E-05
Psed_2129	DNA binding domain protein, excisionase family	-1.36	4.80E-05	5.17E-04
Psed_0580	highly repetitive protein	-1.37	2.41E-04	1.88E-03
Psed_1107	peptidase C60 sortase A and B	-1.38	2.89E-05	3.33E-04
Psed_0081	ABC-type transporter, integral membrane subunit	-1.38	6.48E-06	9.98E-05
Psed_3863	hypothetical protein	-1.39	1.96E-05	2.41E-04
Psed_5317	Error-prone DNA polymerase	-1.39	1.84E-06	3.57E-05
Psed_4688	polysaccharide deacetylase	-1.40	4.77E-07	1.16E-05
Psed_3977	Ureidoglycolate lyase	-1.41	7.52E-04	4.76E-03
Psed_3163	Glycine dehydrogenase [decarboxylating]	-1.44	1.60E-06	3.24E-05
Psed_1772	Electron transfer flavoprotein alpha subunit	-1.44	1.02E-04	9.61E-04

Appendix 7. Genes differentially expressed on succinate relative to pyruvate.

Psed_2846	L-aspartate oxidase	-1.45	3.56E-07	9.12E-06
Psed_3124	Methylmalonyl-CoA mutase	-1.46	1.72E-07	5.02E-06
Psed_1694	ectoine/hydroxyectoine ABC transporter, permease protein EhuC	-1.46	1.11E-05	1.54E-04
Psed_3928	major facilitator superfamily MFS_1	-1.48	1.09E-07	3.63E-06
Psed_1771	Electron transfer flavoprotein alpha/beta-subunit	-1.48	7.33E-05	7.36E-04
Psed_0080	ABC-type transporter, integral membrane subunit	-1.49	1.24E-08	6.03E-07
Psed_0958	Fumarate hydratase class II	-1.50	3.40E-07	8.89E-06
Psed_2112	Cupin 2 conserved barrel domain protein	-1.50	3.90E-05	4.31E-04
Psed_5324	hypothetical protein	-1.52	5.68E-09	3.05E-07
Psed_6606	hypothetical protein	-1.52	6.00E-07	1.42E-05
Psed_6554	Protein grpE	-1.54	1.79E-04	1.49E-03
Psed_3174	hypothetical protein	-1.55	1.83E-06	3.56E-05
Psed_5209	Peroxiredoxin	-1.56	2.06E-09	1.35E-07
Psed_4109	HpcH/HpaI aldolase	-1.58	3.69E-06	6.14E-05
Psed_6892	hypothetical protein	-1.59	1.66E-08	7.69E-07
Psed_6914	amidohydrolase	-1.59	1.42E-07	4.32E-06
Psed_1693	ectoine/hydroxyectoine ABC transporter solute-binding protein	-1.59	1.09E-03	6.39E-03
Psed_3675	PucR family transcriptional regulator	-1.63	1.86E-06	3.59E-05
Psed_0967	RmuC-domain protein	-1.64	4.60E-08	1.78E-06
Psed_2763	hypothetical protein	-1.65	4.67E-07	1.14E-05
Psed_0951	hypothetical protein	-1.65	2.55E-06	4.55E-05
Psed_5472	regulatory protein LuxR	-1.67	1.22E-08	5.99E-07
Psed_5355	regulatory protein LuxR	-1.67	1.38E-06	2.84E-05
Psed_1825	D-3-phosphoglycerate dehydrogenase	-1.70	1.17E-04	1.07E-03
Psed_1060	magnesium and cobalt transport protein CorA	-1.70	3.77E-08	1.50E-06
Psed_3023	cobaltochelatase, CobN subunit	-1.70	9.84E-10	7.54E-08
Psed_2122	LexA repressor	-1.71	2.01E-05	2.46E-04
Psed_5870	hypothetical protein	-1.75	9.21E-08	3.16E-06
Psed_3995	ABC-type transporter, periplasmic subunit	-1.75	1.82E-07	5.23E-06
Psed_5868	hypothetical protein	-1.82	9.38E-06	1.35E-04
Psed_2318	regulatory protein MerR	-1.83	5.98E-06	9.29E-05
Psed_4110	MmgE/PrpD family protein	-1.83	2.13E-06	3.96E-05

Psed_5488	FAD-dependent pyridine nucleotide-disulphide oxidoreductase	-1.84	1.15E-06	2.45E-05
Psed_1117	hypothetical protein	-1.84	2.93E-07	7.88E-06
Psed_1653	hypothetical protein	-1.84	3.92E-08	1.55E-06
Psed_5825	diaminobutyrate/2-oxoglutarate amino-transferase	-1.87	1.11E-04	1.03E-03
Psed_4176	GntR domain protein	-1.88	1.93E-06	3.67E-05
Psed_2284	CBS domain containing protein	-1.89	4.86E-04	3.33E-03
Psed_6096	ABC-type transporter, integral mem-brane subunit	-1.89	2.49E-06	4.46E-05
Psed_1654	hypothetical protein	-1.93	3.65E-08	1.47E-06
Psed_0950	hypothetical protein	-1.94	6.76E-05	6.90E-04
Psed_0106	putative Fe-S oxidoreductase	-1.96	1.14E-05	1.57E-04
Psed_5826	L-2,4-diaminobutyric acid acetyltrans-ferase	-1.97	1.62E-08	7.53E-07
Psed_0902	NADH dehydrogenase (ubiquinone)	-1.98	5.72E-08	2.11E-06
Psed_6645	hypothetical protein	-1.99	7.24E-08	2.60E-06
Psed_3037	ABC-type metal ion transporter, peri-plasmic subunit	-1.99	1.35E-07	4.15E-06
Psed_2123	Peptidoglycan-binding lysin domain	-2.03	1.76E-09	1.22E-07
Psed_1116	transcription factor WhiB	-2.06	3.51E-09	2.08E-07
Psed_3591	hypothetical protein	-2.10	2.93E-08	1.23E-06
Psed_3123	cytochrome bd ubiquinol oxidase sub-unit I	-2.11	1.24E-04	1.12E-03
Psed_3047	hypothetical protein	-2.12	1.30E-03	7.34E-03
Psed_3048	AmiS/UreI transporter	-2.12	7.53E-04	4.76E-03
Psed_6168	Phosphomethylpyrimidine synthase	-2.17	7.53E-06	1.12E-04
Psed_0137	copper resistance D domain protein	-2.18	9.12E-08	3.14E-06
Psed_1656	hypothetical protein	-2.19	2.56E-09	1.59E-07
Psed_2789	hypothetical protein	-2.22	5.89E-09	3.12E-07
Psed_6805	hypothetical protein	-2.23	1.67E-04	1.41E-03
Psed_5843	hypothetical protein	-2.24	2.26E-06	4.19E-05
Psed_1023	hypothetical protein	-2.27	2.09E-08	9.46E-07
Psed_3903	hypothetical protein	-2.29	3.56E-07	9.12E-06
Psed_5872	hypothetical protein	-2.33	1.46E-07	4.42E-06
Psed_1657	Cobalt transporter subunit CbtB putative	-2.34	1.25E-08	6.03E-07
Psed_2845	Quinolinate synthase A	-2.37	2.07E-07	5.84E-06
Psed_4532	band 7 protein	-2.38	5.73E-07	1.37E-05
Psed_3571	Radical SAM domain protein	-2.43	1.11E-08	5.50E-07
Psed_1655	hypothetical protein	-2.50	2.87E-11	3.53E-09

Appendix 7. Genes differentially expressed on succinate relative to pyruvate.

Psed_0140	hypothetical protein	-2.57	3.15E-07	8.31E-06
Psed_2296	hypothetical protein	-2.60	1.79E-03	9.51E-03
Psed_4840	CBS domain containing protein	-2.60	2.11E-04	1.70E-03
Psed_6641	ABC-type transporter, integral membrane subunit	-2.65	9.73E-07	2.13E-05
Psed_4844	glyceraldehyde-3-phosphate dehydrogenase, type I	-2.66	2.18E-04	1.74E-03
Psed_3440	SH3 type 3 domain protein	-2.66	1.37E-07	4.18E-06
Psed_3594	Tellurite resistance TerB	-2.67	1.37E-06	2.84E-05
Psed_5844	hypothetical protein	-2.68	6.25E-07	1.47E-05
Psed_6095	Fe(3+)-transporting ATPase	-2.70	1.00E-05	1.41E-04
Psed_3904	CO dehydrogenase maturation factor-like protein	-2.75	1.64E-07	4.83E-06
Psed_3589	stress protein	-2.78	8.64E-10	6.70E-08
Psed_0138	copper resistance protein CopC	-2.83	6.33E-07	1.49E-05
Psed_2203	cobalamin synthesis CobW domain protein	-2.89	1.56E-08	7.31E-07
Psed_3590	Tellurium resistance	-3.16	2.47E-08	1.08E-06
Psed_0276	major facilitator superfamily MFS_1	-3.20	1.47E-11	1.99E-09
Psed_0139	nuclear export factor GLE1	-3.23	7.09E-07	1.64E-05
Psed_6094	ABC-type metal ion transporter, periplasmic subunit	-3.31	3.48E-08	1.43E-06
Psed_2204	50S ribosomal protein L33	-3.54	1.96E-09	1.30E-07
Psed_1421	ferric-uptake regulator	-3.56	1.44E-09	1.04E-07
Psed_0079	ABC-type transporter, periplasmic subunit	-3.80	5.47E-11	5.84E-09
Psed_6640	extracellular solute-binding protein family 1	-3.81	5.77E-09	3.08E-07
Psed_5195	alanine dehydrogenase	-3.92	6.31E-09	3.31E-07
Psed_5471	hypothetical protein	-4.00	2.54E-10	2.43E-08
Psed_0078	Methyltransferase type 12	-4.21	9.77E-14	6.36E-11
Psed_1422	Ferritin Dps family protein	-4.23	4.53E-12	7.55E-10
Psed_3670	Butyryl-CoA dehydrogenase	-5.33	2.96E-07	7.91E-06

Appendix 8

Genes differentially expressed on succinate relative to pyruvate.

Appendix 8. Genes differentially expressed on succinate relative to pyruvate. Differentially expressed genes have an adjusted p-value < 0.01 and have a $log_2FC > 1$ or $log_2FC < -1$ relative to pyruvate and are sorted by log_2FC.

Genes	Protein	log_2FC	p-value	adjusted p-value
Psed_4939	lipase	6.70	1.02E-16	2.21E-13
Psed_4940	fumarylacetoacetate (FAA) hydrolase	5.21	1.18E-12	6.82E-10
Psed_3486	hypothetical protein	5.04	7.13E-11	1.33E-08
Psed_3500	glucose-6-phosphate 1-dehydrogenase	4.90	1.14E-11	3.36E-09
Psed_4700	Cyclopropane-fatty-acyl-phospholipid synthase	4.86	9.82E-10	9.98E-08
Psed_3496	hypothetical protein	4.78	3.74E-12	1.43E-09
Psed_3481	ATP-dependent chaperone ClpB	4.62	1.69E-10	2.62E-08
Psed_3485	Chaperone protein dnaK	4.60	3.18E-11	7.15E-09
Psed_3451	Domain of unknown function DUF1931	4.47	2.06E-11	4.85E-09
Psed_3446	Ferritin Dps family protein	4.47	1.70E-12	7.91E-10
Psed_3484	Protein grpE	4.47	1.52E-11	3.97E-09
Psed_3508	intracellular protease, PfpI family	4.39	1.31E-11	3.71E-09
Psed_5371	hypothetical protein	4.37	1.30E-06	2.64E-05
Psed_5248	nitroreductase	4.37	9.09E-14	9.86E-11
Psed_3497	transport-associated	4.36	4.80E-13	3.42E-10
Psed_3460	formaldehyde dehydrogenase, glutathione-independent	4.25	1.04E-11	3.21E-09
Psed_3487	heat shock protein Hsp20	4.19	2.18E-10	3.02E-08
Psed_3483	chaperone DnaJ domain protein	4.19	3.94E-12	1.43E-09
Psed_5783	hypothetical protein	4.12	5.99E-09	4.19E-07
Psed_3473	Pyruvate dehydrogenase (cytochrome)	4.07	1.12E-10	1.92E-08
Psed_3461	transport-associated	3.89	2.08E-10	3.02E-08
Psed_3463	hypothetical protein	3.89	1.50E-10	2.44E-08
Psed_5787	hypothetical protein	3.87	4.27E-11	8.96E-09
Psed_5799	hypothetical protein	3.86	3.98E-11	8.63E-09
Psed_5795	hypothetical protein	3.83	2.22E-09	1.92E-07
Psed_5800	hypothetical protein	3.80	4.49E-11	9.13E-09
Psed_5784	hypothetical protein	3.79	1.04E-10	1.82E-08
Psed_5811	hypothetical protein	3.75	1.09E-09	1.08E-07
Psed_3477	Erythromycin esterase	3.72	1.26E-12	6.82E-10
Psed_1555	peptidase S1 and S6 chymotrypsin/Hap	3.71	1.50E-05	1.68E-04
Psed_5814	hypothetical protein	3.71	5.00E-08	2.10E-06
Psed_5801	hypothetical protein	3.68	3.17E-09	2.43E-07
Psed_3480	thioredoxin	3.66	2.29E-10	3.11E-08

Psed_5804	hypothetical protein	3.66	9.54E-09	6.21E-07
Psed_3482	hypothetical protein	3.61	2.43E-09	2.02E-07
Psed_5796	hypothetical protein	3.57	1.44E-08	8.36E-07
Psed_3490	major facilitator superfamily MFS_1	3.56	7.66E-11	1.38E-08
Psed_6982	Mn2+/Fe2+ transporter, NRAMP family	3.46	6.49E-08	2.56E-06
Psed_5249	NADPH:quinone reductase	3.45	1.31E-10	2.19E-08
Psed_5792	hypothetical protein	3.45	1.01E-07	3.56E-06
Psed_5786	phage terminase	3.43	1.33E-08	7.96E-07
Psed_3453	hypothetical protein	3.42	8.06E-10	8.32E-08
Psed_3495	protein of unknown function DUF308 membrane	3.40	2.14E-09	1.88E-07
Psed_5782	Collagen triple helix repeat-containing protein	3.39	4.77E-08	2.02E-06
Psed_5788	peptidase S14 ClpP	3.39	1.23E-09	1.16E-07
Psed_4701	Cyclopropane-fatty-acyl-phospholipid synthase	3.38	8.77E-07	1.95E-05
Psed_3513	Helix-turn-helix, AraC domain	3.36	4.92E-09	3.56E-07
Psed_6981	Aldehyde Dehydrogenase	3.35	7.10E-10	7.63E-08
Psed_3256	hypothetical protein	3.34	2.08E-07	6.17E-06
Psed_3443	hypothetical protein	3.34	1.41E-08	8.30E-07
Psed_3447	hypothetical protein	3.32	2.85E-09	2.32E-07
Psed_3462	1-deoxy-D-xylulose-5-phosphate synthase	3.32	6.81E-12	2.33E-09
Psed_3780	conserved hypothetical protein	3.28	3.84E-10	4.81E-08
Psed_3472	protein of unknown function (DUF461)	3.26	1.48E-11	3.97E-09
Psed_5798	hypothetical protein	3.24	1.88E-08	1.01E-06
Psed_4702	protein of unknown function DUF1295	3.20	1.01E-06	2.18E-05
Psed_5797	hypothetical protein	3.12	1.45E-08	8.36E-07
Psed_5809	hypothetical protein	3.02	1.09E-08	6.63E-07
Psed_4703	alkyl hydroperoxide reductase/ Thiol specific antioxidant/ Mal allergen	2.96	2.13E-06	3.87E-05
Psed_6980	hypothetical protein	2.93	4.85E-10	5.64E-08
Psed_3456	Thioredoxin-disulfide reductase	2.91	2.09E-10	3.02E-08
Psed_5791	hypothetical protein	2.90	2.18E-10	3.02E-08
Psed_5815	hypothetical protein	2.88	1.99E-07	6.01E-06
Psed_5794	hypothetical protein	2.87	9.74E-09	6.22E-07
Psed_3503	PDZ/DHR/GLGF domain-containing protein	2.84	6.48E-08	2.56E-06
Psed_4938	regulatory protein MarR	2.83	4.31E-08	1.92E-06
Psed_6807	hypothetical protein	2.79	1.41E-06	2.82E-05

Appendix 8. Genes differentially expressed on succinate relative to pyruvate.

Psed_5802	hypothetical protein	2.79	2.53E-08	1.26E-06
Psed_5789	hypothetical protein	2.79	1.31E-06	2.67E-05
Psed_4698	amine oxidase	2.78	4.51E-06	7.00E-05
Psed_4941	Formyl-CoA transferase	2.78	4.30E-08	1.92E-06
Psed_3478	RNA polymerase sigma-70 factor, sigma-B/F/G subfamily	2.75	3.69E-09	2.76E-07
Psed_0922	hypothetical protein	2.74	2.58E-10	3.42E-08
Psed_5805	hypothetical protein	2.72	9.47E-07	2.07E-05
Psed_6970	D-lactate dehydrogenase (cytochrome)	2.71	2.74E-06	4.71E-05
Psed_3476	dienelactone hydrolase	2.68	6.70E-09	4.58E-07
Psed_5539	hypothetical protein	2.67	2.09E-05	2.17E-04
Psed_3475	hypothetical protein	2.63	5.05E-13	3.42E-10
Psed_5812	hypothetical protein	2.62	7.60E-07	1.74E-05
Psed_3444	hypothetical protein	2.59	1.67E-10	2.62E-08
Psed_3448	hypothetical protein	2.59	6.25E-11	1.20E-08
Psed_5793	hypothetical protein	2.59	8.90E-08	3.27E-06
Psed_5790	hypothetical protein	2.58	1.98E-06	3.63E-05
Psed_0934	YCII-related	2.58	9.97E-09	6.30E-07
Psed_0531	G3E family GTPase	2.54	1.27E-07	4.29E-06
Psed_3918	FAD linked oxidase domain protein	2.54	5.78E-04	3.03E-03
Psed_4697	hypothetical protein	2.52	6.71E-05	5.45E-04
Psed_3493	ParB domain protein nuclease	2.50	6.87E-09	4.61E-07
Psed_6977	Ferredoxin--NAD(+) reductase	2.48	7.64E-10	8.02E-08
Psed_3504	dienelactone hydrolase	2.48	1.00E-07	3.56E-06
Psed_3470	MscS Mechanosensitive ion channel	2.47	1.74E-11	4.37E-09
Psed_0094	YCII-related	2.46	3.95E-08	1.82E-06
Psed_3514	glycosyl transferase group 1	2.42	3.04E-09	2.39E-07
Psed_5808	hypothetical protein	2.40	2.32E-06	4.15E-05
Psed_3450	hypothetical protein	2.39	2.11E-06	3.85E-05
Psed_5759	hypothetical protein	2.39	1.65E-06	3.16E-05
Psed_3510	transcription elongation factor GreA	2.33	1.72E-06	3.24E-05
Psed_5736	Transglycosylase-like domain protein	2.30	4.07E-06	6.47E-05
Psed_3501	hypothetical protein	2.30	1.25E-08	7.51E-07
Psed_3669	hypothetical protein	2.28	1.13E-07	3.92E-06
Psed_5758	hypothetical protein	2.27	1.90E-07	5.81E-06
Psed_5785	hypothetical protein	2.24	5.15E-08	2.14E-06
Psed_6979	monooxygenase component MmoB/ DmpM	2.23	1.07E-09	1.07E-07
Psed_5760	hypothetical protein	2.22	2.71E-06	4.68E-05

Appendix 8. Genes differentially expressed on succinate relative to pyruvate.

Psed_4322	Dihydrolipoyl dehydrogenase	2.21	4.29E-10	5.18E-08
Psed_5810	hypothetical protein	2.20	2.64E-06	4.59E-05
Psed_6665	NLP/P60 protein	2.19	1.42E-05	1.63E-04
Psed_5813	hypothetical protein	2.18	2.83E-06	4.85E-05
Psed_0130	amidohydrolase 2	2.17	1.45E-06	2.87E-05
Psed_6971	Hydroxyacid-oxoacid transhydrogenase	2.17	1.52E-06	3.00E-05
Psed_4958	hypothetical protein	2.13	1.23E-06	2.54E-05
Psed_1968	hypothetical protein	2.11	6.69E-10	7.38E-08
Psed_3458	hypothetical protein	2.11	2.30E-08	1.17E-06
Psed_3522	response regulator receiver	2.11	1.68E-06	3.20E-05
Psed_5247	hypothetical protein	2.10	3.61E-07	9.49E-06
Psed_4268	membrane protein of uknown function UCP014873	2.06	3.83E-05	3.52E-04
Psed_0554	hypothetical protein	2.06	5.33E-04	2.85E-03
Psed_5761	hypothetical protein	2.02	1.30E-07	4.35E-06
Psed_5779	hypothetical protein	2.01	1.53E-09	1.40E-07
Psed_5803	hypothetical protein	2.01	4.74E-07	1.19E-05
Psed_0131	Alcohol dehydrogenase GroES domain protein	1.99	5.99E-07	1.44E-05
Psed_3515	hypothetical protein	1.95	1.41E-08	8.30E-07
Psed_3457	polyphosphate:nucleotide phosphotransferase, PPK2 family	1.95	1.72E-08	9.39E-07
Psed_4478	hypothetical protein	1.94	3.33E-09	2.52E-07
Psed_3474	membrane protein	1.91	4.23E-07	1.09E-05
Psed_1967	major facilitator superfamily MFS_1	1.85	7.15E-10	7.63E-08
Psed_4755	ammonium transporter	1.85	1.45E-05	1.64E-04
Psed_5747	hypothetical protein	1.83	8.93E-09	5.87E-07
Psed_1178	hypothetical protein	1.82	1.48E-05	1.66E-04
Psed_6974	Ethyl tert-butyl ether degradation EthD	1.82	4.45E-09	3.25E-07
Psed_6879	S-adenosylmethionine synthase	1.82	1.10E-04	8.09E-04
Psed_5757	hypothetical protein	1.79	5.66E-06	8.17E-05
Psed_0288	S-(hydroxymethyl)glutathione dehydrogenase	1.76	1.58E-08	8.80E-07
Psed_5753	hypothetical protein	1.73	7.68E-08	2.97E-06
Psed_3111	Hemerythrin HHE cation binding domain protein	1.67	2.29E-05	2.32E-04
Psed_5621	beta-Ig-H3/fasciclin	1.66	2.81E-04	1.69E-03
Psed_6972	GntR domain protein	1.66	9.09E-05	6.94E-04
Psed_3564	Aromatic-amino-acid transaminase	1.66	1.43E-05	1.63E-04

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Psed_2039	RNA polymerase sigma factor, sigma-70 family	1.65	4.18E-06	6.62E-05
Psed_5246	uncharacterized peroxidase-related enzyme	1.64	3.71E-06	6.01E-05
Psed_5762	hypothetical protein	1.63	9.96E-08	3.56E-06
Psed_6975	Betaine-aldehyde dehydrogenase	1.63	4.77E-10	5.64E-08
Psed_4699	protein of unknown function DUF1365	1.62	4.87E-05	4.24E-04
Psed_0129	protein of unknown function DUF59	1.62	4.75E-06	7.26E-05
Psed_6600	acyl-CoA dehydrogenase domain-containing protein	1.61	1.17E-03	5.17E-03
Psed_5176	hypothetical protein	1.61	2.15E-05	2.21E-04
Psed_6978	methane/phenol/toluene hydroxylase	1.59	1.72E-09	1.55E-07
Psed_1982	hypothetical protein	1.59	4.78E-05	4.16E-04
Psed_6780	hypothetical protein	1.57	2.93E-07	7.91E-06
Psed_5769	hypothetical protein	1.55	2.63E-07	7.37E-06
Psed_4321	hypothetical protein	1.55	1.50E-05	1.68E-04
Psed_3652	hypothetical protein	1.52	1.05E-05	1.32E-04
Psed_0095	RNA polymerase sigma factor, sigma-70 family	1.52	2.62E-08	1.28E-06
Psed_5767	hypothetical protein	1.50	2.04E-07	6.11E-06
Psed_6650	hypothetical protein	1.48	5.86E-05	4.96E-04
Psed_6426	Copper amine oxidase domain-containing protein	1.47	5.34E-07	1.30E-05
Psed_5766	hypothetical protein	1.43	2.82E-07	7.73E-06
Psed_5768	hypothetical protein	1.43	5.19E-09	3.67E-07
Psed_2843	peptidase S16 lon domain protein	1.42	1.78E-06	3.32E-05
Psed_5681	Radical SAM domain protein	1.39	6.23E-06	8.77E-05
Psed_3489	Protein of unknown function DUF2267	1.38	1.77E-08	9.62E-07
Psed_5405	nicotinamide nucleotide transhydrogenase alpha subunit 2 PntAB	1.37	1.27E-04	8.91E-04
Psed_5780	hypothetical protein	1.37	2.28E-05	2.32E-04
Psed_6812	hypothetical protein	1.37	8.28E-07	1.85E-05
Psed_5763	hypothetical protein	1.35	2.19E-05	2.24E-04
Psed_3502	hypothetical protein	1.35	2.00E-08	1.07E-06
Psed_4754	nitrogen regulatory protein P-II	1.34	4.42E-05	3.94E-04
Psed_5778	cell divisionFtsK/SpoIIIE	1.33	5.35E-07	1.30E-05
Psed_0551	Glutaryl-CoA dehydrogenase	1.33	7.81E-04	3.85E-03
Psed_6427	amino acid permease-associated region	1.32	1.69E-05	1.84E-04
Psed_3449	NADH dehydrogenase (ubiquinone)	1.31	1.13E-07	3.92E-06
Psed_6806	hypothetical protein	1.31	3.95E-05	3.62E-04

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Psed_5749	hypothetical protein	1.31	1.00E-04	7.51E-04
Psed_5764	hypothetical protein	1.31	7.23E-05	5.77E-04
Psed_5404	NAD(P)(+) transhydrogenase (AB-specific)	1.29	2.95E-06	5.03E-05
Psed_5770	hypothetical protein	1.29	5.93E-05	4.99E-04
Psed_5781	hypothetical protein	1.29	5.85E-04	3.07E-03
Psed_5081	DoxX family protein	1.28	2.68E-04	1.63E-03
Psed_3445	hypothetical protein	1.28	7.41E-08	2.89E-06
Psed_0473	hypothetical protein	1.28	2.91E-09	2.34E-07
Psed_3042	NADH dehydrogenase (quinone)	1.27	1.55E-04	1.04E-03
Psed_0355	hypothetical protein	1.26	3.33E-07	8.93E-06
Psed_6160	heavy metal translocating P-type ATPase	1.24	3.16E-05	2.99E-04
Psed_4083	hypothetical protein	1.23	1.26E-04	8.85E-04
Psed_5135	malic protein NAD-binding	1.23	3.17E-04	1.86E-03
Psed_0088	protein of unknown function DUF485	1.22	1.00E-05	1.27E-04
Psed_2321	ABC-type sugar transport system periplasmic component-like	1.22	8.99E-05	6.89E-04
Psed_5755	hypothetical protein	1.21	8.20E-08	3.14E-06
Psed_5082	YVTN beta-propeller repeat-containing protein	1.20	2.95E-09	2.34E-07
Psed_5224	hypothetical protein	1.19	2.52E-03	9.71E-03
Psed_0382	hypothetical protein	1.16	1.80E-03	7.36E-03
Psed_5756	hypothetical protein	1.16	2.08E-04	1.31E-03
Psed_6066	4-hydroxyphenylacetate 3-hydroxylase	1.16	3.15E-05	2.98E-04
Psed_6878	hypothetical protein	1.16	1.37E-07	4.54E-06
Psed_6249	Ribulose bisphosphate carboxylase large chain	1.15	7.30E-05	5.81E-04
Psed_4753	nitrogen regulatory protein P-II	1.15	2.38E-05	2.38E-04
Psed_4578	Stearoyl-CoA 9-desaturase	1.14	2.29E-03	8.96E-03
Psed_4115	Protein of unknown function DUF2236	1.14	6.01E-04	3.14E-03
Psed_5245	regulatory protein TetR	1.13	2.37E-05	2.38E-04
Psed_3043	formate dehydrogenase, alpha subunit	1.11	2.96E-04	1.77E-03
Psed_6847	hypothetical protein	1.11	5.39E-05	4.62E-04
Psed_5748	hypothetical protein	1.10	5.00E-07	1.23E-05
Psed_0357	helix-turn-helix domain protein	1.08	1.54E-07	4.94E-06
Psed_6067	flavin reductase domain protein FMN-binding	1.07	1.46E-04	1.00E-03
Psed_4937	hypothetical protein	1.06	6.07E-05	5.06E-04
Psed_1303	Heavy metal transport/detoxification protein	1.05	4.88E-04	2.65E-03

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Psed_5358	transcription factor WhiB	1.05	3.90E-04	2.21E-03
Psed_1584	6-phosphofructokinase	1.05	5.82E-06	8.35E-05
Psed_6006	hypothetical protein	1.05	2.85E-06	4.86E-05
Psed_3442	protein of unknown function DUF939	1.05	6.10E-06	8.63E-05
Psed_6357	hydrogenase accessory protein HypB	1.05	9.85E-04	4.57E-03
Psed_3986	Peptidase M23	1.05	5.88E-04	3.08E-03
Psed_3794	Methyltransferase type 11	1.04	9.27E-04	4.37E-03
Psed_5752	hypothetical protein	1.04	3.36E-06	5.54E-05
Psed_5250	major facilitator superfamily MFS_1	1.04	2.18E-08	1.13E-06
Psed_6857	protein of unknown function DUF156	1.03	5.15E-05	4.44E-04
Psed_4236	hypothetical protein	1.03	4.06E-05	3.68E-04
Psed_2040	putative transmembrane anti-sigma factor	1.03	1.36E-04	9.46E-04
Psed_3041	NADH dehydrogenase (ubiquinone) 24 kDa subunit	1.03	1.56E-03	6.55E-03
Psed_1301	Redoxin domain protein	1.03	1.28E-03	5.57E-03
Psed_5816	hypothetical protein	1.02	1.50E-04	1.02E-03
Psed_6362	Cytochrome-c3 hydrogenase	1.02	1.64E-04	1.09E-03
Psed_5316	hypothetical protein	1.01	3.14E-04	1.86E-03
Psed_5765	hypothetical protein	1.01	2.86E-07	7.80E-06
Psed_7017	hypothetical protein	-1.00	1.66E-06	3.18E-05
Psed_5273	Dihydrolipoyl dehydrogenase	-1.00	1.44E-05	1.64E-04
Psed_4685	Homoserine O-acetyltransferase	-1.00	2.38E-03	9.25E-03
Psed_7016	hypothetical protein	-1.00	1.53E-04	1.03E-03
Psed_2862	anthranilate synthase component I	-1.00	2.94E-05	2.80E-04
Psed_2847	nicotinate-nucleotide pyrophosphorylase	-1.00	1.10E-06	2.33E-05
Psed_2705	hypothetical protein	-1.00	8.81E-06	1.15E-04
Psed_5566	NAD(P)H-quinone oxidoreductase subunit H	-1.00	1.06E-03	4.81E-03
Psed_0541	Ethyl tert-butyl ether degradation EthD	-1.01	9.66E-06	1.23E-04
Psed_5369	Alanine racemase	-1.01	1.69E-06	3.20E-05
Psed_4802	alpha amylase catalytic region	-1.01	2.42E-04	1.49E-03
Psed_4713	hypothetical protein	-1.01	1.40E-03	6.00E-03
Psed_0438	Phosphoribosylformylglycinamidine synthase 2	-1.01	1.95E-04	1.24E-03
Psed_3671	GntR domain protein	-1.01	1.86E-07	5.79E-06
Psed_1869	polysaccharide biosynthesis protein CelD	-1.01	3.69E-06	5.98E-05
Psed_3071	Dihydroxy-acid dehydratase	-1.01	1.53E-05	1.70E-04
Psed_1652	protein of unknown function DUF477	-1.01	9.70E-09	6.22E-07

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Psed_2499	Carbon-monoxide dehydrogenase (acceptor)	-1.01	2.29E-05	2.32E-04
Psed_3355	band 7 protein	-1.01	2.57E-05	2.55E-04
Psed_2867	Prolipoprotein diacylglyceryl transferase	-1.01	3.84E-06	6.16E-05
Psed_3576	beta-lactamase domain protein	-1.01	8.63E-05	6.65E-04
Psed_6616	hypothetical protein	-1.01	6.87E-05	5.55E-04
Psed_3937	hypothetical protein	-1.01	6.69E-05	5.45E-04
Psed_4845	gluconate kinase	-1.02	1.15E-03	5.08E-03
Psed_5743	thiamine biosynthesis protein ThiS	-1.02	5.81E-08	2.35E-06
Psed_0205	GtrA family protein	-1.02	5.26E-04	2.82E-03
Psed_2498	Carbon-monoxide dehydrogenase (acceptor)	-1.02	4.52E-04	2.48E-03
Psed_3541	aminodeoxychorismate lyase	-1.02	1.18E-06	2.45E-05
Psed_3158	OsmC family protein	-1.02	2.34E-07	6.80E-06
Psed_5856	alkylhydroperoxidase like protein, AhpD family	-1.02	1.13E-06	2.37E-05
Psed_1632	regulatory protein MarR	-1.02	8.28E-04	4.04E-03
Psed_4409	methylmalonyl-CoA mutase, large subunit	-1.02	3.47E-04	2.01E-03
Psed_4080	protein of unknown function DUF574	-1.03	5.65E-04	2.98E-03
Psed_3543	alanyl-tRNA synthetase	-1.03	9.18E-04	4.34E-03
Psed_2805	cell division protein FtsW	-1.03	4.58E-08	1.96E-06
Psed_1888	oxidoreductase domain protein	-1.03	5.88E-05	4.97E-04
Psed_1813	glucose dehydrogenase	-1.03	2.54E-06	4.45E-05
Psed_0332	Amidase	-1.03	6.33E-06	8.90E-05
Psed_3651	hypothetical protein	-1.03	1.18E-03	5.22E-03
Psed_3964	Lysine exporter protein (LYSE/YGGA)	-1.03	1.66E-04	1.10E-03
Psed_0165	hypothetical protein	-1.03	1.77E-05	1.91E-04
Psed_6656	Deoxyribonuclease IV (phage-T(4)-induced)	-1.03	8.82E-04	4.23E-03
Psed_1086	Conserved hypothetical protein CHP02569	-1.03	8.72E-07	1.94E-05
Psed_2429	hypothetical protein	-1.04	8.85E-08	3.27E-06
Psed_6661	Peptidoglycan glycosyltransferase	-1.04	1.45E-03	6.21E-03
Psed_1663	diaminopimelate decarboxylase	-1.04	1.91E-05	2.03E-04
Psed_2875	Malate dehydrogenase	-1.04	2.28E-04	1.41E-03
Psed_0172	protein of unknown function DUF350	-1.04	7.62E-07	1.74E-05
Psed_4598	hypothetical protein	-1.04	2.61E-07	7.37E-06
Psed_5875	2-nitropropane dioxygenase NPD	-1.04	2.64E-06	4.59E-05
Psed_2021	DoxX family protein	-1.04	1.83E-04	1.18E-03

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Psed_6892	hypothetical protein	-1.04	2.75E-06	4.72E-05
Psed_0038	regulatory protein ArsR	-1.04	2.16E-05	2.22E-04
Psed_3808	segregation and condensation protein B	-1.04	1.34E-04	9.38E-04
Psed_5464	preprotein translocase, SecE subunit	-1.04	9.16E-04	4.34E-03
Psed_0109	Peptidylprolyl isomerase	-1.04	8.69E-04	4.19E-03
Psed_2282	protein of unknown function UPF0089	-1.04	1.06E-03	4.81E-03
Psed_2866	Tryptophan synthase alpha chain	-1.04	4.29E-05	3.85E-04
Psed_2122	LexA repressor	-1.05	1.80E-03	7.37E-03
Psed_4562	amino acid permease-associated region	-1.05	2.73E-07	7.57E-06
Psed_2430	3-oxoacyl-[acyl-carrier-protein] reductase	-1.05	1.64E-06	3.16E-05
Psed_1420	hypothetical protein	-1.05	5.86E-05	4.96E-04
Psed_4765	protein of unknown function DUF177	-1.05	6.31E-04	3.26E-03
Psed_1762	Carbon-monoxide dehydrogenase (acceptor)	-1.05	9.42E-04	4.42E-03
Psed_3610	lipid A biosynthesis acyltransferase	-1.05	1.09E-05	1.36E-04
Psed_2826	pseudouridine synthase, RluA family	-1.05	6.40E-05	5.29E-04
Psed_2933	protein of unknown function DUF164	-1.05	1.53E-04	1.03E-03
Psed_5745	Thiamine-phosphate pyrophosphorylase	-1.05	1.40E-07	4.59E-06
Psed_0432	phosphoribosylformylglycinamidine synthase, purS	-1.05	2.83E-05	2.72E-04
Psed_5697	Carbon-monoxide dehydrogenase (acceptor)	-1.05	7.84E-05	6.16E-04
Psed_1680	ATP synthase subunit b	-1.05	1.11E-03	4.97E-03
Psed_4014	exodeoxyribonuclease V, gamma subunit	-1.05	2.31E-06	4.13E-05
Psed_1122	UPF0182 protein	-1.05	5.83E-05	4.94E-04
Psed_1166	hypothetical protein	-1.05	1.33E-05	1.56E-04
Psed_3371	3-oxoacyl-(acyl-carrier-protein) reductase	-1.06	1.51E-04	1.02E-03
Psed_6103	protein of unknown function DUF461	-1.06	6.36E-04	3.28E-03
Psed_4711	peptidase M50	-1.06	6.52E-04	3.33E-03
Psed_6961	hypothetical protein	-1.06	7.90E-07	1.79E-05
Psed_5201	phosphoglucomutase/phosphomannomutase alpha/beta/alpha domain II	-1.06	2.41E-07	6.98E-06
Psed_6128	hypothetical protein	-1.06	1.21E-03	5.34E-03
Psed_0180	glycosyl transferase family 2	-1.06	8.39E-06	1.11E-04
Psed_6149	Enoyl-CoA hydratase	-1.06	3.50E-04	2.02E-03
Psed_6602	VanW family protein	-1.06	1.38E-03	5.94E-03
Psed_4722	hypothetical protein	-1.06	1.34E-05	1.57E-04
Psed_5302	tryptophanyl-tRNA synthetase	-1.07	7.56E-05	5.98E-04

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Psed_3960	Phosphonate-transporting ATPase	-1.07	3.31E-05	3.11E-04
Psed_2305	hypothetical protein	-1.07	3.54E-04	2.04E-03
Psed_1623	argininosuccinate lyase	-1.07	3.67E-07	9.60E-06
Psed_3761	Glutamate synthase (NADPH)	-1.07	1.89E-04	1.21E-03
Psed_2130	transglutaminase domain-containing protein	-1.07	7.90E-04	3.89E-03
Psed_0958	Fumarate hydratase class II	-1.07	1.53E-05	1.70E-04
Psed_0325	Glyoxalase/bleomycin resistance protein/dioxygenase	-1.07	1.38E-03	5.96E-03
Psed_5330	antigen 34 kDa family protein	-1.07	3.21E-04	1.88E-03
Psed_3951	hypothetical protein	-1.08	1.11E-04	8.12E-04
Psed_4535	response regulator receiver	-1.08	8.15E-06	1.08E-04
Psed_0362	aspartate kinase	-1.08	1.09E-03	4.92E-03
Psed_4563	cytosol aminopeptidase	-1.08	1.45E-04	9.98E-04
Psed_1677	ATP synthase I	-1.08	7.69E-04	3.80E-03
Psed_1513	Glutamate racemase	-1.08	7.00E-06	9.63E-05
Psed_0021	isochorismatase hydrolase	-1.08	1.32E-05	1.55E-04
Psed_1829	glutamyl-tRNA synthetase	-1.08	4.48E-04	2.47E-03
Psed_3928	major facilitator superfamily MFS_1	-1.08	4.44E-06	6.93E-05
Psed_3349	acyl-CoA dehydrogenase domain-containing protein	-1.08	1.10E-05	1.36E-04
Psed_3614	hypothetical protein	-1.08	2.11E-05	2.18E-04
Psed_1966	Formyl-CoA transferase	-1.08	7.15E-07	1.67E-05
Psed_5466	hypothetical protein	-1.09	1.11E-05	1.36E-04
Psed_6662	hypothetical protein	-1.09	8.69E-08	3.25E-06
Psed_0183	hypothetical protein	-1.09	1.69E-07	5.34E-06
Psed_2329	Peroxidase	-1.09	1.20E-03	5.29E-03
Psed_4709	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase	-1.09	1.25E-05	1.48E-04
Psed_4725	phosphatidate cytidylyltransferase	-1.09	1.99E-05	2.09E-04
Psed_0876	Bifunctional protein glmU	-1.09	1.53E-04	1.03E-03
Psed_1877	lipopolysaccharide biosynthesis protein	-1.09	5.55E-04	2.94E-03
Psed_5091	Undecaprenyl-diphosphatase	-1.09	7.13E-05	5.71E-04
Psed_0902	NADH dehydrogenase (ubiquinone)	-1.09	5.03E-05	4.36E-04
Psed_6524	RNA polymerase sigma-70 factor, sigma-E family	-1.09	1.00E-07	3.56E-06
Psed_2985	regulatory protein ArsR	-1.09	1.30E-04	9.15E-04
Psed_1495	cytochrome c oxidase, subunit I	-1.10	3.26E-04	1.90E-03
Psed_1615	Conserved hypothetical protein CHP00268	-1.10	2.82E-07	7.73E-06

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Psed_5198	mannose-6-phosphate isomerase, class I	-1.10	1.93E-05	2.04E-04
Psed_3282	peptidase M24	-1.10	9.85E-05	7.43E-04
Psed_4623	Ribosomal protein S12 methylthiotransferase rimO	-1.10	5.33E-05	4.58E-04
Psed_5355	regulatory protein LuxR	-1.10	1.20E-04	8.60E-04
Psed_3124	Methylmalonyl-CoA mutase	-1.10	4.52E-06	7.00E-05
Psed_0301	Alcohol dehydrogenase	-1.11	6.45E-07	1.53E-05
Psed_1710	3-hydroxyacyl-CoA dehydrogenase., 3-hydroxybutyryl-CoA dehydrogenase	-1.11	1.05E-03	4.77E-03
Psed_6302	hypothetical protein	-1.11	4.63E-05	4.06E-04
Psed_5092	NADP-dependent oxidoreductase domain	-1.11	5.19E-09	3.67E-07
Psed_1320	hypothetical protein	-1.11	4.39E-09	3.25E-07
Psed_3887	Transcriptional regulator IclR	-1.11	2.05E-06	3.74E-05
Psed_0875	ribose-phosphate pyrophosphokinase	-1.11	2.61E-04	1.59E-03
Psed_5161	3-phosphoshikimate 1-carboxyvinyltransferase	-1.11	1.30E-07	4.35E-06
Psed_4487	PhoH family protein	-1.11	5.50E-04	2.92E-03
Psed_3358	hypothetical protein	-1.11	2.54E-08	1.26E-06
Psed_4756	signal recognition particle-docking protein FtsY	-1.11	1.17E-05	1.41E-04
Psed_1001	ABC-type transporter, periplasmic subunit	-1.12	1.56E-06	3.03E-05
Psed_2849	Histidinol-phosphate aminotransferase	-1.12	6.28E-07	1.50E-05
Psed_3383	glutamate/cysteine ligase family protein	-1.12	1.42E-05	1.63E-04
Psed_5899	hypothetical protein	-1.12	9.34E-06	1.21E-04
Psed_0545	Cna B domain protein	-1.12	1.96E-07	5.95E-06
Psed_5217	threonine dehydratase	-1.12	1.86E-05	2.00E-04
Psed_1761	S-adenosylhomocysteine deaminase	-1.12	1.50E-04	1.02E-03
Psed_3865	Protein of unknown function DUF2029	-1.12	6.38E-05	5.29E-04
Psed_1189	amidohydrolase 2	-1.12	1.17E-04	8.44E-04
Psed_3176	helix-turn-helix HxlR type	-1.12	1.27E-06	2.60E-05
Psed_1942	Lipoprotein signal peptidase	-1.12	1.69E-05	1.84E-04
Psed_0166	Prephenate dehydratase	-1.12	1.48E-07	4.79E-06
Psed_3198	hypothetical protein	-1.12	1.56E-03	6.54E-03
Psed_3323	ATP phosphoribosyltransferase	-1.12	5.92E-05	4.98E-04
Psed_3829	Argininosuccinate lyase	-1.13	2.34E-05	2.35E-04
Psed_1993	ATP-dependent DNA helicase, RecQ family	-1.13	7.23E-06	9.89E-05
Psed_0300	Pyridoxine 4-dehydrogenase	-1.13	2.10E-05	2.17E-04

Appendix 8. Genes differentially expressed on succinate relative to pyruvate.

Psed_4547	asparagine synthase (glutamine-hydrolyzing)	-1.13	9.65E-07	2.09E-05
Psed_3678	squalene-associated FAD-dependent desaturase	-1.13	1.08E-05	1.35E-04
Psed_5626	hypothetical protein	-1.13	2.41E-03	9.35E-03
Psed_3917	nitrile hydratase, alpha subunit	-1.13	3.36E-07	8.95E-06
Psed_4799	Prolyl oligopeptidase	-1.13	7.95E-08	3.06E-06
Psed_4459	protein of unknown function DUF182	-1.13	2.65E-07	7.37E-06
Psed_1878	glycosyl transferase group 1	-1.13	8.97E-04	4.29E-03
Psed_2024	aminoglycoside phosphotransferase	-1.13	2.21E-06	3.97E-05
Psed_3847	Polyketide cyclase/dehydrase	-1.13	2.53E-03	9.73E-03
Psed_3401	Fe(3+)-transporting ATPase	-1.14	7.62E-07	1.74E-05
Psed_3370	Enoyl-[acyl-carrier-protein] reductase (NADH)	-1.14	2.34E-06	4.18E-05
Psed_1769	xanthine permease	-1.14	3.26E-04	1.90E-03
Psed_1457	alpha/beta hydrolase fold	-1.14	4.25E-07	1.09E-05
Psed_3695	ABC-type transporter, integral membrane subunit	-1.15	9.70E-07	2.10E-05
Psed_3717	Transcriptional regulator IclR	-1.15	1.66E-04	1.10E-03
Psed_1712	DivIVA domain	-1.16	7.64E-07	1.74E-05
Psed_6068	beta-ketoadipate pathway transcriptional regulators, PcaR/PcaU/PobR family	-1.16	1.62E-05	1.78E-04
Psed_6522	hypothetical protein	-1.16	1.79E-05	1.93E-04
Psed_0187	galactofuranosyl transferase	-1.16	1.49E-04	1.02E-03
Psed_1003	ABC-type transporter, integral membrane subunit	-1.16	1.54E-06	3.01E-05
Psed_3408	glucose-6-phosphate isomerase	-1.16	1.06E-04	7.85E-04
Psed_5327	Bifunctional purine biosynthesis protein purH	-1.16	4.63E-06	7.09E-05
Psed_0174	phosphoesterase PA-phosphatase related	-1.16	6.46E-04	3.31E-03
Psed_4770	hypothetical protein	-1.16	1.35E-06	2.72E-05
Psed_0896	Enolase	-1.17	6.40E-04	3.29E-03
Psed_6526	SNARE associated Golgi protein	-1.17	1.68E-04	1.10E-03
Psed_1519	Ribonuclease PH	-1.17	4.06E-06	6.45E-05
Psed_3621	hypothetical protein	-1.17	1.42E-07	4.65E-06
Psed_3578	RelA/SpoT family protein	-1.17	7.66E-05	6.04E-04
Psed_3821	hypothetical protein	-1.17	1.57E-05	1.73E-04
Psed_3719	cyclase family protein	-1.17	1.35E-03	5.85E-03
Psed_6508	hypothetical protein	-1.18	7.92E-06	1.05E-04
Psed_1509	Mov34/MPN/PAD-1 family protein	-1.18	5.47E-04	2.90E-03

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Psed_0157	Rhodanese-like protein	-1.18	6.44E-04	3.31E-03
Psed_0233	putative transglycosylase associated protein	-1.18	1.09E-05	1.36E-04
Psed_3521	hypothetical protein	-1.18	4.47E-05	3.96E-04
Psed_4468	glycyl-tRNA synthetase	-1.18	1.24E-04	8.79E-04
Psed_6168	Phosphomethylpyrimidine synthase	-1.18	2.20E-03	8.69E-03
Psed_3784	NAD(+) synthase (glutamine-hydrolyzing)	-1.18	1.62E-07	5.15E-06
Psed_2757	Cytochrome-c oxidase	-1.18	1.69E-03	7.01E-03
Psed_4654	Thymidylate synthase thyX	-1.19	1.24E-04	8.77E-04
Psed_5844	hypothetical protein	-1.19	2.03E-03	8.13E-03
Psed_5019	protein of unknown function DUF1707	-1.19	9.87E-05	7.44E-04
Psed_2929	Protein-tyrosine phosphatase, low molecular weight	-1.19	1.70E-05	1.84E-04
Psed_5312	isocitrate dehydrogenase, NADP-dependent	-1.19	1.07E-04	7.92E-04
Psed_0284	pyridoxamine 5'-phosphate oxidase-related FMN-binding	-1.19	7.10E-07	1.67E-05
Psed_3337	peptidase M48 Ste24p	-1.19	1.24E-05	1.48E-04
Psed_3697	ABC-type transporter, periplasmic subunit	-1.19	4.34E-07	1.10E-05
Psed_5110	Xenobiotic-transporting ATPase., Peptide-transporting ATPase	-1.19	8.17E-06	1.08E-04
Psed_6211	NLP/P60 protein	-1.19	1.48E-08	8.40E-07
Psed_3327	protein of unknown function DUF75	-1.19	3.76E-07	9.79E-06
Psed_3854	Antibiotic biosynthesis monooxygenase	-1.20	1.72E-04	1.12E-03
Psed_4749	signal recognition particle protein	-1.20	2.94E-05	2.80E-04
Psed_2112	Cupin 2 conserved barrel domain protein	-1.20	3.39E-04	1.97E-03
Psed_3836	N-acetyl-gamma-glutamyl-phosphate reductase	-1.20	3.74E-04	2.14E-03
Psed_0175	UbiA prenyltransferase	-1.20	5.13E-08	2.14E-06
Psed_6525	hypothetical protein	-1.20	3.55E-08	1.68E-06
Psed_3341	putative F420-dependent oxidoreductase	-1.20	9.80E-08	3.56E-06
Psed_5175	(S)-2-hydroxy-acid oxidase	-1.20	4.01E-04	2.26E-03
Psed_4212	tyrosyl-tRNA synthetase	-1.20	2.65E-05	2.59E-04
Psed_3536	Shikimate kinase	-1.21	4.81E-04	2.61E-03
Psed_4300	nitrate reductase, alpha subunit	-1.21	1.14E-03	5.07E-03
Psed_4736	Protein of unknown function DUF2469	-1.21	2.71E-05	2.63E-04
Psed_3903	hypothetical protein	-1.21	3.19E-04	1.87E-03
Psed_4067	protein of unknown function DUF1470	-1.21	4.85E-06	7.36E-05

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Psed_5479	beta-lactamase	-1.21	2.43E-05	2.43E-04
Psed_2991	type III restriction protein res subunit	-1.21	2.69E-04	1.63E-03
Psed_5208	LPPG:FO 2-phospho-L-lactate transferase	-1.21	1.99E-05	2.09E-04
Psed_2778	phospho-2-dehydro-3-deoxyheptonate aldolase	-1.21	2.61E-05	2.57E-04
Psed_1115	hypothetical protein	-1.21	2.43E-06	4.31E-05
Psed_3894	ErfK/YbiS/YcfS/YnhG family protein	-1.21	1.28E-06	2.63E-05
Psed_3721	alpha/beta hydrolase fold	-1.21	1.45E-03	6.18E-03
Psed_3723	oxidoreductase domain protein	-1.22	3.25E-04	1.90E-03
Psed_2972	dihydroxyacetone kinase, DhaK subunit	-1.22	2.05E-05	2.13E-04
Psed_3613	threonyl-tRNA synthetase	-1.22	1.79E-04	1.16E-03
Psed_4548	GCN5-related N-acetyltransferase	-1.22	6.74E-04	3.41E-03
Psed_3405	Protoheme IX farnesyltransferase	-1.22	1.21E-05	1.45E-04
Psed_6804	hypothetical protein	-1.22	2.63E-07	7.37E-06
Psed_0967	RmuC-domain protein	-1.22	1.55E-06	3.02E-05
Psed_3286	Sec-independent protein translocase, TatC subunit	-1.22	3.00E-06	5.08E-05
Psed_4486	metalloprotease ybeY	-1.22	2.02E-04	1.28E-03
Psed_1912	hypothetical protein	-1.23	6.02E-07	1.44E-05
Psed_3427	6,7-dimethyl-8-ribityllumazine synthase	-1.23	5.92E-04	3.10E-03
Psed_0160	3-oxoacyl-[acyl-carrier-protein] reductase	-1.23	7.16E-06	9.82E-05
Psed_6269	hypothetical protein	-1.24	9.37E-05	7.13E-04
Psed_4517	Alkanesulfonate monooxygenase	-1.24	9.38E-06	1.21E-04
Psed_1887	DegT/DnrJ/EryC1/StrS aminotransferase	-1.24	1.18E-04	8.53E-04
Psed_1186	cell division ATP-binding protein FtsE	-1.24	1.37E-04	9.52E-04
Psed_1785	Serine--pyruvate transaminase	-1.24	1.36E-05	1.58E-04
Psed_1794	Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit C	-1.24	1.40E-03	6.00E-03
Psed_1886	DegT/DnrJ/EryC1/StrS aminotransferase	-1.25	2.03E-04	1.28E-03
Psed_1095	alpha/beta hydrolase fold	-1.25	1.35E-06	2.73E-05
Psed_4505	Sulfite reductase (ferredoxin)	-1.25	1.26E-03	5.51E-03
Psed_1871	polysaccharide deacetylase	-1.25	2.14E-04	1.34E-03
Psed_5472	regulatory protein LuxR	-1.25	4.49E-07	1.13E-05
Psed_0023	regulatory protein GntR HTH	-1.25	5.39E-10	6.16E-08
Psed_1922	major facilitator superfamily MFS_1	-1.25	4.36E-06	6.84E-05
Psed_1185	Peptide chain release factor 2	-1.25	1.94E-05	2.05E-04

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Psed_5335	ATP-dependent DNA helicase PcrA	-1.25	1.87E-07	5.79E-06
Psed_5116	hypothetical protein	-1.25	1.72E-05	1.86E-04
Psed_3728	Pyruvate dehydrogenase (acetyl-transferring)	-1.26	4.58E-04	2.51E-03
Psed_1675	glycosyl transferase family 4	-1.26	5.44E-08	2.23E-06
Psed_5618	Porphobilinogen deaminase	-1.26	1.08E-03	4.88E-03
Psed_0250	Xenobiotic-transporting ATPase	-1.26	4.50E-05	3.98E-04
Psed_0282	esterase	-1.26	5.53E-07	1.34E-05
Psed_4571	Lipoyl synthase	-1.27	1.58E-08	8.80E-07
Psed_3727	Pyruvate dehydrogenase (acetyl-transferring)	-1.27	5.99E-05	5.03E-04
Psed_0207	Teichoic-acid-transporting ATPase	-1.27	2.70E-05	2.63E-04
Psed_1447	Phosphoribosylaminoimidazolecarboxamide formyltransferase	-1.27	2.29E-07	6.69E-06
Psed_5153	transcription factor WhiB	-1.27	3.92E-06	6.25E-05
Psed_4805	NAD-glutamate dehydrogenase	-1.28	1.43E-05	1.63E-04
Psed_2976	flavin reductase domain protein FMN-binding	-1.28	6.68E-04	3.38E-03
Psed_4712	1-deoxy-D-xylulose 5-phosphate reductoisomerase	-1.28	2.55E-07	7.29E-06
Psed_1187	protein of unknown function DUF214	-1.28	1.44E-07	4.68E-06
Psed_5742	Thiazole synthase	-1.29	1.18E-04	8.48E-04
Psed_5194	hypothetical protein	-1.29	2.69E-05	2.62E-04
Psed_2766	Long-chain-fatty-acid--CoA ligase	-1.29	1.09E-07	3.82E-06
Psed_0428	adenylosuccinate lyase	-1.29	1.48E-05	1.66E-04
Psed_4807	ATP-binding cassette protein, ChvD family	-1.29	5.04E-06	7.51E-05
Psed_3658	RNA polymerase sigma-70 factor	-1.30	2.11E-09	1.88E-07
Psed_5564	NADH-quinone oxidoreductase, F subunit	-1.30	9.84E-04	4.57E-03
Psed_1752	Antibiotic biosynthesis monooxygenase	-1.30	6.36E-05	5.29E-04
Psed_3066	hypothetical protein	-1.31	2.49E-06	4.40E-05
Psed_3577	peptidyl-prolyl cis-trans isomerase cyclophilin type	-1.31	8.48E-05	6.56E-04
Psed_4628	cell divisionFtsK/SpoIIIE	-1.31	4.30E-05	3.85E-04
Psed_5014	esterase/lipase	-1.31	2.48E-05	2.47E-04
Psed_3713	helix-turn-helix domain-containing protein AraC type	-1.31	1.50E-09	1.39E-07
Psed_4806	hypothetical protein	-1.31	2.32E-05	2.34E-04
Psed_1828	monooxygenase FAD-binding	-1.31	3.37E-07	8.95E-06

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Psed_5314	Bifunctional protein folD	-1.32	1.10E-06	2.32E-05
Psed_5264	Thiosulfate sulfurtransferase	-1.32	1.46E-05	1.65E-04
Psed_3962	polar amino acid ABC transporter, inner membrane subunit	-1.32	5.22E-06	7.72E-05
Psed_1612	alpha/beta hydrolase fold	-1.32	3.20E-05	3.01E-04
Psed_6121	hypothetical protein	-1.32	4.61E-06	7.09E-05
Psed_6286	hypothetical protein	-1.33	1.91E-05	2.03E-04
Psed_1842	HNH endonuclease	-1.33	1.00E-07	3.56E-06
Psed_6615	uncharacterized peroxidase-related enzyme	-1.33	4.43E-04	2.44E-03
Psed_0550	Rhodanese-like protein	-1.33	3.56E-08	1.68E-06
Psed_6914	amidohydrolase	-1.33	1.15E-06	2.40E-05
Psed_3857	hypothetical protein	-1.33	1.44E-05	1.64E-04
Psed_4566	2-oxoglutarate dehydrogenase, E2 component, dihydrolipoamide succinyltransferase	-1.33	7.15E-05	5.71E-04
Psed_3174	hypothetical protein	-1.33	9.78E-06	1.24E-04
Psed_6500	Endoribonuclease L-PSP	-1.34	4.98E-07	1.23E-05
Psed_1872	glycosyl transferase group 1	-1.34	1.03E-06	2.19E-05
Psed_2723	Magnesium chelatase	-1.34	3.14E-09	2.43E-07
Psed_1676	hypothetical protein	-1.34	1.41E-05	1.62E-04
Psed_2690	DSBA oxidoreductase	-1.34	4.95E-06	7.46E-05
Psed_2721	hypothetical protein	-1.34	4.32E-07	1.10E-05
Psed_3831	Arginine repressor	-1.34	9.46E-06	1.22E-04
Psed_2141	2-oxo-acid dehydrogenase E1 subunit, homodimeric type	-1.35	3.25E-05	3.06E-04
Psed_0185	UDP-galactopyranose mutase	-1.35	2.67E-06	4.63E-05
Psed_0312	Sulfate transporter/antisigma-factor antagonist STAS	-1.35	4.46E-05	3.96E-04
Psed_2809	cell division protein FtsZ	-1.35	4.71E-05	4.12E-04
Psed_5647	hypothetical protein	-1.35	3.93E-08	1.82E-06
Psed_1002	ABC-type transporter, integral membrane subunit	-1.36	5.02E-07	1.23E-05
Psed_0138	copper resistance protein CopC	-1.36	1.05E-03	4.77E-03
Psed_5267	protein of unknown function DUF692	-1.37	3.97E-05	3.62E-04
Psed_1024	protein of unknown function DUF1470	-1.37	2.03E-06	3.72E-05
Psed_4449	DNA primase	-1.37	5.28E-06	7.77E-05
Psed_6523	fructose-bisphosphate aldolase, class II	-1.37	1.47E-04	1.01E-03
Psed_2941	regulatory protein MarR	-1.37	2.46E-05	2.46E-04
Psed_1685	ATP synthase subunit beta	-1.37	2.85E-04	1.71E-03

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Psed_5368	alpha/beta hydrolase fold	-1.38	1.16E-05	1.41E-04
Psed_1046	Citryl-CoA lyase	-1.38	6.65E-05	5.44E-04
Psed_1978	Polyamine-transporting ATPase	-1.38	1.72E-06	3.24E-05
Psed_1825	D-3-phosphoglycerate dehydrogenase	-1.39	6.76E-04	3.41E-03
Psed_3963	polar amino acid ABC transporter, inner membrane subunit	-1.40	3.01E-06	5.08E-05
Psed_5565	NADH dehydrogenase (quinone)	-1.40	1.10E-05	1.36E-04
Psed_5874	ErfK/YbiS/YcfS/YnhG family protein	-1.40	4.27E-08	1.92E-06
Psed_4992	hypothetical protein	-1.40	6.20E-09	4.29E-07
Psed_1686	ATP synthase epsilon chain	-1.40	3.07E-06	5.16E-05
Psed_2434	GAF domain protein	-1.40	5.37E-05	4.61E-04
Psed_2831	DNA polymerase III, alpha subunit	-1.40	4.43E-06	6.93E-05
Psed_4109	HpcH/HpaI aldolase	-1.40	1.38E-05	1.60E-04
Psed_1370	regulatory protein LacI	-1.40	2.76E-08	1.34E-06
Psed_3594	Tellurite resistance TerB	-1.40	8.69E-04	4.19E-03
Psed_5265	Fe-S metabolism associated SufE	-1.40	1.88E-05	2.01E-04
Psed_6511	NUDIX hydrolase	-1.41	5.26E-08	2.17E-06
Psed_5624	Long-chain-fatty-acid--CoA ligase	-1.41	5.20E-07	1.27E-05
Psed_1662	Arginyl-tRNA synthetase	-1.41	5.10E-05	4.41E-04
Psed_0897	Septum formation initiator	-1.41	4.27E-05	3.84E-04
Psed_5471	hypothetical protein	-1.41	6.63E-05	5.44E-04
Psed_5659	(R)-benzylsuccinyl-CoA dehydrogenase	-1.42	1.42E-05	1.63E-04
Psed_5207	F420-0:gamma-glutamyl ligase	-1.42	2.10E-08	1.10E-06
Psed_1830	Heat shock protein 70	-1.42	5.91E-05	4.98E-04
Psed_1691	methylmalonyl-CoA mutase, large subunit	-1.43	1.93E-04	1.23E-03
Psed_3545	secreted protein	-1.43	6.00E-06	8.52E-05
Psed_5197	cation diffusion facilitator family transporter	-1.43	6.52E-04	3.33E-03
Psed_2812	Cell division protein sepF	-1.44	6.72E-05	5.46E-04
Psed_1060	magnesium and cobalt transport protein CorA	-1.44	2.89E-07	7.85E-06
Psed_3730	Carboxymuconolactone decarboxylase	-1.44	7.79E-04	3.85E-03
Psed_0140	hypothetical protein	-1.44	1.63E-04	1.08E-03
Psed_3575	Histidyl-tRNA synthetase	-1.45	7.26E-09	4.83E-07
Psed_1117	hypothetical protein	-1.45	4.82E-06	7.33E-05
Psed_5211	Mannose-1-phosphate guanylyltransferase	-1.45	2.63E-09	2.17E-07
Psed_0252	amino acid adenylation domain protein	-1.45	1.33E-06	2.70E-05

Psed_4983	Aminocarboxymuconate-semialdehyde decarboxylase	-1.45	1.77E-06	3.29E-05
Psed_2289	hypothetical protein	-1.45	5.09E-04	2.75E-03
Psed_3585	Crossover junction endodeoxyribonuclease ruvC	-1.45	8.87E-08	3.27E-06
Psed_6110	Antibiotic biosynthesis monooxygenase	-1.45	7.91E-06	1.05E-04
Psed_2888	1-deoxy-D-xylulose-5-phosphate synthase	-1.46	4.33E-06	6.81E-05
Psed_1689	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	-1.46	2.22E-07	6.54E-06
Psed_5346	GMP synthase [glutamine-hydrolyzing]	-1.46	1.12E-09	1.08E-07
Psed_4976	NADP oxidoreductase coenzyme F420-dependent	-1.46	9.93E-06	1.26E-04
Psed_5570	geranylgeranyl reductase	-1.47	7.13E-07	1.67E-05
Psed_6565	protein of unknown function DUF224 cysteine-rich region domain protein	-1.47	1.10E-05	1.36E-04
Psed_1880	glycosyl transferase family 2	-1.47	3.11E-04	1.84E-03
Psed_3953	(Dimethylallyl)adenosine tRNA methylthiotransferase miaB	-1.48	1.14E-07	3.94E-06
Psed_6521	phospholipid/glycerol acyltransferase	-1.48	5.96E-08	2.40E-06
Psed_5562	NADH dehydrogenase (quinone)	-1.48	1.12E-04	8.19E-04
Psed_3681	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	-1.48	2.02E-06	3.70E-05
Psed_2845	Quinolinate synthase A	-1.48	4.03E-05	3.66E-04
Psed_2799	Ribosomal RNA small subunit methyltransferase H	-1.48	1.04E-05	1.31E-04
Psed_5563	NADH-quinone oxidoreductase, chain G	-1.49	1.97E-05	2.08E-04
Psed_3591	hypothetical protein	-1.49	1.85E-06	3.42E-05
Psed_5555	Trans-hexaprenyltranstransferase	-1.50	6.55E-05	5.39E-04
Psed_1671	Sua5/YciO/YrdC/YwlC family protein	-1.50	1.02E-08	6.37E-07
Psed_1772	Electron transfer flavoprotein alpha subunit	-1.51	6.69E-05	5.45E-04
Psed_1819	hypothetical protein	-1.51	1.06E-05	1.33E-04
Psed_3833	Acetylornithine/succinyldiaminopimelate aminotransferase	-1.52	6.38E-05	5.29E-04
Psed_0389	hypothetical protein	-1.52	1.07E-03	4.85E-03
Psed_3667	SSS sodium solute transporter superfamily	-1.52	2.60E-05	2.56E-04
Psed_5568	NAD(P)H-quinone oxidoreductase subunit K	-1.53	1.55E-06	3.02E-05

Psed_2291	pyridoxamine 5'-phosphate oxidase-related FMN-binding protein	-1.54	4.59E-06	7.08E-05
Psed_6640	extracellular solute-binding protein family 1	-1.54	1.79E-04	1.16E-03
Psed_3720	amidohydrolase 2	-1.55	5.77E-06	8.30E-05
Psed_1818	Dihydroxy-acid dehydratase	-1.56	6.86E-05	5.55E-04
Psed_1693	ectoine/hydroxyectoine ABC transporter solute-binding protein	-1.56	1.28E-03	5.57E-03
Psed_6641	ABC-type transporter, integral membrane subunit	-1.56	2.50E-04	1.53E-03
Psed_5115	2-oxoglutarate dehydrogenase, E1 subunit	-1.57	1.87E-04	1.20E-03
Psed_4100	hypothetical protein	-1.58	4.36E-08	1.93E-06
Psed_3961	ABC-type transporter, periplasmic subunit family 3	-1.58	1.59E-06	3.07E-05
Psed_3812	Tyrosine recombinase xerC	-1.58	6.35E-10	7.13E-08
Psed_1633	hypothetical protein	-1.58	7.71E-06	1.04E-04
Psed_5240	GtrA family protein	-1.58	1.32E-07	4.40E-06
Psed_4632	hypothetical protein	-1.59	1.52E-04	1.03E-03
Psed_5548	Enoyl-CoA hydratase/isomerase	-1.60	6.04E-08	2.41E-06
Psed_4557	hypothetical protein	-1.60	3.91E-06	6.25E-05
Psed_3410	OpcA protein	-1.60	2.13E-05	2.20E-04
Psed_3871	hypothetical protein	-1.60	2.29E-05	2.32E-04
Psed_0139	nuclear export factor GLE1	-1.61	8.65E-04	4.18E-03
Psed_4682	Prolyl-tRNA synthetase	-1.62	2.45E-04	1.50E-03
Psed_1731	alpha-glucan phosphorylase	-1.62	9.57E-06	1.23E-04
Psed_1879	exopolysaccharide biosynthesis polyprenyl glycosylphosphotransferase	-1.62	5.21E-04	2.80E-03
Psed_5465	Aspartate transaminase	-1.62	6.97E-06	9.62E-05
Psed_3876	ABC-type transporter, integral membrane subunit	-1.63	1.76E-07	5.50E-06
Psed_2887	diguanylate cyclase	-1.64	3.04E-07	8.18E-06
Psed_3377	aconitate hydratase 1	-1.65	5.62E-05	4.79E-04
Psed_1908	hypothetical protein	-1.65	9.41E-05	7.14E-04
Psed_2995	monosaccharide-transporting ATPase	-1.65	1.75E-06	3.27E-05
Psed_6612	ABC-type transporter, periplasmic subunit family 3	-1.66	1.50E-07	4.82E-06
Psed_3680	squalene-hopene cyclase	-1.68	4.42E-08	1.94E-06
Psed_2119	GTP-binding proten HflX	-1.68	1.71E-04	1.12E-03
Psed_3875	Extracellular ligand-binding receptor	-1.70	1.05E-06	2.24E-05

Appendix 8. Genes differentially expressed on succinate relative to pyruvate.

Psed_1023	hypothetical protein	-1.70	7.60E-07	1.74E-05
Psed_4483	FAD dependent oxidoreductase	-1.70	5.13E-06	7.63E-05
Psed_0391	metallophosphoesterase	-1.70	4.97E-06	7.47E-05
Psed_3682	hopanoid biosynthesis associated radical SAM protein HpnH	-1.70	9.43E-07	2.07E-05
Psed_1881	glycosyl transferase group 1	-1.71	4.66E-08	1.98E-06
Psed_6999	hypothetical protein	-1.71	8.47E-08	3.19E-06
Psed_5556	NAD(P)H-quinone oxidoreductase subunit 2	-1.71	9.08E-05	6.94E-04
Psed_3278	LmbE family protein	-1.72	1.64E-05	1.80E-04
Psed_0251	Xenobiotic-transporting ATPase	-1.72	1.31E-07	4.38E-06
Psed_1494	LmbE family protein	-1.72	3.57E-07	9.40E-06
Psed_3544	hypothetical protein	-1.72	1.60E-06	3.08E-05
Psed_2281	ABC transporter related	-1.72	6.61E-06	9.17E-05
Psed_4861	cell shape determining protein MreB/Mrl	-1.73	4.22E-05	3.80E-04
Psed_1722	Thioredoxin domain-containing protein	-1.74	7.73E-06	1.04E-04
Psed_5560	NADH-ubiquinone/plastoquinone oxidoreductase chain 6	-1.74	2.27E-03	8.92E-03
Psed_6301	dienelactone hydrolase	-1.75	5.43E-06	7.93E-05
Psed_4874	Glyoxalase/bleomycin resistance protein/dioxygenase	-1.76	9.28E-04	4.37E-03
Psed_1674	Glycine hydroxymethyltransferase	-1.76	1.48E-04	1.01E-03
Psed_5567	NAD(P)H-quinone oxidoreductase subunit J	-1.77	3.12E-06	5.21E-05
Psed_0078	Methyltransferase type 12	-1.77	1.06E-08	6.48E-07
Psed_5209	Peroxiredoxin	-1.77	3.70E-10	4.73E-08
Psed_4631	LexA DNA-binding domain protein	-1.78	1.69E-04	1.11E-03
Psed_3589	stress protein	-1.78	2.53E-07	7.24E-06
Psed_2763	hypothetical protein	-1.78	1.89E-07	5.80E-06
Psed_4843	UspA domain-containing protein	-1.79	6.38E-04	3.29E-03
Psed_5823	ectoine hydroxylase	-1.79	9.03E-04	4.30E-03
Psed_3172	Serine/threonine protein kinase-related	-1.79	1.12E-09	1.08E-07
Psed_6509	endonuclease III	-1.80	1.56E-05	1.72E-04
Psed_5922	Carboxylesterase	-1.81	1.62E-04	1.08E-03
Psed_5826	L-2,4-diaminobutyric acid acetyltransferase	-1.81	4.52E-08	1.96E-06
Psed_3832	Ornithine carbamoyltransferase	-1.82	3.08E-06	5.16E-05
Psed_1682	ATP synthase subunit delta	-1.82	7.52E-06	1.02E-04
Psed_3338	Penicillinase repressor	-1.83	1.74E-07	5.48E-06

Appendix 8. Genes differentially expressed on succinate relative to pyruvate.

Psed_0886	hypothetical protein	-1.83	5.37E-06	7.88E-05
Psed_5824	L-ectoine synthase	-1.83	6.17E-04	3.21E-03
Psed_0013	hypothetical protein	-1.85	2.23E-08	1.15E-06
Psed_5362	Aryl-alcohol dehydrogenase (NADP(+))	-1.86	7.09E-08	2.78E-06
Psed_5557	proton-translocating NADH-quinone oxidoreductase, chain M	-1.86	6.65E-05	5.44E-04
Psed_0397	Pyrophosphate-energized proton pump	-1.86	3.40E-05	3.18E-04
Psed_4021	Bifunctional protein folD	-1.87	7.44E-06	1.01E-04
Psed_3693	oligopeptide/dipeptide ABC transporter, ATPase subunit	-1.89	1.47E-08	8.37E-07
Psed_3679	Dimethylallyltranstransferase	-1.90	1.40E-05	1.61E-04
Psed_3676	squalene synthase HpnC	-1.90	2.38E-08	1.20E-06
Psed_5608	ResB family protein	-1.91	9.61E-07	2.09E-05
Psed_2286	hypothetical protein	-1.92	1.87E-03	7.60E-03
Psed_1771	Electron transfer flavoprotein alpha/ beta-subunit	-1.92	4.93E-06	7.45E-05
Psed_2143	alkyl hydroperoxide reductase/ Thiol specific antioxidant/ Mal allergen	-1.92	7.86E-06	1.05E-04
Psed_2436	nitroreductase	-1.93	5.55E-04	2.94E-03
Psed_6803	hypothetical protein	-1.95	9.03E-06	1.18E-04
Psed_5843	hypothetical protein	-1.98	9.41E-06	1.22E-04
Psed_4655	protein of unknown function DUF1778	-1.98	1.19E-07	4.06E-06
Psed_2290	hypothetical protein	-1.98	6.12E-04	3.19E-03
Psed_3995	ABC-type transporter, periplasmic sub-unit	-1.98	4.01E-08	1.84E-06
Psed_5872	hypothetical protein	-1.99	1.00E-06	2.16E-05
Psed_1421	ferric-uptake regulator	-2.00	1.85E-06	3.42E-05
Psed_2437	CBS domain containing protein	-2.01	1.48E-03	6.30E-03
Psed_4110	MmgE/PrpD family protein	-2.01	7.43E-07	1.72E-05
Psed_1763	molybdopterin dehydrogenase FAD-binding	-2.03	3.00E-06	5.08E-05
Psed_1713	crotonyl-CoA reductase	-2.07	6.18E-06	8.72E-05
Psed_2297	hypothetical protein	-2.11	6.84E-04	3.44E-03
Psed_1116	transcription factor WhiB	-2.12	2.38E-09	2.01E-07
Psed_4841	UspA domain-containing protein	-2.13	9.11E-04	4.33E-03
Psed_1562	hypothetical protein	-2.14	6.43E-06	8.98E-05
Psed_4688	polysaccharide deacetylase	-2.14	2.31E-09	1.98E-07
Psed_4176	GntR domain protein	-2.14	4.18E-07	1.08E-05
Psed_5825	diaminobutyrate/2-oxoglutarate amino-transferase	-2.19	2.26E-05	2.31E-04

Appendix 8. Genes differentially expressed on succinate relative to pyruvate.

Psed_1730	alpha amylase catalytic region	-2.19	3.35E-08	1.61E-06
Psed_0011	hypothetical protein	-2.24	6.75E-09	4.58E-07
Psed_2298	UspA domain-containing protein	-2.35	2.22E-04	1.39E-03
Psed_1198	DoxX family protein	-2.38	8.42E-08	3.19E-06
Psed_4532	band 7 protein	-2.38	5.74E-07	1.38E-05
Psed_4656	hypothetical protein	-2.39	5.50E-08	2.24E-06
Psed_1726	hypothetical protein	-2.40	2.09E-10	3.02E-08
Psed_2318	regulatory protein MerR	-2.40	2.44E-07	7.04E-06
Psed_5609	cytochrome c biogenesis protein transmembrane region	-2.46	4.55E-08	1.96E-06
Psed_3590	Tellurium resistance	-2.49	4.79E-07	1.20E-05
Psed_3675	PucR family transcriptional regulator	-2.49	1.03E-08	6.38E-07
Psed_3123	cytochrome bd ubiquinol oxidase subunit I	-2.50	2.28E-05	2.32E-04
Psed_1727	RNA polymerase sigma factor, sigma-70 family	-2.50	8.34E-08	3.18E-06
Psed_3122	cytochrome d ubiquinol oxidase, subunit II	-2.58	5.24E-05	4.51E-04
Psed_3120	ABC transporter, CydDC cysteine exporter (CydDC-E) family, permease/ ATP-binding protein CydC	-2.60	4.33E-05	3.87E-04
Psed_2299	hypothetical protein	-2.75	2.58E-07	7.34E-06
Psed_4844	glyceraldehyde-3-phosphate dehydrogenase, type I	-2.84	1.15E-04	8.35E-04
Psed_3121	ABC transporter, CydDC cysteine exporter (CydDC-E) family, permease/ ATP-binding protein CydD	-2.85	9.72E-06	1.24E-04
Psed_0276	major facilitator superfamily MFS_1	-3.12	2.08E-11	4.85E-09
Psed_5195	alanine dehydrogenase	-3.56	2.15E-08	1.12E-06
Psed_3670	Butyryl-CoA dehydrogenase	-4.90	8.16E-07	1.83E-05

Appendix 9

Genes differentially expressed on 1,4-dioxane verus glycolate.

Appendix 9. Genes differentially expressed on 1,4-dioxane verus glycolate. Differentially expressed genes have an adjusted p-value < 0.01 and have a $log_2FC > 1$ or $log_2FC < -1$ and are sorted by log_2FC.

Genes	Protein	log_2FC	p-value	adjusted p-value
Psed_2204	50S ribosomal protein L33	5.34	3.31E-12	3.60E-09
Psed_6982	Mn2+/Fe2+ transporter, NRAMP family	3.95	5.18E-09	5.35E-07
Psed_6749	hypothetical protein	3.78	1.06E-09	1.77E-07
Psed_6981	Aldehyde Dehydrogenase	3.70	7.82E-11	2.69E-08
Psed_0249	L-lysine 6-monooxygenase (NADPH)	3.48	2.51E-09	3.06E-07
Psed_6970	D-lactate dehydrogenase (cytochrome)	3.39	8.46E-08	4.92E-06
Psed_6746	hypothetical protein	3.38	4.06E-11	1.97E-08
Psed_3903	hypothetical protein	3.32	1.40E-09	2.22E-07
Psed_3904	CO dehydrogenase maturation factor-like protein	3.31	6.99E-09	6.81E-07
Psed_6980	hypothetical protein	3.22	5.84E-11	2.53E-08
Psed_0164	hypothetical protein	3.22	1.56E-09	2.31E-07
Psed_6743	hypothetical protein	3.19	1.86E-10	4.83E-08
Psed_0011	hypothetical protein	3.19	2.61E-11	1.65E-08
Psed_1658	Trimethylamine-N-oxide reductase (cytochrome c)	3.17	1.37E-06	4.58E-05
Psed_6094	ABC-type metal ion transporter, periplasmic subunit	3.10	3.48E-08	2.33E-06
Psed_6971	Hydroxyacid-oxoacid transhydrogenase	3.02	1.16E-08	1.01E-06
Psed_0250	Xenobiotic-transporting ATPase	3.00	6.25E-10	1.23E-07
Psed_6748	hypothetical protein	2.99	2.78E-10	6.47E-08
Psed_6751	Transglycosylase-like domain protein	2.99	1.77E-09	2.39E-07
Psed_6161	Siderophore-interacting protein	2.84	1.02E-09	1.75E-07
Psed_3996	Methionyl-tRNA formyltransferase	2.82	8.71E-07	3.22E-05
Psed_0251	Xenobiotic-transporting ATPase	2.78	1.10E-10	3.13E-08
Psed_5844	hypothetical protein	2.76	1.96E-07	9.69E-06
Psed_6977	Ferredoxin--NAD(+) reductase	2.70	1.06E-10	3.13E-08
Psed_6095	Fe(3+)-transporting ATPase	2.68	5.10E-06	1.32E-04
Psed_6972	GntR domain protein	2.64	2.65E-07	1.20E-05
Psed_0247	ABC-type transporter, integral membrane subunit	2.57	6.29E-08	3.86E-06
Psed_0252	amino acid adenylation domain protein	2.54	5.02E-10	1.05E-07
Psed_0038	regulatory protein ArsR	2.53	1.80E-10	4.83E-08
Psed_2032	50S ribosomal protein L31 type B	2.47	3.66E-06	1.04E-04
Psed_6747	hypothetical protein	2.46	3.18E-10	7.14E-08

Psed_3667	SSS sodium solute transporter superfamily	2.46	4.72E-08	3.01E-06
Psed_6752	hypothetical protein	2.44	8.42E-09	7.83E-07
Psed_0248	ABC-type transporter, periplasmic subunit	2.43	7.85E-11	2.69E-08
Psed_6162	MbtH domain protein	2.37	2.40E-08	1.79E-06
Psed_6979	monooxygenase component MmoB/DmpM	2.36	2.07E-10	5.19E-08
Psed_6750	hypothetical protein	2.34	1.56E-09	2.31E-07
Psed_6974	Ethyl tert-butyl ether degradation EthD	2.32	7.24E-11	2.69E-08
Psed_5471	hypothetical protein	2.29	1.40E-07	7.28E-06
Psed_6754	hypothetical protein	2.23	5.04E-09	5.30E-07
Psed_5843	hypothetical protein	2.22	1.17E-06	4.14E-05
Psed_6744	hypothetical protein	2.22	7.10E-10	1.36E-07
Psed_0982	fibronectin-attachment family protein	2.13	1.18E-05	2.63E-04
Psed_6973	hypothetical protein	2.09	4.09E-07	1.76E-05
Psed_7002	transcription factor WhiB	2.08	2.11E-08	1.60E-06
Psed_6745	ATP-binding protein	2.06	5.92E-13	9.64E-10
Psed_2203	cobalamin synthesis CobW domain protein	2.04	5.07E-07	2.09E-05
Psed_6730	Luciferase-like, subgroup	2.02	7.13E-06	1.69E-04
Psed_0013	hypothetical protein	1.97	4.05E-09	4.36E-07
Psed_6755	hypothetical protein	1.96	5.76E-08	3.60E-06
Psed_6096	ABC-type transporter, integral membrane subunit	1.95	8.19E-07	3.06E-05
Psed_6968	transposase mutator type	1.94	4.19E-10	9.09E-08
Psed_0021	isochorismatase hydrolase	1.91	6.31E-09	6.32E-07
Psed_1656	hypothetical protein	1.90	7.01E-09	6.81E-07
Psed_6978	methane/phenol/toluene hydroxylase	1.89	7.10E-11	2.69E-08
Psed_6975	Betaine-aldehyde dehydrogenase	1.88	2.82E-11	1.65E-08
Psed_6805	hypothetical protein	1.88	4.33E-04	5.05E-03
Psed_6729	hypothetical protein	1.88	1.09E-07	6.11E-06
Psed_3679	Dimethylallyltranstransferase	1.86	8.55E-06	1.97E-04
Psed_6753	hypothetical protein	1.82	6.41E-05	1.10E-03
Psed_1657	Cobalt transporter subunit CbtB putative	1.82	1.21E-07	6.59E-06
Psed_2329	Peroxidase	1.82	4.40E-06	1.17E-04
Psed_0246	ABC-type transporter, integral membrane subunit	1.82	2.27E-07	1.08E-05
Psed_6756	hypothetical protein	1.80	8.57E-08	4.93E-06
Psed_3440	SH3 type 3 domain protein	1.80	6.26E-06	1.54E-04

Appendix 9. Genes differentially expressed on 1,4-dioxane verus glycolate.

Psed_3174	hypothetical protein	1.79	1.47E-07	7.57E-06
Psed_0106	putative Fe-S oxidoreductase	1.76	1.82E-05	3.66E-04
Psed_6599	N-acetylmuramoyl-L-alanine amidase family 2	1.69	1.35E-07	7.08E-06
Psed_3682	hopanoid biosynthesis associated radical SAM protein HpnH	1.68	5.03E-07	2.09E-05
Psed_0580	highly repetitive protein	1.67	1.79E-05	3.65E-04
Psed_6757	hypothetical protein	1.67	2.48E-08	1.83E-06
Psed_6889	hypothetical protein	1.67	4.36E-08	2.84E-06
Psed_6202	pyrimidine utilization protein A	1.65	3.79E-06	1.06E-04
Psed_2033	ABC-type transporter, periplasmic subunit	1.64	1.73E-04	2.42E-03
Psed_6914	amidohydrolase	1.64	4.07E-08	2.67E-06
Psed_6890	hypothetical protein	1.64	2.35E-07	1.11E-05
Psed_0998	hypothetical protein	1.60	3.23E-08	2.21E-06
Psed_7003	hypothetical protein	1.57	1.63E-09	2.35E-07
Psed_3726	Xylose isomerase domain-containing protein TIM barrel	1.56	5.32E-04	5.90E-03
Psed_3681	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	1.55	5.48E-07	2.19E-05
Psed_2812	Cell division protein sepF	1.53	1.72E-05	3.54E-04
Psed_3653	hypothetical protein	1.53	3.70E-05	7.02E-04
Psed_0010	hypothetical protein	1.53	7.71E-07	2.94E-05
Psed_3680	squalene-hopene cyclase	1.52	6.49E-08	3.91E-06
Psed_0282	esterase	1.52	2.56E-08	1.88E-06
Psed_6948	transposase mutator type	1.51	1.68E-07	8.46E-06
Psed_4146	Potassium-transporting ATPase B chain	1.48	6.67E-05	1.13E-03
Psed_3894	ErfK/YbiS/YcfS/YnhG family protein	1.47	5.61E-08	3.54E-06
Psed_0012	hypothetical protein	1.46	5.82E-06	1.46E-04
Psed_3725	Extracellular ligand-binding receptor	1.44	6.90E-05	1.15E-03
Psed_6742	NLP/P60 protein	1.42	8.79E-10	1.55E-07
Psed_3971	glutamate--cysteine ligase GCS2	1.42	5.72E-06	1.44E-04
Psed_3676	squalene synthase HpnC	1.41	4.22E-07	1.80E-05
Psed_6888	C-5 cytosine-specific DNA methylase	1.38	2.76E-07	1.24E-05
Psed_6263	Extracellular ligand-binding receptor	1.38	1.18E-06	4.14E-05
Psed_2914	Iron-chelate-transporting ATPase	1.37	2.78E-06	8.45E-05
Psed_1654	hypothetical protein	1.37	1.04E-06	3.82E-05
Psed_5355	regulatory protein LuxR	1.37	6.43E-06	1.57E-04
Psed_2913	ABC-type transporter, integral membrane subunit	1.36	4.39E-06	1.17E-04

Appendix 9. Genes differentially expressed on 1,4-dioxane verus glycolate.

Psed_6114	FAD-binding 9 siderophore-interacting domain protein	1.36	2.76E-06	8.43E-05
Psed_0951	hypothetical protein	1.35	1.20E-05	2.66E-04
Psed_2328	high-affinity nickel-transporter	1.35	1.82E-05	3.66E-04
Psed_0950	hypothetical protein	1.34	1.03E-03	9.77E-03
Psed_3972	nitroreductase	1.34	2.90E-07	1.29E-05
Psed_5881	Heme oxygenase	1.33	3.77E-06	1.06E-04
Psed_6261	ABC-type transporter, integral membrane subunit	1.32	6.63E-06	1.61E-04
Psed_6728	Amidase	1.30	6.64E-06	1.61E-04
Psed_6259	FMN-dependent oxidoreductase, nitrilo-triacetate monooxygenase family	1.29	4.09E-05	7.58E-04
Psed_7008	hypothetical protein	1.29	4.09E-09	4.36E-07
Psed_6913	hypothetical protein	1.28	1.13E-06	4.08E-05
Psed_2995	monosaccharide-transporting ATPase	1.27	1.49E-05	3.18E-04
Psed_1713	crotonyl-CoA reductase	1.27	4.10E-04	4.87E-03
Psed_3935	GntR domain protein	1.24	1.38E-04	2.01E-03
Psed_2800	penicillin-binding protein transpeptidase	1.24	8.95E-05	1.42E-03
Psed_2799	Ribosomal RNA small subunit methyl-transferase H	1.24	3.52E-05	6.72E-04
Psed_1655	hypothetical protein	1.23	1.19E-07	6.50E-06
Psed_5496	regulatory protein MerR	1.23	3.26E-04	4.07E-03
Psed_0889	Tat-translocated protein	1.23	1.03E-03	9.76E-03
Psed_3564	Aromatic-amino-acid transaminase	1.22	1.58E-04	2.24E-03
Psed_7007	hypothetical protein	1.22	1.14E-07	6.30E-06
Psed_2809	cell division protein FtsZ	1.22	6.90E-05	1.15E-03
Psed_6737	alpha/beta hydrolase fold	1.22	2.01E-04	2.73E-03
Psed_6892	hypothetical protein	1.21	2.07E-07	1.01E-05
Psed_3729	3-oxoacyl-[acyl-carrier-protein] reductase	1.21	6.35E-04	6.75E-03
Psed_6262	ABC-type transporter, integral membrane subunit	1.21	8.73E-06	2.00E-04
Psed_3995	ABC-type transporter, periplasmic subunit	1.20	6.83E-06	1.63E-04
Psed_6732	MaoC domain protein dehydratase	1.19	1.01E-04	1.57E-03
Psed_5293	hypothetical protein	1.19	3.95E-06	1.09E-04
Psed_2715	sporulation and cell division protein SsgA	1.19	1.48E-06	4.90E-05
Psed_3043	formate dehydrogenase, alpha subunit	1.17	9.00E-05	1.42E-03
Psed_7019	hypothetical protein	1.17	8.56E-05	1.37E-03

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Psed_6758	hypothetical protein	1.17	9.47E-07	3.48E-05
Psed_4147	Potassium-transporting ATPase A chain	1.17	1.41E-04	2.04E-03
Psed_6994	Cobyrinic acid ac-diamide synthase	1.17	6.44E-05	1.10E-03
Psed_2887	diguanylate cyclase	1.17	7.15E-06	1.69E-04
Psed_3271	BFD domain protein [2Fe-2S]-binding domain protein	1.17	1.16E-05	2.58E-04
Psed_1691	methylmalonyl-CoA mutase, large subunit	1.16	6.64E-04	6.98E-03
Psed_7018	putative partitioning protein ParA	1.14	1.61E-05	3.36E-04
Psed_6958	hypothetical protein	1.13	2.97E-06	8.88E-05
Psed_6987	Integrase catalytic region	1.13	1.33E-07	7.07E-06
Psed_0079	ABC-type transporter, periplasmic subunit	1.13	6.12E-05	1.06E-03
Psed_6203	Luciferase-like, subgroup	1.13	5.16E-06	1.33E-04
Psed_0023	regulatory protein GntR HTH	1.13	8.69E-10	1.55E-07
Psed_3817	hypothetical protein	1.12	8.36E-05	1.34E-03
Psed_3722	hypothetical protein	1.12	5.55E-04	6.07E-03
Psed_3678	squalene-associated FAD-dependent desaturase	1.12	5.95E-06	1.49E-04
Psed_3042	NADH dehydrogenase (quinone)	1.11	2.84E-04	3.67E-03
Psed_2132	hypothetical protein	1.11	1.84E-04	2.54E-03
Psed_4688	polysaccharide deacetylase	1.10	3.46E-06	1.00E-04
Psed_2893	hypothetical protein	1.09	5.35E-07	2.18E-05
Psed_0022	hypothetical protein	1.09	8.31E-10	1.54E-07
Psed_6891	Relaxase/mobilization nuclease family protein	1.09	3.23E-07	1.42E-05
Psed_6961	hypothetical protein	1.09	2.62E-07	1.20E-05
Psed_3728	Pyruvate dehydrogenase (acetyl-transferring)	1.08	9.49E-04	9.19E-03
Psed_6988	IstB domain protein ATP-binding protein	1.06	2.11E-06	6.72E-05
Psed_5215	cell envelope-related function transcriptional attenuator, LytR/CpsA family	1.06	5.80E-05	1.01E-03
Psed_6951	hypothetical protein	1.05	2.18E-05	4.30E-04
Psed_2491	hypothetical protein	1.05	5.93E-04	6.42E-03
Psed_2763	hypothetical protein	1.05	3.47E-05	6.66E-04
Psed_3375	NLP/P60 protein	1.05	4.94E-05	8.87E-04
Psed_6893	hypothetical protein	1.03	3.62E-05	6.89E-04
Psed_0078	Methyltransferase type 12	1.03	3.41E-06	9.97E-05
Psed_3175	hypothetical protein	1.02	2.00E-06	6.44E-05
Psed_5710	hypothetical protein	1.01	1.44E-04	2.07E-03

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Psed_3723	oxidoreductase domain protein	1.01	8.92E-04	8.74E-03
Psed_0195	FAD linked oxidase domain protein	-1.00	1.49E-04	2.13E-03
Psed_3266	ABC transporter related	-1.01	1.57E-04	2.24E-03
Psed_4486	metalloprotease ybeY	-1.01	6.17E-04	6.59E-03
Psed_2965	Creatininase	-1.01	2.82E-04	3.64E-03
Psed_6425	hypothetical protein	-1.02	3.52E-04	4.32E-03
Psed_6551	Nitric oxide dioxygenase	-1.02	8.25E-07	3.07E-05
Psed_0381	hypothetical protein	-1.03	4.18E-04	4.92E-03
Psed_6812	hypothetical protein	-1.03	9.70E-06	2.22E-04
Psed_5749	hypothetical protein	-1.04	4.54E-04	5.25E-03
Psed_0425	Threonine aldolase	-1.04	2.84E-08	2.03E-06
Psed_4662	processing peptidase	-1.05	3.95E-05	7.34E-04
Psed_1116	transcription factor WhiB	-1.05	5.46E-06	1.39E-04
Psed_5773	hypothetical protein	-1.06	1.44E-05	3.08E-04
Psed_4257	regulatory protein LuxR	-1.06	4.71E-05	8.60E-04
Psed_0390	hypothetical protein	-1.06	8.01E-04	8.04E-03
Psed_6609	Pyruvate dehydrogenase (acetyl-transferring)	-1.07	7.49E-05	1.23E-03
Psed_4401	DoxX family protein	-1.07	1.44E-05	3.08E-04
Psed_3513	Helix-turn-helix, AraC domain	-1.08	7.04E-04	7.22E-03
Psed_0310	Xenobiotic-transporting ATPase	-1.09	1.33E-06	4.49E-05
Psed_4753	nitrogen regulatory protein P-II	-1.09	1.96E-05	3.88E-04
Psed_4689	RIO-like kinase	-1.11	2.12E-07	1.02E-05
Psed_5780	hypothetical protein	-1.12	8.85E-05	1.41E-03
Psed_5748	hypothetical protein	-1.12	1.84E-07	9.14E-06
Psed_6857	protein of unknown function DUF156	-1.13	1.05E-05	2.37E-04
Psed_3267	Glyoxalase/bleomycin resistance protein/dioxygenase	-1.13	1.74E-04	2.43E-03
Psed_4051	hypothetical protein	-1.13	2.40E-07	1.12E-05
Psed_5746	hypothetical protein	-1.13	5.67E-07	2.24E-05
Psed_5374	hypothetical protein	-1.13	4.00E-04	4.77E-03
Psed_5763	hypothetical protein	-1.13	6.71E-05	1.13E-03
Psed_5747	hypothetical protein	-1.14	1.41E-06	4.71E-05
Psed_5283	regulatory protein TetR	-1.15	8.15E-06	1.89E-04
Psed_0388	Acetylcholinesterase	-1.15	8.35E-05	1.34E-03
Psed_6811	type IV secretory pathway VirD4 protein-like protein	-1.15	3.15E-04	3.97E-03
Psed_2631	DNA topoisomerase IB	-1.16	4.77E-04	5.44E-03
Psed_1824	Methyltransferase type 11	-1.16	3.39E-05	6.55E-04
Psed_4549	Stearoyl-CoA 9-desaturase	-1.17	3.16E-08	2.21E-06

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Psed_2550	Glycine hydroxymethyltransferase	-1.18	3.33E-08	2.26E-06
Psed_3590	Tellurium resistance	-1.18	5.49E-04	6.02E-03
Psed_6666	protein of unknown function UPF0158	-1.20	4.46E-06	1.18E-04
Psed_0355	hypothetical protein	-1.20	2.62E-07	1.20E-05
Psed_3500	glucose-6-phosphate 1-dehydrogenase	-1.20	1.30E-04	1.92E-03
Psed_3198	hypothetical protein	-1.20	5.25E-04	5.82E-03
Psed_6636	Nitric-oxide synthase	-1.20	1.22E-04	1.82E-03
Psed_1420	hypothetical protein	-1.20	6.84E-06	1.63E-04
Psed_1791	fatty acid desaturase	-1.21	3.96E-04	4.76E-03
Psed_2293	heat shock protein DnaJ domain protein	-1.21	1.50E-04	2.14E-03
Psed_6426	Copper amine oxidase domain-containing protein	-1.21	2.33E-06	7.22E-05
Psed_5760	hypothetical protein	-1.22	5.79E-04	6.30E-03
Psed_5756	hypothetical protein	-1.22	6.69E-05	1.13E-03
Psed_1973	Valyl-tRNA synthetase	-1.24	1.90E-04	2.62E-03
Psed_6427	amino acid permease-associated region	-1.25	1.51E-05	3.22E-04
Psed_5802	hypothetical protein	-1.25	1.07E-04	1.65E-03
Psed_6853	protein of unknown function DUF302	-1.26	2.54E-09	3.06E-07
Psed_6553	Chaperone protein dnaJ	-1.26	3.45E-05	6.65E-04
Psed_2660	regulatory protein TetR	-1.28	1.33E-07	7.07E-06
Psed_2288	hypothetical protein	-1.28	9.36E-04	9.09E-03
Psed_4106	Catalase	-1.28	5.07E-05	9.03E-04
Psed_5826	L-2,4-diaminobutyric acid acetyltransferase	-1.28	1.32E-06	4.47E-05
Psed_2553	Glycine dehydrogenase [decarboxylating]	-1.29	2.66E-09	3.15E-07
Psed_4487	PhoH family protein	-1.29	7.51E-05	1.23E-03
Psed_5781	hypothetical protein	-1.30	2.99E-04	3.82E-03
Psed_3456	Thioredoxin-disulfide reductase	-1.30	2.23E-06	7.01E-05
Psed_3686	integral membrane protein	-1.31	1.29E-05	2.81E-04
Psed_6608	Dihydrolipoyllysine-residue acetyltransferase	-1.31	4.15E-05	7.67E-04
Psed_3472	protein of unknown function (DUF461)	-1.33	6.81E-07	2.66E-05
Psed_2974	phosphoenolpyruvate-protein phosphotransferase	-1.33	5.46E-06	1.39E-04
Psed_6158	hypothetical protein	-1.33	4.35E-06	1.17E-04
Psed_1113	glutaredoxin-like protein	-1.34	1.20E-06	4.21E-05
Psed_6856	Rhodanese-like protein	-1.36	1.68E-06	5.51E-05
Psed_3481	ATP-dependent chaperone ClpB	-1.40	1.19E-04	1.79E-03

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Psed_1168	3-oxoacyl-[acyl-carrier-protein] reductase	-1.40	2.65E-06	8.14E-05
Psed_2661	drug resistance transporter, EmrB/QacA subfamily	-1.42	1.69E-04	2.38E-03
Psed_6506	major facilitator superfamily MFS_1	-1.45	2.61E-08	1.89E-06
Psed_2299	hypothetical protein	-1.45	1.37E-04	2.01E-03
Psed_5758	hypothetical protein	-1.45	1.52E-05	3.23E-04
Psed_0385	Radical SAM domain protein	-1.47	2.16E-06	6.88E-05
Psed_2119	GTP-binding proten HflX	-1.47	3.15E-04	3.97E-03
Psed_2011	threonine synthase	-1.49	3.90E-09	4.30E-07
Psed_5195	alanine dehydrogenase	-1.50	1.78E-04	2.48E-03
Psed_3967	GCN5-related N-acetyltransferase	-1.50	7.57E-07	2.90E-05
Psed_1303	Heavy metal transport/detoxification protein	-1.52	6.85E-06	1.63E-04
Psed_2434	GAF domain protein	-1.52	1.13E-05	2.53E-04
Psed_4754	nitrogen regulatory protein P-II	-1.57	3.74E-06	1.06E-04
Psed_3675	PucR family transcriptional regulator	-1.57	1.25E-06	4.29E-05
Psed_5736	Transglycosylase-like domain protein	-1.58	1.08E-04	1.65E-03
Psed_3858	protein of unknown function DUF6 transmembrane	-1.58	7.28E-07	2.82E-05
Psed_4860	hypothetical protein	-1.61	8.37E-04	8.32E-03
Psed_2291	pyridoxamine 5'-phosphate oxidase-related FMN-binding protein	-1.62	1.17E-06	4.14E-05
Psed_0311	regulatory protein TetR	-1.63	8.13E-07	3.06E-05
Psed_0091	regulatory protein MerR	-1.64	1.03E-08	9.19E-07
Psed_3463	hypothetical protein	-1.66	2.91E-06	8.76E-05
Psed_6552	regulatory protein MerR	-1.66	7.96E-06	1.85E-04
Psed_5632	pyrroline-5-carboxylate reductase	-1.66	2.47E-09	3.06E-07
Psed_2080	GCN5-related N-acetyltransferase	-1.66	2.08E-06	6.66E-05
Psed_5192	Uncharacterised protein family UPF0324	-1.67	6.60E-06	1.61E-04
Psed_2386	cyclase/dehydrase	-1.68	1.80E-06	5.85E-05
Psed_3964	Lysine exporter protein (LYSE/YGGA)	-1.68	4.14E-07	1.77E-05
Psed_1302	protein of unknown function DUF156	-1.68	3.14E-04	3.97E-03
Psed_5872	hypothetical protein	-1.69	3.10E-06	9.21E-05
Psed_4847	CBS domain containing protein	-1.70	4.65E-04	5.35E-03
Psed_4861	cell shape determining protein MreB/Mrl	-1.70	2.51E-05	4.92E-04
Psed_2385	hypothetical protein	-1.71	2.28E-06	7.14E-05
Psed_4300	nitrate reductase, alpha subunit	-1.76	1.81E-05	3.65E-04
Psed_4333	UBA/THIF-type NAD/FAD binding protein	-1.77	3.91E-05	7.30E-04

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Psed_5809	hypothetical protein	-1.78	2.97E-06	8.88E-05
Psed_2283	UspA domain-containing protein	-1.79	3.39E-04	4.20E-03
Psed_5803	hypothetical protein	-1.80	7.85E-07	2.97E-05
Psed_0903	hypothetical protein	-1.82	1.00E-05	2.28E-04
Psed_0382	hypothetical protein	-1.83	1.56E-05	3.28E-04
Psed_2292	response regulator receiver	-1.83	3.24E-04	4.06E-03
Psed_1530	transcription factor WhiB	-1.83	1.34E-07	7.07E-06
Psed_2973	dihydroxyacetone kinase, L subunit	-1.84	2.22E-10	5.36E-08
Psed_1421	ferric-uptake regulator	-1.84	2.23E-06	7.01E-05
Psed_2271	GCN5-related N-acetyltransferase	-1.85	1.56E-07	7.98E-06
Psed_1304	heavy metal translocating P-type ATPase	-1.85	4.66E-05	8.55E-04
Psed_5812	hypothetical protein	-1.86	1.71E-05	3.54E-04
Psed_6855	beta-lactamase domain protein	-1.86	4.59E-08	2.96E-06
Psed_4872	hypothetical protein	-1.87	3.31E-06	9.71E-05
Psed_2298	UspA domain-containing protein	-1.87	8.91E-04	8.74E-03
Psed_4299	major facilitator superfamily MFS_1	-1.88	7.22E-05	1.20E-03
Psed_5187	Adenosylhomocysteinase	-1.90	3.14E-06	9.28E-05
Psed_5810	hypothetical protein	-1.91	6.38E-06	1.57E-04
Psed_5458	hypothetical protein	-1.91	1.01E-07	5.72E-06
Psed_2630	Xenobiotic-transporting ATPase	-1.91	7.11E-09	6.81E-07
Psed_3453	hypothetical protein	-1.94	4.52E-07	1.90E-05
Psed_2289	hypothetical protein	-1.94	1.61E-05	3.36E-04
Psed_2290	hypothetical protein	-1.94	4.01E-04	4.78E-03
Psed_5800	hypothetical protein	-1.94	1.14E-07	6.30E-06
Psed_4984	isochorismatase hydrolase	-1.96	7.00E-08	4.17E-06
Psed_3496	hypothetical protein	-1.98	1.61E-07	8.21E-06
Psed_2297	hypothetical protein	-1.99	6.30E-04	6.70E-03
Psed_1422	Ferritin Dps family protein	-2.00	3.71E-08	2.46E-06
Psed_3484	Protein grpE	-2.01	2.08E-07	1.01E-05
Psed_4301	nitrate reductase, beta subunit	-2.02	5.48E-04	6.02E-03
Psed_5813	hypothetical protein	-2.02	3.17E-06	9.34E-05
Psed_0554	hypothetical protein	-2.02	3.46E-04	4.27E-03
Psed_5185	Thymidylate kinase	-2.03	4.25E-06	1.15E-04
Psed_3483	chaperone DnaJ domain protein	-2.05	2.05E-08	1.60E-06
Psed_2972	dihydroxyacetone kinase, DhaK subunit	-2.05	2.03E-08	1.60E-06
Psed_3669	hypothetical protein	-2.06	1.76E-07	8.81E-06
Psed_4332	hypothetical protein	-2.09	2.30E-06	7.15E-05
Psed_5520	hypothetical protein	-2.10	3.97E-06	1.09E-04
Psed_2281	ABC transporter related	-2.11	2.86E-07	1.27E-05

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Psed_4840	CBS domain containing protein	-2.13	6.80E-04	7.12E-03
Psed_6547	ATP-dependent chaperone ClpB	-2.13	5.79E-09	5.89E-07
Psed_0350	Bile acid:sodium symporter	-2.15	1.25E-06	4.29E-05
Psed_4755	ammonium transporter	-2.15	1.24E-06	4.29E-05
Psed_4874	Glyoxalase/bleomycin resistance protein/ dioxygenase	-2.15	8.28E-05	1.33E-03
Psed_5808	hypothetical protein	-2.17	3.43E-06	9.98E-05
Psed_3497	transport-associated	-2.19	2.13E-09	2.71E-07
Psed_1927	hypothetical protein	-2.21	1.26E-08	1.07E-06
Psed_4842	UspA domain-containing protein	-2.23	8.11E-05	1.31E-03
Psed_1117	hypothetical protein	-2.24	1.11E-08	9.80E-07
Psed_3451	Domain of unknown function DUF1931	-2.24	7.04E-08	4.17E-06
Psed_5814	hypothetical protein	-2.26	7.91E-06	1.85E-04
Psed_5186	NUDIX hydrolase	-2.29	5.23E-07	2.14E-05
Psed_4841	UspA domain-containing protein	-2.31	2.57E-04	3.38E-03
Psed_3460	formaldehyde dehydrogenase, glutathi-one-independent	-2.31	1.33E-08	1.11E-06
Psed_2436	nitroreductase	-2.32	5.25E-05	9.26E-04
Psed_3508	intracellular protease, PfpI family	-2.35	2.07E-08	1.60E-06
Psed_2679	NADH dehydrogenase (ubiquinone)	-2.35	3.29E-09	3.69E-07
Psed_5797	hypothetical protein	-2.35	2.16E-07	1.03E-05
Psed_2978	RNA polymerase sigma factor, sigma-70 family	-2.36	8.73E-09	7.91E-07
Psed_5784	hypothetical protein	-2.37	2.03E-08	1.60E-06
Psed_4548	GCN5-related N-acetyltransferase	-2.37	3.34E-07	1.46E-05
Psed_5786	phage terminase	-2.38	5.41E-07	2.18E-05
Psed_3109	hypothetical protein	-2.42	5.62E-07	2.23E-05
Psed_5358	transcription factor WhiB	-2.47	1.48E-08	1.20E-06
Psed_5783	hypothetical protein	-2.47	1.44E-06	4.77E-05
Psed_0389	hypothetical protein	-2.49	4.71E-06	1.23E-04
Psed_3446	Ferritin Dps family protein	-2.52	1.51E-09	2.31E-07
Psed_4843	UspA domain-containing protein	-2.53	1.16E-05	2.58E-04
Psed_2295	Domain of unknown function DUF1918	-2.54	4.20E-06	1.15E-04
Psed_5811	hypothetical protein	-2.72	2.93E-08	2.07E-06
Psed_6665	NLP/P60 protein	-2.77	4.78E-07	2.00E-05
Psed_5815	hypothetical protein	-2.84	1.01E-07	5.72E-06
Psed_4848	hypothetical protein	-2.89	4.81E-05	8.73E-04
Psed_2433	DoxX family protein	-2.96	4.50E-04	5.23E-03
Psed_0312	Sulfate transporter/antisigma-factor an-tagonist STAS	-2.96	1.80E-09	2.39E-07

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Psed_5801	hypothetical protein	-2.97	2.10E-08	1.60E-06
Psed_3108	hypothetical protein	-3.02	3.96E-07	1.72E-05
Psed_4873	Domain of unknown function DUF1876	-3.04	5.50E-06	1.39E-04
Psed_3486	hypothetical protein	-3.19	1.24E-08	1.06E-06
Psed_3485	Chaperone protein dnaK	-3.25	1.30E-09	2.11E-07
Psed_3487	heat shock protein Hsp20	-3.30	2.11E-09	2.71E-07
Psed_2437	CBS domain containing protein	-3.30	7.01E-06	1.67E-04
Psed_5793	hypothetical protein	-3.30	1.67E-09	2.37E-07
Psed_5792	hypothetical protein	-3.34	6.41E-08	3.90E-06
Psed_5787	hypothetical protein	-3.43	8.92E-11	2.90E-08
Psed_5798	hypothetical protein	-3.52	2.77E-09	3.22E-07
Psed_0090	hypothetical protein	-3.58	8.61E-13	1.12E-09
Psed_5799	hypothetical protein	-3.59	4.24E-11	1.97E-08
Psed_2680	Cysteine desulfurase	-3.59	1.06E-11	8.66E-09
Psed_2554	Xenobiotic-transporting ATPase	-3.61	1.00E-10	3.11E-08
Psed_5782	Collagen triple helix repeat-containing protein	-3.64	8.34E-09	7.83E-07
Psed_5804	hypothetical protein	-3.74	2.96E-09	3.39E-07
Psed_5789	hypothetical protein	-3.92	8.75E-09	7.91E-07
Psed_5796	hypothetical protein	-3.92	1.79E-09	2.39E-07
Psed_5788	peptidase S14 ClpP	-4.23	2.68E-11	1.65E-08
Psed_5791	hypothetical protein	-4.39	3.17E-13	6.87E-10
Psed_5794	hypothetical protein	-4.58	8.16E-12	7.59E-09
Psed_5790	hypothetical protein	-4.67	5.29E-10	1.08E-07
Psed_5795	hypothetical protein	-4.94	3.05E-11	1.65E-08
Psed_0088	protein of unknown function DUF485	-5.61	1.15E-14	3.91E-11
Psed_0089	SSS sodium solute transporter superfamily	-6.25	1.20E-14	3.91E-11

Appendix 10

Genes differentially expressed on THF versus succinate.

Appendix 10. Genes differentially expressed on THF versus succinate. Differentially expressed genes have an adjusted p-value < 0.01 and have a $log_2FC > 1$ or $log_2FC < -1$ and are sorted by log_2FC.

Genes	Protein	log_2FC	p-value	adjusted p-value
Psed_4939	lipase	6.88	7.00E-17	2.28E-13
Psed_4940	fumarylacetoacetate (FAA) hydrolase	5.23	1.14E-12	6.76E-10
Psed_5248	nitroreductase	4.41	8.03E-14	8.72E-11
Psed_4700	Cyclopropane-fatty-acyl-phospholipid synthase	3.85	2.04E-08	1.09E-06
Psed_5249	NADPH:quinone reductase	3.70	5.13E-11	1.52E-08
Psed_1422	Ferritin Dps family protein	3.54	4.97E-11	1.52E-08
Psed_0079	ABC-type transporter, periplasmic subunit	3.41	2.31E-10	4.56E-08
Psed_6982	Mn2+/Fe2+ transporter, NRAMP family	3.21	1.63E-07	4.90E-06
Psed_2204	50S ribosomal protein L33	3.02	1.58E-08	9.08E-07
Psed_4938	regulatory protein MarR	2.99	2.14E-08	1.11E-06
Psed_6970	D-lactate dehydrogenase (cytochrome)	2.91	1.20E-06	2.16E-05
Psed_6807	hypothetical protein	2.90	8.82E-07	1.69E-05
Psed_3049	Formamidase	2.87	9.70E-05	6.43E-04
Psed_6094	ABC-type metal ion transporter, periplasmic subunit	2.79	2.91E-07	7.69E-06
Psed_4941	Formyl-CoA transferase	2.76	4.71E-08	2.00E-06
Psed_0934	YCII-related	2.74	4.70E-09	4.13E-07
Psed_4702	protein of unknown function DUF1295	2.69	7.48E-06	8.05E-05
Psed_4322	Dihydrolipoyl dehydrogenase	2.63	4.30E-11	1.47E-08
Psed_5471	hypothetical protein	2.59	7.12E-08	2.65E-06
Psed_2789	hypothetical protein	2.51	1.18E-09	1.58E-07
Psed_6981	Aldehyde Dehydrogenase	2.48	3.59E-08	1.67E-06
Psed_5783	hypothetical protein	2.47	3.10E-06	4.22E-05
Psed_2203	cobalamin synthesis CobW domain protein	2.46	1.20E-07	3.94E-06
Psed_0078	Methyltransferase type 12	2.44	1.64E-10	3.55E-08
Psed_0950	hypothetical protein	2.39	7.81E-06	8.39E-05
Psed_3571	Radical SAM domain protein	2.37	1.56E-08	9.08E-07
Psed_3256	hypothetical protein	2.35	1.21E-05	1.21E-04
Psed_6640	extracellular solute-binding protein family 1	2.27	3.25E-06	4.38E-05
Psed_1655	hypothetical protein	2.24	1.26E-10	3.03E-08
Psed_5247	hypothetical protein	2.23	1.72E-07	5.14E-06

Psed_4321	hypothetical protein	2.18	3.04E-07	7.94E-06
Psed_6753	hypothetical protein	2.12	2.75E-05	2.32E-04
Psed_1657	Cobalt transporter subunit CbtB putative	2.12	4.32E-08	1.89E-06
Psed_4701	Cyclopropane-fatty-acyl-phospholipid synthase	2.11	1.36E-04	8.29E-04
Psed_3048	AmiS/UreI transporter	2.05	9.98E-04	4.03E-03
Psed_6879	S-adenosylmethionine synthase	2.04	3.41E-05	2.74E-04
Psed_5868	hypothetical protein	1.98	3.62E-06	4.77E-05
Psed_3037	ABC-type metal ion transporter, periplasmic subunit	1.97	1.60E-07	4.85E-06
Psed_5760	hypothetical protein	1.96	1.09E-05	1.10E-04
Psed_5759	hypothetical protein	1.94	1.66E-05	1.54E-04
Psed_4268	membrane protein of uknown function UCP014873	1.94	7.01E-05	4.87E-04
Psed_5779	hypothetical protein	1.93	2.61E-09	2.74E-07
Psed_6665	NLP/P60 protein	1.92	5.64E-05	4.08E-04
Psed_3440	SH3 type 3 domain protein	1.90	6.92E-06	7.62E-05
Psed_3038	Sulfate-transporting ATPase	1.86	2.76E-08	1.37E-06
Psed_5317	Error-prone DNA polymerase	1.85	5.96E-08	2.35E-06
Psed_1968	hypothetical protein	1.84	4.01E-09	3.68E-07
Psed_6096	ABC-type transporter, integral membrane subunit	1.82	3.72E-06	4.84E-05
Psed_4703	alkyl hydroperoxide reductase/ Thiol specific antioxidant/ Mal allergen	1.82	3.13E-04	1.57E-03
Psed_6095	Fe(3+)-transporting ATPase	1.80	5.30E-04	2.41E-03
Psed_6751	Transglycosylase-like domain protein	1.78	2.39E-06	3.49E-05
Psed_6977	Ferredoxin--NAD(+) reductase	1.78	5.58E-08	2.29E-06
Psed_3652	hypothetical protein	1.77	1.92E-06	3.01E-05
Psed_1303	Heavy metal transport/detoxification protein	1.77	2.55E-06	3.69E-05
Psed_1967	major facilitator superfamily MFS_1	1.76	1.38E-09	1.76E-07
Psed_6980	hypothetical protein	1.76	3.31E-07	8.23E-06
Psed_4819	integral membrane protein	1.75	3.24E-05	2.63E-04
Psed_5224	hypothetical protein	1.74	1.01E-04	6.65E-04
Psed_3904	CO dehydrogenase maturation factor-like protein	1.73	3.14E-05	2.58E-04
Psed_6971	Hydroxyacid-oxoacid transhydrogenase	1.73	1.93E-05	1.76E-04
Psed_1302	protein of unknown function DUF156	1.71	4.80E-04	2.23E-03
Psed_0982	fibronectin-attachment family protein	1.70	2.20E-04	1.21E-03
Psed_0094	YCII-related	1.69	3.63E-06	4.77E-05

Appendix 10. Genes differentially expressed on THF versus succinate.

Psed_1107	peptidase C60 sortase A and B	1.68	3.32E-06	4.48E-05
Psed_2265	hypothetical protein	1.68	3.06E-04	1.55E-03
Psed_5758	hypothetical protein	1.68	6.68E-06	7.46E-05
Psed_4937	hypothetical protein	1.67	3.83E-07	9.03E-06
Psed_2129	DNA binding domain protein, excisionase family	1.67	5.05E-06	6.04E-05
Psed_0355	hypothetical protein	1.67	9.85E-09	6.86E-07
Psed_6780	hypothetical protein	1.66	1.43E-07	4.51E-06
Psed_3985	hypothetical protein	1.65	8.63E-06	9.11E-05
Psed_1656	hypothetical protein	1.64	1.02E-07	3.51E-06
Psed_5246	uncharacterized peroxidase-related enzyme	1.63	4.02E-06	5.08E-05
Psed_3669	hypothetical protein	1.63	6.01E-06	6.93E-05
Psed_0139	nuclear export factor GLE1	1.62	8.09E-04	3.41E-03
Psed_5757	hypothetical protein	1.61	1.84E-05	1.68E-04
Psed_5784	hypothetical protein	1.60	4.87E-06	5.90E-05
Psed_0016	Integrase catalytic region	1.60	4.38E-04	2.07E-03
Psed_0106	putative Fe-S oxidoreductase	1.59	9.76E-05	6.45E-04
Psed_0137	copper resistance D domain protein	1.59	4.03E-06	5.09E-05
Psed_0288	S-(hydroxymethyl)glutathione dehydrogenase	1.58	6.29E-08	2.44E-06
Psed_2397	putative acyl-CoA dehydrogenase-related protein	1.57	7.16E-04	3.09E-03
Psed_3023	cobaltochelatase, CobN subunit	1.56	3.07E-09	3.07E-07
Psed_0580	highly repetitive protein	1.56	7.21E-05	4.98E-04
Psed_1421	ferric-uptake regulator	1.56	2.87E-05	2.40E-04
Psed_2652	carbon-monoxide dehydrogenase, large subunit	1.55	3.59E-09	3.43E-07
Psed_6979	monooxygenase component MmoB/DmpM	1.55	1.11E-07	3.72E-06
Psed_1584	6-phosphofructokinase	1.54	6.20E-08	2.43E-06
Psed_0082	Monosaccharide-transporting ATPase	1.53	1.44E-08	8.77E-07
Psed_5320	thioredoxin	1.51	7.44E-07	1.49E-05
Psed_2321	ABC-type sugar transport system periplasmic component-like	1.51	9.98E-06	1.02E-04
Psed_6544	hypothetical protein	1.51	1.34E-06	2.30E-05
Psed_5844	hypothetical protein	1.50	2.96E-04	1.50E-03
Psed_6799	transposase IS4 family protein	1.49	2.54E-04	1.34E-03
Psed_1653	hypothetical protein	1.48	5.74E-07	1.21E-05
Psed_0138	copper resistance protein CopC	1.47	5.72E-04	2.57E-03

Appendix 10. Genes differentially expressed on THF versus succinate.

Psed_6409	Endonuclease/exonuclease/phosphatase	1.46	1.96E-04	1.10E-03
Psed_5324	hypothetical protein	1.46	9.58E-09	6.78E-07
Psed_5753	hypothetical protein	1.45	6.84E-07	1.40E-05
Psed_5814	hypothetical protein	1.45	1.07E-03	4.25E-03
Psed_1585	hypothetical protein	1.43	1.35E-05	1.31E-04
Psed_6806	hypothetical protein	1.43	1.57E-05	1.48E-04
Psed_5761	hypothetical protein	1.41	8.92E-06	9.36E-05
Psed_4359	hypothetical protein	1.39	9.54E-06	9.90E-05
Psed_0131	Alcohol dehydrogenase GroES domain protein	1.39	3.35E-05	2.71E-04
Psed_2491	hypothetical protein	1.37	9.71E-05	6.43E-04
Psed_2123	Peptidoglycan-binding lysin domain	1.37	2.54E-07	7.05E-06
Psed_5769	hypothetical protein	1.37	1.22E-06	2.18E-05
Psed_3017	precorrin-6y C5,15-methyltransferase (decarboxylating), CbiE subunit	1.36	2.00E-05	1.80E-04
Psed_2650	hypothetical protein	1.36	9.09E-08	3.23E-06
Psed_5767	hypothetical protein	1.36	6.85E-07	1.40E-05
Psed_5736	Transglycosylase-like domain protein	1.36	7.47E-04	3.19E-03
Psed_0951	hypothetical protein	1.36	2.26E-05	1.98E-04
Psed_6737	alpha/beta hydrolase fold	1.36	1.36E-04	8.31E-04
Psed_1268	Radical SAM domain protein	1.35	5.99E-09	5.00E-07
Psed_6972	GntR domain protein	1.35	5.91E-04	2.65E-03
Psed_6249	Ribulose bisphosphate carboxylase large chain	1.34	1.45E-05	1.39E-04
Psed_0130	amidohydrolase 2	1.34	2.27E-04	1.23E-03
Psed_2688	major facilitator superfamily MFS_1	1.34	3.80E-06	4.90E-05
Psed_2788	hypothetical protein	1.32	2.82E-04	1.45E-03
Psed_0095	RNA polymerase sigma factor, sigma-70 family	1.32	1.63E-07	4.90E-06
Psed_2939	putative acyltransferase	1.31	2.75E-06	3.86E-05
Psed_0369	GCN5-related N-acetyltransferase	1.31	2.95E-04	1.50E-03
Psed_1362	hypothetical protein	1.31	2.25E-03	7.69E-03
Psed_5323	hypothetical protein	1.30	2.25E-06	3.35E-05
Psed_5762	hypothetical protein	1.30	1.54E-06	2.58E-05
Psed_5747	hypothetical protein	1.30	6.40E-07	1.34E-05
Psed_6951	hypothetical protein	1.30	4.74E-06	5.76E-05
Psed_3069	ErfK/YbiS/YcfS/YnhG family protein	1.29	5.44E-05	3.98E-04
Psed_6732	MaoC domain protein dehydratase	1.28	9.72E-05	6.43E-04
Psed_5245	regulatory protein TetR	1.28	6.35E-06	7.24E-05
Psed_3594	Tellurite resistance TerB	1.26	2.00E-03	6.98E-03

Appendix 10. Genes differentially expressed on THF versus succinate.

Psed_0055	non-ribosomal peptide synthetase domain protein	1.26	4.74E-05	3.58E-04
Psed_6856	Rhodanese-like protein	1.26	8.38E-06	8.87E-05
Psed_5967	4Fe-4S ferredoxin iron-sulfur binding domain-containing protein	1.26	9.61E-04	3.92E-03
Psed_6855	beta-lactamase domain protein	1.26	1.05E-05	1.07E-04
Psed_4276	hypothetical protein	1.25	1.10E-04	7.07E-04
Psed_4360	heavy metal translocating P-type ATPase	1.23	5.76E-05	4.15E-04
Psed_4583	hypothetical protein	1.22	3.49E-07	8.52E-06
Psed_6193	Xenobiotic-transporting ATPase	1.22	6.83E-06	7.57E-05
Psed_6754	hypothetical protein	1.22	1.57E-05	1.48E-04
Psed_5778	cell divisionFtsK/SpoIIIE	1.22	1.58E-06	2.59E-05
Psed_3059	hypothetical protein	1.20	4.31E-05	3.30E-04
Psed_2647	hypothetical protein	1.20	4.66E-07	1.04E-05
Psed_6730	Luciferase-like, subgroup	1.20	1.83E-03	6.54E-03
Psed_3564	Aromatic-amino-acid transaminase	1.19	3.78E-04	1.83E-03
Psed_0081	ABC-type transporter, integral membrane subunit	1.19	3.38E-05	2.72E-04
Psed_2937	HNH endonuclease	1.18	3.55E-07	8.55E-06
Psed_2411	FAD-dependent pyridine nucleotide-disulphide oxidoreductase	1.18	3.50E-04	1.72E-03
Psed_0080	ABC-type transporter, integral membrane subunit	1.18	2.31E-07	6.59E-06
Psed_4627	YceI family protein	1.18	3.06E-05	2.53E-04
Psed_5811	hypothetical protein	1.17	6.00E-04	2.68E-03
Psed_5166	hypothetical protein	1.15	4.74E-04	2.21E-03
Psed_2444	PDZ/DHR/GLGF domain protein	1.15	1.63E-04	9.55E-04
Psed_6755	hypothetical protein	1.15	5.17E-05	3.81E-04
Psed_6606	hypothetical protein	1.15	1.43E-05	1.38E-04
Psed_5786	phage terminase	1.15	1.58E-03	5.78E-03
Psed_6401	heavy metal translocating P-type ATPase	1.14	1.24E-03	4.75E-03
Psed_6645	hypothetical protein	1.14	4.25E-05	3.26E-04
Psed_5800	hypothetical protein	1.13	1.03E-04	6.72E-04
Psed_4339	hypothetical protein	1.13	3.92E-07	9.18E-06
Psed_3317	hypothetical protein	1.13	1.61E-05	1.51E-04
Psed_0140	hypothetical protein	1.12	1.42E-03	5.31E-03
Psed_4881	hypothetical protein	1.12	2.52E-04	1.33E-03
Psed_0037	hypothetical protein	1.11	3.02E-03	9.73E-03
Psed_5688	hypothetical protein	1.11	6.45E-05	4.57E-04

Appendix 10. Genes differentially expressed on THF versus succinate.

Psed_3105	membrane protein AbrB duplication	1.10	3.11E-04	1.57E-03
Psed_5764	hypothetical protein	1.09	3.92E-04	1.89E-03
Psed_1292	alkyl hydroperoxide reductase/ Thiol specific antioxidant/ Mal allergen	1.09	1.23E-06	2.19E-05
Psed_6944	Xylose isomerase domain-containing protein TIM barrel	1.09	1.47E-04	8.81E-04
Psed_1037	Asp/Glu/hydantoin racemase	1.08	3.77E-05	2.98E-04
Psed_3903	hypothetical protein	1.08	8.36E-04	3.50E-03
Psed_6735	flavoprotein WrbA	1.08	2.49E-05	2.14E-04
Psed_1571	Glutathione transferase	1.08	1.68E-04	9.78E-04
Psed_4753	nitrogen regulatory protein P-II	1.08	4.34E-05	3.31E-04
Psed_1301	Redoxin domain protein	1.08	8.70E-04	3.62E-03
Psed_4445	regulatory protein TetR	1.07	2.94E-03	9.54E-03
Psed_5516	ferric-uptake regulator	1.07	1.72E-04	10.00E-04
Psed_5809	hypothetical protein	1.07	8.85E-04	3.67E-03
Psed_4390	hypothetical protein	1.07	5.75E-06	6.67E-05
Psed_6008	hypothetical protein	1.06	1.69E-04	9.85E-04
Psed_5870	hypothetical protein	1.06	2.75E-05	2.32E-04
Psed_5488	FAD-dependent pyridine nucleotide-disulphide oxidoreductase	1.06	3.42E-04	1.69E-03
Psed_4443	hypothetical protein	1.06	1.82E-04	1.04E-03
Psed_6197	major facilitator superfamily MFS_1	1.06	1.25E-04	7.78E-04
Psed_5592	hypothetical protein	1.06	1.87E-04	1.06E-03
Psed_5775	hypothetical protein	1.05	3.49E-04	1.72E-03
Psed_6978	methane/phenol/toluene hydroxylase	1.05	3.20E-07	8.17E-06
Psed_5741	hypothetical protein	1.05	7.29E-05	5.02E-04
Psed_6403	Heavy metal transport/detoxification protein	1.05	1.16E-05	1.17E-04
Psed_0096	Uncharacterised protein family UPF0126	1.05	2.83E-04	1.46E-03
Psed_2202	class II aldolase/adducin family protein	1.05	1.49E-07	4.58E-06
Psed_6963	replicative DNA helicase	1.05	1.66E-04	9.73E-04
Psed_5802	hypothetical protein	1.04	9.63E-04	3.92E-03
Psed_1898	CBS domain containing protein	1.04	4.16E-06	5.22E-05
Psed_5480	small multidrug resistance protein	1.04	1.23E-04	7.73E-04
Psed_4236	hypothetical protein	1.04	3.73E-05	2.96E-04
Psed_0289	hypothetical protein	1.04	9.82E-05	6.48E-04
Psed_6213	2'-5' RNA ligase	1.03	6.36E-05	4.52E-04
Psed_4382	hypothetical protein	1.03	3.76E-04	1.83E-03
Psed_6746	hypothetical protein	1.03	1.37E-04	8.31E-04

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Psed_5788	peptidase S14 ClpP	1.03	7.74E-04	3.29E-03
Psed_6736	Amidase	1.03	1.58E-03	5.78E-03
Psed_1129	hypothetical protein	1.02	1.50E-03	5.54E-03
Psed_1300	cytochrome c biogenesis protein transmembrane region	1.02	1.86E-06	2.92E-05
Psed_6897	hypothetical protein	1.02	4.95E-05	3.68E-04
Psed_6847	hypothetical protein	1.02	1.18E-04	7.43E-04
Psed_0357	helix-turn-helix domain protein	1.02	3.10E-07	8.08E-06
Psed_2206	hypothetical protein	1.02	1.25E-06	2.20E-05
Psed_1297	haloacid dehalogenase, type II	1.01	1.61E-03	5.86E-03
Psed_1284	hypothetical protein	1.01	9.12E-04	3.77E-03
Psed_1654	hypothetical protein	1.01	5.95E-05	4.27E-04
Psed_6818	hypothetical protein	1.01	1.47E-03	5.45E-03
Psed_2791	hypothetical protein	1.01	1.58E-04	9.33E-04
Psed_1102	ROK family protein	1.01	2.32E-03	7.87E-03
Psed_5527	hypothetical protein	1.01	3.31E-04	1.65E-03
Psed_4899	hypothetical protein	1.01	8.12E-04	3.42E-03
Psed_2549	L-serine dehydratase 1	1.00	5.45E-05	3.98E-04
Psed_5763	hypothetical protein	1.00	3.95E-04	1.90E-03
Psed_3589	stress protein	1.00	1.35E-04	8.28E-04
Psed_3569	hypothetical protein	-1.00	7.05E-06	7.71E-05
Psed_3520	Guanylate kinase	-1.01	2.26E-04	1.23E-03
Psed_1910	ribose 5-phosphate isomerase	-1.01	2.32E-03	7.87E-03
Psed_5699	Carbon-monoxide dehydrogenase (acceptor)	-1.01	1.77E-03	6.34E-03
Psed_1095	alpha/beta hydrolase fold	-1.01	1.48E-05	1.41E-04
Psed_0256	MOSC domain containing protein	-1.01	2.30E-06	3.40E-05
Psed_1829	glutamyl-tRNA synthetase	-1.01	7.98E-04	3.37E-03
Psed_5743	thiamine biosynthesis protein ThiS	-1.01	6.63E-08	2.53E-06
Psed_2972	dihydroxyacetone kinase, DhaK subunit	-1.01	1.37E-04	8.31E-04
Psed_2795	transglutaminase domain-containing protein	-1.01	2.67E-06	3.81E-05
Psed_6125	dihydropteroate synthase	-1.01	7.28E-05	5.02E-04
Psed_3704	Muconolactone delta-isomerase	-1.01	5.26E-07	1.14E-05
Psed_3475	hypothetical protein	-1.01	1.42E-07	4.51E-06
Psed_1896	C-methyltransferase	-1.01	5.13E-05	3.79E-04
Psed_0454	protein of unknown function DUF1416	-1.01	2.74E-04	1.43E-03
Psed_1830	Heat shock protein 70	-1.01	1.22E-03	4.71E-03
Psed_3998	Long-chain-acyl-CoA dehydrogenase	-1.02	2.42E-05	2.10E-04

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Psed_0116	Peptidoglycan glycosyltransferase	-1.02	1.05E-04	6.85E-04
Psed_4724	Methyltransferase type 11	-1.02	3.21E-06	4.35E-05
Psed_1795	Glutamyl-tRNA(Gln) amidotransferase subunit A	-1.02	4.95E-07	1.09E-05
Psed_3784	NAD(+) synthase (glutamine-hydrolyzing)	-1.02	9.69E-07	1.81E-05
Psed_2906	hypothetical protein	-1.02	2.27E-05	1.99E-04
Psed_1862	protein of unknown function DUF477	-1.02	4.16E-04	1.99E-03
Psed_2965	Creatininase	-1.02	4.68E-04	2.18E-03
Psed_3291	hypothetical protein	-1.03	1.24E-05	1.23E-04
Psed_1528	anti-sigma factor	-1.03	1.76E-05	1.62E-04
Psed_1028	RNA polymerase sigma factor, sigma-70 family	-1.03	5.95E-04	2.66E-03
Psed_0428	adenylosuccinate lyase	-1.03	1.42E-04	8.56E-04
Psed_5733	molybdenum cofactor biosynthesis protein C	-1.04	2.85E-04	1.46E-03
Psed_0195	FAD linked oxidase domain protein	-1.04	2.02E-04	1.13E-03
Psed_3887	Transcriptional regulator IclR	-1.04	4.32E-06	5.37E-05
Psed_5914	Hydantoinase B/oxoprolinase	-1.04	1.15E-04	7.30E-04
Psed_3403	cytochrome oxidase assembly	-1.04	3.70E-05	2.94E-04
Psed_0011	hypothetical protein	-1.04	5.16E-05	3.80E-04
Psed_3541	aminodeoxychorismate lyase	-1.05	9.01E-07	1.72E-05
Psed_1841	hypothetical protein	-1.05	2.97E-04	1.50E-03
Psed_1540	Phosphonate-transporting ATPase	-1.05	1.72E-05	1.60E-04
Psed_4598	hypothetical protein	-1.05	2.36E-07	6.72E-06
Psed_3298	Nitrate reductase	-1.05	3.67E-07	8.78E-06
Psed_5114	Phosphoglycerate mutase	-1.05	8.07E-06	8.59E-05
Psed_1685	ATP synthase subunit beta	-1.05	2.53E-03	8.45E-03
Psed_5019	protein of unknown function DUF1707	-1.05	3.03E-04	1.53E-03
Psed_3461	transport-associated	-1.05	6.76E-04	2.94E-03
Psed_0169	hypothetical protein	-1.05	1.79E-04	1.03E-03
Psed_1370	regulatory protein LacI	-1.05	9.26E-07	1.75E-05
Psed_3878	Monosaccharide-transporting ATPase	-1.05	4.48E-07	1.01E-05
Psed_5690	response regulator receiver	-1.05	3.36E-06	4.51E-05
Psed_2799	Ribosomal RNA small subunit methyltransferase H	-1.06	3.09E-04	1.56E-03
Psed_0541	Ethyl tert-butyl ether degradation EthD	-1.06	5.67E-06	6.60E-05
Psed_5289	lipolytic protein G-D-S-L family	-1.06	3.33E-06	4.48E-05
Psed_5344	hypothetical protein	-1.06	6.70E-05	4.69E-04
Psed_3323	ATP phosphoribosyltransferase	-1.06	1.08E-04	6.97E-04

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Psed_0835	Transglycosylase-like domain protein	-1.06	1.52E-05	1.44E-04
Psed_4018	ATP-binding region ATPase domain protein	-1.06	2.44E-03	8.22E-03
Psed_1852	lipoprotein	-1.06	7.05E-04	3.05E-03
Psed_3243	nitrile hydratase, alpha subunit	-1.06	6.44E-09	5.04E-07
Psed_3680	squalene-hopene cyclase	-1.06	9.92E-06	1.02E-04
Psed_5311	hypothetical protein	-1.06	8.70E-06	9.15E-05
Psed_5336	chorismate mutase	-1.06	4.22E-05	3.25E-04
Psed_1527	RNA polymerase sigma-70 factor, sigma-B/F/G subfamily	-1.06	1.40E-04	8.46E-04
Psed_3699	Dimethylmenaquinone methyltransferase	-1.06	1.22E-05	1.21E-04
Psed_4969	Hydroxyquinol 1,2-dioxygenase	-1.06	1.56E-06	2.59E-05
Psed_0233	putative transglycosylase associated protein	-1.06	3.20E-05	2.61E-04
Psed_3060	nucleic acid binding OB-fold tRNA/helicase-type	-1.06	6.81E-05	4.75E-04
Psed_3145	beta-phosphoglucomutase family hydrolase	-1.06	6.61E-06	7.45E-05
Psed_5275	Carbamoyl-phosphate synthase L chain ATP-binding	-1.06	2.00E-05	1.80E-04
Psed_4670	phosphoesterase RecJ domain protein	-1.07	3.32E-07	8.23E-06
Psed_0227	hypothetical protein	-1.07	4.25E-07	9.75E-06
Psed_5548	Enoyl-CoA hydratase/isomerase	-1.07	6.99E-06	7.68E-05
Psed_5211	Mannose-1-phosphate guanylyltransferase	-1.07	1.21E-07	3.96E-06
Psed_5014	esterase/lipase	-1.07	1.74E-04	1.01E-03
Psed_6562	hypothetical protein	-1.08	3.30E-07	8.23E-06
Psed_4739	50S ribosomal protein L19	-1.08	2.12E-04	1.18E-03
Psed_0208	ABC-2 type transporter	-1.08	2.03E-03	7.05E-03
Psed_1712	DivIVA domain	-1.08	1.70E-06	2.73E-05
Psed_4561	branched-chain amino acid aminotransferase	-1.08	1.87E-04	1.07E-03
Psed_4671	Ribosome-binding factor A	-1.08	1.78E-04	1.03E-03
Psed_3161	protein of unknown function DUF151	-1.08	6.24E-04	2.75E-03
Psed_4468	glycyl-tRNA synthetase	-1.08	2.75E-04	1.43E-03
Psed_1911	DNA-(apurinic or apyrimidinic site) lyase	-1.08	4.70E-06	5.73E-05
Psed_0311	regulatory protein TetR	-1.08	1.31E-04	8.09E-04

Psed_3156	Sulfate transporter/antisigma-factor antagonist STAS	-1.09	1.31E-05	1.28E-04
Psed_5875	2-nitropropane dioxygenase NPD	-1.09	1.56E-06	2.59E-05
Psed_4949	Hydroxypyruvate isomerase	-1.09	1.13E-06	2.06E-05
Psed_3116	YbhB YbcL family protein	-1.09	9.77E-04	3.97E-03
Psed_6358	hydrogenase expression/synthesis HypA	-1.09	1.31E-04	8.08E-04
Psed_5265	Fe-S metabolism associated SufE	-1.09	2.30E-04	1.24E-03
Psed_0109	Peptidylprolyl isomerase	-1.09	5.96E-04	2.66E-03
Psed_1086	Conserved hypothetical protein CHP02569	-1.09	4.45E-07	1.01E-05
Psed_3508	intracellular protease, PfpI family	-1.09	2.25E-04	1.23E-03
Psed_1912	hypothetical protein	-1.10	2.22E-06	3.33E-05
Psed_3497	transport-associated	-1.10	1.89E-05	1.72E-04
Psed_2785	5,10-methylenetetrahydrofolate reductase	-1.10	3.10E-05	2.55E-04
Psed_2849	Histidinol-phosphate aminotransferase	-1.10	7.58E-07	1.50E-05
Psed_3898	3-oxoacyl-[acyl-carrier-protein] reductase	-1.10	2.18E-06	3.30E-05
Psed_3103	Microsomal epoxide hydrolase	-1.10	1.65E-05	1.54E-04
Psed_6374	hydrogenase assembly chaperone hypC/hupF	-1.10	4.24E-04	2.02E-03
Psed_1050	ABC-type transporter, integral membrane subunit	-1.10	2.45E-03	8.25E-03
Psed_4331	protein of unknown function DUF955	-1.10	1.78E-03	6.38E-03
Psed_1624	CoA-disulfide reductase	-1.10	5.40E-05	3.96E-04
Psed_3615	hypothetical protein	-1.11	1.35E-03	5.07E-03
Psed_4007	acyl-CoA dehydrogenase domain-containing protein	-1.11	1.99E-05	1.79E-04
Psed_3007	regulatory protein LuxR	-1.11	1.38E-04	8.34E-04
Psed_3694	ABC-type transporter, integral membrane subunit	-1.11	1.50E-05	1.42E-04
Psed_0180	glycosyl transferase family 2	-1.11	5.21E-06	6.18E-05
Psed_2500	Carbon-monoxide dehydrogenase (acceptor)	-1.11	4.87E-08	2.06E-06
Psed_6563	hypothetical protein	-1.11	7.54E-05	5.16E-04
Psed_4459	protein of unknown function DUF182	-1.11	3.32E-07	8.23E-06
Psed_1845	Glutamate-1-semialdehyde 2,1-aminomutase	-1.11	1.35E-05	1.31E-04
Psed_6682	Kynurenine--oxoglutarate transaminase	-1.11	2.40E-05	2.09E-04
Psed_5341	response regulator receiver	-1.11	1.07E-08	7.11E-07

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Psed_4552	regulatory protein TetR	-1.11	1.77E-05	1.62E-04
Psed_4763	Ribonuclease 3	-1.11	1.45E-03	5.41E-03
Psed_3536	Shikimate kinase	-1.11	9.41E-04	3.87E-03
Psed_0312	Sulfate transporter/antisigma-factor antagonist STAS	-1.12	2.82E-04	1.45E-03
Psed_1587	Long-chain-fatty-acid--CoA ligase	-1.12	2.53E-06	3.67E-05
Psed_3667	SSS sodium solute transporter superfamily	-1.12	5.05E-04	2.32E-03
Psed_4107	hypothetical protein	-1.12	1.21E-03	4.68E-03
Psed_5624	Long-chain-fatty-acid--CoA ligase	-1.12	7.26E-06	7.90E-05
Psed_3577	peptidyl-prolyl cis-trans isomerase cyclophilin type	-1.12	3.57E-04	1.75E-03
Psed_5198	mannose-6-phosphate isomerase, class I	-1.12	1.54E-05	1.46E-04
Psed_6927	amidohydrolase 2	-1.12	1.29E-04	8.01E-04
Psed_3172	Serine/threonine protein kinase-related	-1.12	4.06E-07	9.37E-06
Psed_1663	diaminopimelate decarboxylase	-1.12	8.08E-06	8.59E-05
Psed_1819	hypothetical protein	-1.12	2.10E-04	1.16E-03
Psed_6539	hypothetical protein	-1.12	4.50E-05	3.41E-04
Psed_0488	Cyclohexanone monooxygenase	-1.12	1.30E-06	2.26E-05
Psed_3953	(Dimethylallyl)adenosine tRNA methylthiotransferase miaB	-1.12	2.90E-06	4.00E-05
Psed_1855	hypothetical protein	-1.13	1.93E-05	1.76E-04
Psed_2991	type III restriction protein res subunit	-1.13	5.06E-04	2.33E-03
Psed_5659	(R)-benzylsuccinyl-CoA dehydrogenase	-1.13	1.45E-04	8.70E-04
Psed_1524	alkyl hydroperoxide reductase/ Thiol specific antioxidant/ Mal allergen	-1.13	1.37E-04	8.32E-04
Psed_5629	NAD-dependent epimerase/dehydratase	-1.13	2.69E-06	3.81E-05
Psed_6091	Alpha,alpha-trehalose-phosphate synthase (UDP-forming)	-1.13	1.14E-05	1.15E-04
Psed_1671	Sua5/YciO/YrdC/YwlC family protein	-1.13	3.44E-07	8.43E-06
Psed_5419	ribosomal protein L14	-1.13	1.94E-03	6.82E-03
Psed_3239	regulatory protein ArsR	-1.13	1.91E-05	1.74E-04
Psed_1689	UDP-N-acetylglucosamine 1-carboxyvinyltransferase	-1.13	4.25E-06	5.30E-05
Psed_5555	Trans-hexaprenyltranstransferase	-1.14	8.22E-04	3.45E-03
Psed_5720	PspC domain protein	-1.14	3.71E-06	4.84E-05
Psed_5042	Oligoribonuclease	-1.14	7.29E-06	7.91E-05
Psed_0297	hypothetical protein	-1.14	1.03E-08	7.08E-07
Psed_1456	RNA polymerase sigma factor, sigma-70 family	-1.14	1.83E-06	2.88E-05

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Psed_5456	hypothetical protein	-1.14	5.63E-05	4.08E-04
Psed_3264	hypothetical protein	-1.14	3.53E-07	8.55E-06
Psed_5257	Acyl-CoA dehydrogenase	-1.14	1.04E-04	6.78E-04
Psed_1168	3-oxoacyl-[acyl-carrier-protein] reductase	-1.14	4.74E-05	3.58E-04
Psed_2019	Glucose-6-phosphate isomerase	-1.15	1.45E-07	4.55E-06
Psed_5302	tryptophanyl-tRNA synthetase	-1.15	3.77E-05	2.98E-04
Psed_6526	SNARE associated Golgi protein	-1.15	2.00E-04	1.12E-03
Psed_6320	helix-turn-helix domain-containing protein AraC type	-1.15	2.87E-06	3.97E-05
Psed_3821	hypothetical protein	-1.15	1.95E-05	1.77E-04
Psed_3203	regulatory protein MerR	-1.15	2.83E-04	1.46E-03
Psed_5089	Protein of unknown function DUF3090	-1.15	2.19E-06	3.31E-05
Psed_6509	endonuclease III	-1.15	1.06E-03	4.21E-03
Psed_6565	protein of unknown function DUF224 cysteine-rich region domain protein	-1.15	1.34E-04	8.25E-04
Psed_5091	Undecaprenyl-diphosphatase	-1.15	4.16E-05	3.22E-04
Psed_2852	hypothetical protein	-1.15	2.41E-07	6.80E-06
Psed_4330	hypothetical protein	-1.15	1.46E-04	8.74E-04
Psed_5463	transcription termination/antitermination factor NusG	-1.16	1.09E-04	7.03E-04
Psed_1541	histidine kinase dimerisation and phosphoacceptor region	-1.16	8.78E-07	1.69E-05
Psed_3457	polyphosphate:nucleotide phosphotransferase, PPK2 family	-1.16	8.40E-06	8.88E-05
Psed_6210	NLP/P60 protein	-1.16	1.82E-04	1.04E-03
Psed_2106	regulatory protein LysR	-1.16	1.27E-05	1.25E-04
Psed_0185	UDP-galactopyranose mutase	-1.16	1.43E-05	1.38E-04
Psed_1892	C-methyltransferase	-1.16	1.61E-08	9.20E-07
Psed_5727	protein of unknown function ATP binding	-1.16	2.69E-03	8.88E-03
Psed_5466	hypothetical protein	-1.16	5.24E-06	6.20E-05
Psed_3335	Mg-chelatase subunit ChlI-like protein	-1.16	1.96E-03	6.87E-03
Psed_5027	FAD-binding 9 siderophore-interacting domain protein	-1.16	2.74E-04	1.43E-03
Psed_1468	Cupin 2 conserved barrel domain protein	-1.16	3.23E-07	8.17E-06
Psed_6211	NLP/P60 protein	-1.16	2.04E-08	1.09E-06
Psed_3807	pseudouridine synthase Rsu	-1.17	2.39E-07	6.76E-06
Psed_2465	Extradiol ring-cleavage dioxygenase LigAB LigA subunit	-1.17	2.42E-06	3.53E-05

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Psed_4632	hypothetical protein	-1.17	2.05E-03	7.13E-03
Psed_5111	o-succinylbenzoate--CoA ligase	-1.17	1.10E-03	4.33E-03
Psed_3740	Long-chain-fatty-acid--CoA ligase	-1.17	1.53E-06	2.57E-05
Psed_1513	Glutamate racemase	-1.17	2.87E-06	3.97E-05
Psed_1675	glycosyl transferase family 4	-1.17	1.35E-07	4.37E-06
Psed_0110	Peptidase S54, rhomboid domain	-1.17	7.38E-04	3.16E-03
Psed_4077	hypothetical protein	-1.18	1.71E-09	1.98E-07
Psed_1105	exodeoxyribonuclease III Xth	-1.18	7.16E-09	5.36E-07
Psed_3410	OpcA protein	-1.18	4.18E-04	2.00E-03
Psed_3477	Erythromycin esterase	-1.18	3.13E-06	4.26E-05
Psed_1714	methylmalonyl-CoA epimerase	-1.18	2.95E-04	1.50E-03
Psed_6635	tRNA (guanine-N(7)-)-methyltransferase	-1.18	2.23E-09	2.55E-07
Psed_3157	Benzoylformate decarboxylase	-1.18	1.53E-08	9.07E-07
Psed_3460	formaldehyde dehydrogenase, glutathione-independent	-1.18	6.67E-05	4.68E-04
Psed_2108	acyltransferase 3	-1.18	2.84E-07	7.58E-06
Psed_3472	protein of unknown function (DUF461)	-1.19	5.17E-06	6.15E-05
Psed_1698	Enoyl-CoA hydratase/isomerase	-1.19	2.36E-06	3.47E-05
Psed_6111	Silent information regulator protein Sir2	-1.19	6.46E-06	7.33E-05
Psed_4421	alpha/beta hydrolase fold	-1.19	2.55E-06	3.69E-05
Psed_1824	Methyltransferase type 11	-1.19	5.09E-05	3.77E-04
Psed_1662	Arginyl-tRNA synthetase	-1.20	2.48E-04	1.32E-03
Psed_3808	segregation and condensation protein B	-1.20	3.38E-05	2.72E-04
Psed_4137	L-threonine 3-dehydrogenase	-1.20	3.15E-06	4.28E-05
Psed_3408	glucose-6-phosphate isomerase	-1.20	7.95E-05	5.41E-04
Psed_4488	isochorismatase hydrolase	-1.20	2.31E-09	2.59E-07
Psed_1691	methylmalonyl-CoA mutase, large subunit	-1.20	8.67E-04	3.61E-03
Psed_1506	ATP-dependent Clp protease adapter protein clpS	-1.20	3.80E-04	1.84E-03
Psed_3543	alanyl-tRNA synthetase	-1.20	2.36E-04	1.27E-03
Psed_2766	Long-chain-fatty-acid--CoA ligase	-1.20	2.64E-07	7.25E-06
Psed_4578	Stearoyl-CoA 9-desaturase	-1.20	1.55E-03	5.70E-03
Psed_6804	hypothetical protein	-1.20	3.20E-07	8.17E-06
Psed_3829	Argininosuccinate lyase	-1.20	1.15E-05	1.16E-04
Psed_0354	Recombination protein recR	-1.20	5.95E-08	2.35E-06
Psed_3495	protein of unknown function DUF308 membrane	-1.20	3.05E-04	1.54E-03
Psed_4458	protein of unknown function DUF218	-1.20	2.45E-04	1.31E-03
Psed_0889	Tat-translocated protein	-1.21	1.97E-03	6.89E-03

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Psed_3101	Acetamidase/Formamidase	-1.21	1.34E-04	8.25E-04
Psed_2110	Neprilysin	-1.21	1.74E-05	1.60E-04
Psed_3242	Nitrile hydratase	-1.21	1.09E-07	3.66E-06
Psed_4656	hypothetical protein	-1.21	1.15E-04	7.32E-04
Psed_6602	VanW family protein	-1.21	4.71E-04	2.20E-03
Psed_1908	hypothetical protein	-1.21	1.40E-03	5.23E-03
Psed_3044	hypothetical protein	-1.21	6.52E-05	4.60E-04
Psed_5041	protein of unknown function DUF853 NPT hydrolase	-1.21	3.51E-07	8.53E-06
Psed_0250	Xenobiotic-transporting ATPase	-1.21	6.60E-05	4.65E-04
Psed_6882	filamentation induced by cAMP protein Fic	-1.22	6.59E-04	2.88E-03
Psed_6910	helix-turn-helix domain protein	-1.22	3.41E-04	1.69E-03
Psed_1562	hypothetical protein	-1.22	1.33E-03	5.02E-03
Psed_5362	Aryl-alcohol dehydrogenase (NADP(+))	-1.22	9.58E-06	9.93E-05
Psed_1920	class II aldolase/adducin family protein	-1.22	7.60E-07	1.50E-05
Psed_2638	polyphosphate kinase 2	-1.22	4.38E-06	5.41E-05
Psed_4686	O-acetylhomoserine/O-acetylserine sulfhydrylase	-1.23	2.85E-03	9.32E-03
Psed_6527	SNARE associated Golgi protein	-1.23	3.18E-05	2.60E-04
Psed_3682	hopanoid biosynthesis associated radical SAM protein HpnH	-1.23	3.42E-05	2.75E-04
Psed_4212	tyrosyl-tRNA synthetase	-1.23	2.11E-05	1.87E-04
Psed_2640	hypothetical protein	-1.23	7.29E-06	7.91E-05
Psed_0002	DNA polymerase III, beta subunit	-1.24	1.33E-06	2.29E-05
Psed_2656	Carbon-monoxide dehydrogenase (acceptor)	-1.24	1.47E-07	4.57E-06
Psed_1846	lipopolysaccharide biosynthesis protein	-1.24	3.98E-05	3.13E-04
Psed_6802	integrase family protein	-1.24	5.32E-05	3.91E-04
Psed_4682	Prolyl-tRNA synthetase	-1.24	2.17E-03	7.44E-03
Psed_1722	Thioredoxin domain-containing protein	-1.24	2.28E-04	1.23E-03
Psed_3513	Helix-turn-helix, AraC domain	-1.24	3.61E-04	1.76E-03
Psed_2933	protein of unknown function DUF164	-1.25	2.87E-05	2.40E-04
Psed_1166	hypothetical protein	-1.25	2.03E-06	3.13E-05
Psed_3544	hypothetical protein	-1.25	5.31E-05	3.90E-04
Psed_3847	Polyketide cyclase/dehydrase	-1.25	1.20E-03	4.64E-03
Psed_6612	ABC-type transporter, periplasmic subunit family 3	-1.25	3.94E-06	5.02E-05
Psed_3857	hypothetical protein	-1.26	2.59E-05	2.21E-04
Psed_6702	parB-like partition protein	-1.26	8.87E-04	3.68E-03

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Psed_3539	putative F420-dependent enzyme	-1.26	1.30E-05	1.27E-04
Psed_4557	hypothetical protein	-1.26	4.85E-05	3.63E-04
Psed_5021	CHAD domain containing protein	-1.26	1.11E-09	1.58E-07
Psed_6511	NUDIX hydrolase	-1.27	1.89E-07	5.57E-06
Psed_0878	peroxiredoxin, OsmC subfamily	-1.28	1.75E-04	1.01E-03
Psed_4614	hypothetical protein	-1.28	9.91E-09	6.86E-07
Psed_6110	Antibiotic biosynthesis monooxygenase	-1.28	3.03E-05	2.51E-04
Psed_3456	Thioredoxin-disulfide reductase	-1.29	5.50E-06	6.44E-05
Psed_4628	cell divisionFtsK/SpoIIIE	-1.29	4.91E-05	3.66E-04
Psed_3831	Arginine repressor	-1.29	1.49E-05	1.41E-04
Psed_6116	Lysyl-tRNA synthetase	-1.29	2.65E-05	2.26E-04
Psed_6523	fructose-bisphosphate aldolase, class II	-1.29	2.60E-04	1.37E-03
Psed_5625	RNA polymerase sigma factor, sigma-70 family	-1.29	1.11E-03	4.36E-03
Psed_3422	UPF0042 nucleotide-binding protein yhbJ	-1.29	1.52E-04	9.09E-04
Psed_1632	regulatory protein MarR	-1.29	10.00E-05	6.59E-04
Psed_0306	Acetylornithine transaminase	-1.30	1.17E-06	2.11E-05
Psed_2905	hypothetical protein	-1.30	3.99E-08	1.79E-06
Psed_5276	Citryl-CoA lyase	-1.30	2.48E-04	1.32E-03
Psed_0248	ABC-type transporter, periplasmic subunit	-1.30	5.47E-07	1.17E-05
Psed_3833	Acetylornithine/succinyldiaminopimelate aminotransferase	-1.30	2.79E-04	1.44E-03
Psed_4789	FAD linked oxidase domain protein	-1.31	4.35E-07	9.89E-06
Psed_4483	FAD dependent oxidoreductase	-1.31	8.12E-05	5.50E-04
Psed_4554	Adenosine kinase	-1.31	1.11E-06	2.02E-05
Psed_3840	tRNA/rRNA methyltransferase (SpoU)	-1.31	1.09E-04	7.02E-04
Psed_1878	glycosyl transferase group 1	-1.31	2.59E-04	1.37E-03
Psed_1504	isochorismatase hydrolase	-1.31	1.56E-08	9.08E-07
Psed_5449	protein of unknown function DUF140	-1.31	6.42E-07	1.34E-05
Psed_1725	1,4-alpha-glucan-branching enzyme	-1.31	7.65E-07	1.50E-05
Psed_5478	membrane protein of unknown function	-1.32	1.46E-07	4.55E-06
Psed_0381	hypothetical protein	-1.32	7.64E-05	5.22E-04
Psed_1987	ribonuclease, Rne/Rng family	-1.32	4.01E-05	3.14E-04
Psed_4551	Alkylglycerone-phosphate synthase	-1.32	1.48E-07	4.58E-06
Psed_3504	dienelactone hydrolase	-1.32	1.12E-04	7.16E-04
Psed_6525	hypothetical protein	-1.32	1.06E-08	7.11E-07

Psed_6304	FAD-dependent pyridine nucleotide-disulphide oxidoreductase	-1.32	2.27E-04	1.23E-03
Psed_3470	MscS Mechanosensitive ion channel	-1.32	6.23E-08	2.43E-06
Psed_3812	Tyrosine recombinase xerC	-1.32	6.50E-09	5.04E-07
Psed_1977	Nucleoside diphosphate kinase	-1.33	6.04E-04	2.68E-03
Psed_3325	HAD-superfamily hydrolase, subfamily IA, variant 3	-1.33	8.81E-09	6.37E-07
Psed_6595	GTP-binding protein HSR1-related	-1.33	3.74E-07	8.90E-06
Psed_4861	cell shape determining protein MreB/Mrl	-1.33	5.12E-04	2.35E-03
Psed_3834	acetylglutamate kinase	-1.33	2.10E-05	1.87E-04
Psed_5737	ATPase associated with various cellular activities AAA_5	-1.33	4.80E-05	3.61E-04
Psed_5734	molybdenum cofactor synthesis domain protein	-1.33	3.60E-07	8.65E-06
Psed_5188	hypothetical protein	-1.33	3.93E-08	1.78E-06
Psed_1001	ABC-type transporter, periplasmic subunit	-1.33	1.88E-07	5.55E-06
Psed_3532	N utilization substance protein B-like protein	-1.34	4.45E-05	3.39E-04
Psed_5203	hypothetical protein	-1.34	6.78E-07	1.40E-05
Psed_4736	Protein of unknown function DUF2469	-1.34	9.19E-06	9.59E-05
Psed_1848	nucleotide sugar dehydrogenase	-1.34	1.74E-06	2.78E-05
Psed_0253	Pentachlorophenol monooxygenase	-1.34	3.16E-07	8.17E-06
Psed_3854	Antibiotic biosynthesis monooxygenase	-1.34	5.65E-05	4.08E-04
Psed_3338	Penicillinase repressor	-1.34	6.40E-06	7.29E-05
Psed_1879	exopolysaccharide biosynthesis polyprenyl glycosylphosphotransferase	-1.34	2.31E-03	7.84E-03
Psed_1711	hypothetical protein	-1.34	7.05E-07	1.43E-05
Psed_3613	threonyl-tRNA synthetase	-1.34	7.05E-05	4.89E-04
Psed_1686	ATP synthase epsilon chain	-1.34	4.93E-06	5.94E-05
Psed_2891	3'-5' exonuclease	-1.35	1.06E-04	6.92E-04
Psed_4806	hypothetical protein	-1.35	1.73E-05	1.60E-04
Psed_6561	hypothetical protein	-1.35	1.61E-05	1.51E-04
Psed_1794	Aspartyl/glutamyl-tRNA(Asn/Gln) amidotransferase subunit C	-1.35	7.23E-04	3.10E-03
Psed_4402	Glucokinase	-1.35	6.66E-08	2.53E-06
Psed_3484	Protein grpE	-1.35	3.85E-05	3.04E-04
Psed_6687	Ribosomal RNA small subunit methyltransferase G	-1.36	2.67E-07	7.30E-06

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Psed_1420	hypothetical protein	-1.36	3.79E-06	4.90E-05
Psed_4663	Polyribonucleotide nucleotidyltransferase	-1.36	4.12E-05	3.20E-04
Psed_3162	regulatory protein MerR	-1.36	2.79E-04	1.44E-03
Psed_3327	protein of unknown function DUF75	-1.37	7.28E-08	2.69E-06
Psed_5175	(S)-2-hydroxy-acid oxidase	-1.37	1.24E-04	7.76E-04
Psed_6616	hypothetical protein	-1.37	2.79E-06	3.90E-05
Psed_3487	heat shock protein Hsp20	-1.37	1.28E-04	7.95E-04
Psed_1709	Aldehyde Dehydrogenase	-1.37	1.80E-08	9.96E-07
Psed_2892	response regulator receiver	-1.37	2.14E-04	1.18E-03
Psed_5618	Porphobilinogen deaminase	-1.37	5.32E-04	2.41E-03
Psed_5240	GtrA family protein	-1.38	7.25E-07	1.46E-05
Psed_0124	Transcriptional regulator IclR	-1.38	4.75E-11	1.52E-08
Psed_1858	polysaccharide biosynthesis protein	-1.38	3.25E-05	2.64E-04
Psed_3545	secreted protein	-1.38	9.10E-06	9.53E-05
Psed_2527	catechol 2,3 dioxygenase	-1.38	1.95E-04	1.10E-03
Psed_4662	processing peptidase	-1.38	4.09E-06	5.14E-05
Psed_1726	hypothetical protein	-1.38	2.44E-07	6.82E-06
Psed_3761	Glutamate synthase (NADPH)	-1.39	1.45E-05	1.39E-04
Psed_3876	ABC-type transporter, integral membrane subunit	-1.39	1.23E-06	2.19E-05
Psed_2867	Prolipoprotein diacylglyceryl transferase	-1.39	8.99E-08	3.22E-06
Psed_1866	ATP-grasp fold domain protein, DUF201-type	-1.39	3.75E-08	1.73E-06
Psed_1633	hypothetical protein	-1.39	3.11E-05	2.56E-04
Psed_3128	HpcH/HpaI aldolase	-1.39	1.54E-09	1.87E-07
Psed_2831	DNA polymerase III, alpha subunit	-1.39	4.74E-06	5.76E-05
Psed_5116	hypothetical protein	-1.39	5.30E-06	6.23E-05
Psed_4807	ATP-binding cassette protein, ChvD family	-1.40	2.12E-06	3.25E-05
Psed_1179	protein tyrosine/serine phosphatase	-1.40	1.44E-08	8.77E-07
Psed_0930	hypothetical protein	-1.40	2.97E-09	3.07E-07
Psed_1978	Polyamine-transporting ATPase	-1.40	1.54E-06	2.58E-05
Psed_2780	transcriptional regulatory protein	-1.40	6.91E-09	5.27E-07
Psed_1186	cell division ATP-binding protein FtsE	-1.40	4.07E-05	3.17E-04
Psed_1854	glycosyl transferase group 1	-1.41	2.99E-08	1.43E-06
Psed_5450	Fe(3+)-transporting ATPase	-1.41	4.14E-08	1.85E-06
Psed_4685	Homoserine O-acetyltransferase	-1.41	1.35E-04	8.26E-04

Psed_1708	Alcohol dehydrogenase zinc-binding domain protein	-1.41	4.24E-06	5.30E-05
Psed_3695	ABC-type transporter, integral membrane subunit	-1.41	8.03E-08	2.92E-06
Psed_3445	hypothetical protein	-1.41	2.09E-08	1.10E-06
Psed_0560	hypothetical protein	-1.41	4.24E-04	2.02E-03
Psed_4138	Formyl-CoA transferase	-1.41	6.96E-09	5.27E-07
Psed_3193	Cupin 2 conserved barrel domain protein	-1.42	1.46E-08	8.82E-07
Psed_3453	hypothetical protein	-1.42	3.22E-05	2.62E-04
Psed_3889	2-hydroxy-3-oxopropionate reductase	-1.42	2.70E-06	3.82E-05
Psed_4984	isochorismatase hydrolase	-1.42	6.67E-06	7.46E-05
Psed_0207	Teichoic-acid-transporting ATPase	-1.42	7.87E-06	8.43E-05
Psed_0327	Ku protein	-1.42	7.47E-07	1.49E-05
Psed_2869	pyruvate kinase	-1.42	6.84E-05	4.76E-04
Psed_1291	Transcription regulator MerR DNA binding	-1.43	1.49E-06	2.53E-05
Psed_0172	protein of unknown function DUF350	-1.43	1.53E-08	9.07E-07
Psed_2668	hypothetical protein	-1.43	2.38E-05	2.08E-04
Psed_1727	RNA polymerase sigma factor, sigma-70 family	-1.43	4.85E-05	3.63E-04
Psed_5910	Phenylacetate--CoA ligase	-1.43	3.22E-08	1.52E-06
Psed_4983	Aminocarboxymuconate-semialdehyde decarboxylase	-1.43	2.03E-06	3.13E-05
Psed_5647	hypothetical protein	-1.43	1.91E-08	1.04E-06
Psed_6680	cell wall hydrolase/autolysin	-1.44	5.05E-07	1.11E-05
Psed_3578	RelA/SpoT family protein	-1.44	9.13E-06	9.54E-05
Psed_4477	transcription activator effector binding protein	-1.44	5.24E-10	8.98E-08
Psed_1984	Radical SAM domain protein	-1.44	2.30E-04	1.24E-03
Psed_1857	glycosyl transferase family 2	-1.45	2.53E-05	2.17E-04
Psed_1597	regulatory protein TetR	-1.45	6.76E-10	1.07E-07
Psed_2850	Imidazoleglycerol-phosphate dehydratase	-1.45	4.37E-06	5.40E-05
Psed_6692	50S ribosomal protein L34	-1.45	3.00E-04	1.52E-03
Psed_3795	bifunctional deaminase-reductase domain protein	-1.45	4.24E-07	9.75E-06
Psed_3108	hypothetical protein	-1.45	1.30E-03	4.94E-03
Psed_3919	regulatory protein GntR HTH	-1.46	1.23E-05	1.21E-04
Psed_2429	hypothetical protein	-1.46	1.08E-09	1.58E-07
Psed_3449	NADH dehydrogenase (ubiquinone)	-1.46	2.95E-08	1.42E-06

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Psed_5374	hypothetical protein	-1.46	6.84E-05	4.76E-04
Psed_4986	NADP-dependent oxidoreductase domain	-1.47	7.80E-08	2.85E-06
Psed_4478	hypothetical protein	-1.47	1.17E-07	3.87E-06
Psed_0391	metallophosphoesterase	-1.47	2.45E-05	2.12E-04
Psed_2995	monosaccharide-transporting ATPase	-1.47	6.52E-06	7.39E-05
Psed_4329	hypothetical protein	-1.47	6.85E-06	7.59E-05
Psed_4100	hypothetical protein	-1.47	9.91E-08	3.42E-06
Psed_3698	Phthalate 4,5-dioxygenase	-1.48	1.21E-05	1.21E-04
Psed_5113	putative F420-dependent oxidoreductase	-1.48	2.02E-06	3.13E-05
Psed_5609	cytochrome c biogenesis protein transmembrane region	-1.48	1.73E-05	1.60E-04
Psed_5911	Hydantoinase/oxoprolinase	-1.49	3.44E-07	8.43E-06
Psed_5540	hypothetical protein	-1.49	1.60E-07	4.85E-06
Psed_5375	phosphoglucosamine mutase	-1.49	2.84E-05	2.38E-04
Psed_6427	amino acid permease-associated region	-1.49	4.36E-06	5.40E-05
Psed_4220	Luciferase-like, subgroup	-1.49	6.00E-04	2.68E-03
Psed_3462	1-deoxy-D-xylulose-5-phosphate synthase	-1.50	2.15E-07	6.16E-06
Psed_3602	small GTP-binding protein	-1.50	4.54E-04	2.12E-03
Psed_3280	PspC domain protein	-1.50	1.80E-08	9.96E-07
Psed_4631	LexA DNA-binding domain protein	-1.51	7.24E-04	3.11E-03
Psed_1731	alpha-glucan phosphorylase	-1.51	1.99E-05	1.79E-04
Psed_3476	dienelactone hydrolase	-1.52	6.54E-06	7.39E-05
Psed_6594	hypothetical protein	-1.53	1.04E-06	1.93E-05
Psed_3241	Amidase	-1.53	2.73E-08	1.37E-06
Psed_6286	hypothetical protein	-1.54	3.83E-06	4.92E-05
Psed_1453	Amidase	-1.54	4.11E-05	3.20E-04
Psed_4524	Barstar (barnase inhibitor)	-1.54	6.16E-09	5.00E-07
Psed_3515	hypothetical protein	-1.54	2.70E-07	7.36E-06
Psed_0537	short-chain dehydrogenase/reductase SDR	-1.54	2.22E-05	1.95E-04
Psed_5100	hypothetical protein	-1.54	5.90E-08	2.35E-06
Psed_3490	major facilitator superfamily MFS_1	-1.54	2.81E-06	3.91E-05
Psed_3121	ABC transporter, CydDC cysteine exporter (CydDC-E) family, permease/ATP-binding protein CydD	-1.55	2.61E-03	8.65E-03
Psed_3715	Phosphoglycerate dehydrogenase	-1.55	3.31E-07	8.23E-06
Psed_3205	ABC-type transporter, integral membrane subunit	-1.56	5.88E-08	2.35E-06

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Psed_1451	nitrile hydratase, alpha subunit	-1.56	4.30E-05	3.29E-04
Psed_2888	1-deoxy-D-xylulose-5-phosphate synthase	-1.56	1.96E-06	3.06E-05
Psed_0246	ABC-type transporter, integral membrane subunit	-1.57	2.94E-06	4.03E-05
Psed_4693	GCN5-related N-acetyltransferase	-1.57	8.20E-06	8.70E-05
Psed_2119	GTP-binding proten HflX	-1.57	3.17E-04	1.59E-03
Psed_6926	ABC-type transporter, integral membrane subunit	-1.58	3.33E-04	1.65E-03
Psed_1187	protein of unknown function DUF214	-1.58	1.07E-08	7.11E-07
Psed_0392	GatB/YqeY	-1.58	5.81E-06	6.72E-05
Psed_6031	response regulator receiver	-1.58	1.38E-04	8.33E-04
Psed_3875	Extracellular ligand-binding receptor	-1.58	2.43E-06	3.54E-05
Psed_3963	polar amino acid ABC transporter, inner membrane subunit	-1.58	7.15E-07	1.45E-05
Psed_1185	Peptide chain release factor 2	-1.58	1.43E-06	2.44E-05
Psed_2641	ATPase associated with various cellular activities AAA_5	-1.58	2.73E-07	7.39E-06
Psed_1184	transcriptional regulator PadR family protein	-1.58	3.02E-05	2.51E-04
Psed_3614	hypothetical protein	-1.59	2.77E-07	7.46E-06
Psed_1818	Dihydroxy-acid dehydratase	-1.59	5.57E-05	4.06E-04
Psed_3444	hypothetical protein	-1.59	9.18E-08	3.25E-06
Psed_3760	major facilitator superfamily MFS_1	-1.59	1.61E-03	5.87E-03
Psed_3204	extracellular solute-binding protein family 1	-1.59	4.00E-06	5.07E-05
Psed_3448	hypothetical protein	-1.60	3.46E-08	1.62E-06
Psed_3043	formate dehydrogenase, alpha subunit	-1.60	7.32E-06	7.93E-05
Psed_5343	PspC domain protein	-1.60	5.80E-08	2.35E-06
Psed_0280	Alanine--tRNA ligase	-1.61	4.43E-09	3.95E-07
Psed_1844	exopolysaccharide biosynthesis polyprenyl glycosylphosphotransferase	-1.61	6.55E-07	1.36E-05
Psed_1099	regulatory protein MarR	-1.61	1.04E-10	2.61E-08
Psed_3906	Luciferase-like, subgroup	-1.61	4.69E-07	1.05E-05
Psed_0891	iron permease FTR1	-1.61	4.82E-04	2.24E-03
Psed_6550	pyridoxamine 5'-phosphate oxidase-related FMN-binding	-1.62	5.60E-08	2.29E-06
Psed_2008	degV family protein	-1.62	8.40E-12	3.22E-09
Psed_4576	regulatory protein TetR	-1.62	5.25E-06	6.20E-05
Psed_2964	Amidase	-1.63	1.40E-06	2.39E-05

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Psed_1590	Formyl-CoA transferase	-1.63	4.89E-06	5.90E-05
Psed_3575	Histidyl-tRNA synthetase	-1.63	1.55E-09	1.87E-07
Psed_1198	DoxX family protein	-1.63	7.21E-06	7.86E-05
Psed_1002	ABC-type transporter, integral membrane subunit	-1.64	5.09E-08	2.14E-06
Psed_3514	glycosyl transferase group 1	-1.65	3.84E-07	9.03E-06
Psed_3502	hypothetical protein	-1.65	1.45E-09	1.82E-07
Psed_1559	response regulator receiver	-1.66	7.95E-04	3.36E-03
Psed_0914	Luciferase-like, subgroup	-1.66	1.45E-05	1.39E-04
Psed_3414	hypothetical protein	-1.66	1.40E-07	4.48E-06
Psed_4770	hypothetical protein	-1.67	1.62E-08	9.20E-07
Psed_4569	hypothetical protein	-1.68	9.50E-09	6.78E-07
Psed_1457	alpha/beta hydrolase fold	-1.69	3.28E-09	3.24E-07
Psed_4333	UBA/THIF-type NAD/FAD binding protein	-1.69	1.21E-04	7.61E-04
Psed_4052	(S)-2-hydroxy-acid oxidase	-1.70	1.73E-05	1.60E-04
Psed_4306	hypothetical protein	-1.70	3.98E-07	9.27E-06
Psed_5697	Carbon-monoxide dehydrogenase (acceptor)	-1.70	4.06E-07	9.37E-06
Psed_6609	Pyruvate dehydrogenase (acetyl-transferring)	-1.70	9.95E-07	1.84E-05
Psed_6924	ABC-type transporter, periplasmic subunit	-1.71	2.98E-06	4.09E-05
Psed_3489	Protein of unknown function DUF2267	-1.71	1.17E-09	1.58E-07
Psed_1076	short-chain dehydrogenase/reductase SDR	-1.71	9.71E-06	1.00E-04
Psed_3734	ABC-type transporter, integral membrane subunit	-1.71	1.65E-03	5.99E-03
Psed_3521	hypothetical protein	-1.72	6.88E-07	1.40E-05
Psed_6591	Heat shock protein 70	-1.73	1.03E-05	1.05E-04
Psed_3696	4Fe-4S ferredoxin iron-sulfur binding domain-containing protein	-1.73	1.71E-08	9.62E-07
Psed_3751	hypothetical protein	-1.73	5.56E-04	2.51E-03
Psed_4725	phosphatidate cytidylyltransferase	-1.74	8.79E-08	3.16E-06
Psed_2030	Linalool 8-monooxygenase	-1.75	2.11E-06	3.24E-05
Psed_6599	N-acetylmuramoyl-L-alanine amidase family 2	-1.75	1.93E-07	5.63E-06
Psed_1849	asparagine synthase (glutamine-hydrolyzing)	-1.76	1.19E-08	7.64E-07

Psed_3729	3-oxoacyl-[acyl-carrier-protein] reductase	-1.76	3.50E-05	2.80E-04
Psed_1452	Nitrile hydratase	-1.77	1.57E-04	9.28E-04
Psed_3718	3-oxoacyl-[acyl-carrier-protein] reductase	-1.77	1.23E-06	2.19E-05
Psed_0038	regulatory protein ArsR	-1.78	4.30E-08	1.89E-06
Psed_6636	Nitric-oxide synthase	-1.79	3.80E-06	4.90E-05
Psed_0895	Tetratricopeptide TPR_1 repeat-containing protein	-1.80	2.85E-10	5.29E-08
Psed_6196	protein of unknown function DUF151	-1.80	3.46E-09	3.37E-07
Psed_3182	hypothetical protein	-1.80	1.91E-08	1.04E-06
Psed_3836	N-acetyl-gamma-glutamyl-phosphate reductase	-1.82	5.65E-06	6.60E-05
Psed_5024	Mandelate racemase/muconate lactonizing protein	-1.83	1.76E-04	1.01E-03
Psed_5913	5-oxoprolinase (ATP-hydrolyzing)	-1.83	3.03E-09	3.07E-07
Psed_3962	polar amino acid ABC transporter, inner membrane subunit	-1.83	1.06E-07	3.61E-06
Psed_3692	oligopeptide/dipeptide ABC transporter, ATPase subunit	-1.83	1.29E-09	1.68E-07
Psed_3473	Pyruvate dehydrogenase (cytochrome)	-1.84	2.57E-06	3.70E-05
Psed_6063	muconolactone delta-isomerase	-1.84	2.76E-05	2.33E-04
Psed_3726	Xylose isomerase domain-containing protein TIM barrel	-1.84	2.25E-04	1.23E-03
Psed_5912	Acetone carboxylase gamma subunit	-1.84	1.35E-10	3.14E-08
Psed_6923	oligopeptide/dipeptide ABC transporter, ATPase subunit	-1.85	1.18E-07	3.89E-06
Psed_3480	thioredoxin	-1.85	1.27E-06	2.22E-05
Psed_2501	GntR domain protein	-1.86	2.32E-08	1.18E-06
Psed_3522	response regulator receiver	-1.88	6.18E-06	7.10E-05
Psed_6878	hypothetical protein	-1.89	2.64E-10	5.06E-08
Psed_5268	hypothetical protein	-1.89	5.70E-08	2.32E-06
Psed_1178	hypothetical protein	-1.89	9.44E-06	9.83E-05
Psed_3832	Ornithine carbamoyltransferase	-1.92	1.64E-06	2.68E-05
Psed_2026	glutamate decarboxylase	-1.92	2.07E-10	4.34E-08
Psed_1182	acyl-CoA dehydrogenase domain-containing protein	-1.93	1.80E-06	2.86E-05
Psed_2143	alkyl hydroperoxide reductase/ Thiol specific antioxidant/ Mal allergen	-1.93	7.46E-06	8.05E-05
Psed_3478	RNA polymerase sigma-70 factor, sigma-B/F/G subfamily	-1.94	3.02E-07	7.92E-06

Appendix 10. Genes differentially expressed on THF versus succinate.

Psed_5539	hypothetical protein	-1.95	4.35E-04	2.06E-03
Psed_6922	oligopeptide/dipeptide ABC transporter, ATPase subunit	-1.95	7.89E-11	2.05E-08
Psed_3463	hypothetical protein	-1.96	9.15E-07	1.74E-05
Psed_4992	hypothetical protein	-1.97	6.94E-11	1.96E-08
Psed_6062	Phenol 2-monooxygenase	-1.98	2.32E-05	2.03E-04
Psed_5484	peptidase S15	-1.99	8.22E-09	6.08E-07
Psed_3961	ABC-type transporter, periplasmic subunit family 3	-1.99	9.94E-08	3.42E-06
Psed_4577	Cold-shock protein DNA-binding	-2.01	1.16E-04	7.36E-04
Psed_3731	Phosphonate-transporting ATPase	-2.01	6.70E-06	7.47E-05
Psed_0247	ABC-type transporter, integral membrane subunit	-2.01	2.68E-06	3.81E-05
Psed_1713	crotonyl-CoA reductase	-2.02	8.06E-06	8.59E-05
Psed_3458	hypothetical protein	-2.03	3.88E-08	1.78E-06
Psed_2499	Carbon-monoxide dehydrogenase (acceptor)	-2.03	5.69E-09	4.87E-07
Psed_3042	NADH dehydrogenase (quinone)	-2.04	9.80E-07	1.82E-05
Psed_0397	Pyrophosphate-energized proton pump	-2.05	1.22E-05	1.21E-04
Psed_5273	Dihydrolipoyl dehydrogenase	-2.05	2.51E-09	2.72E-07
Psed_3697	ABC-type transporter, periplasmic subunit	-2.06	4.01E-10	7.25E-08
Psed_6921	Xylose isomerase domain-containing protein TIM barrel	-2.07	9.52E-08	3.33E-06
Psed_1791	fatty acid desaturase	-2.07	3.35E-06	4.49E-05
Psed_3960	Phosphonate-transporting ATPase	-2.09	1.26E-08	7.94E-07
Psed_3722	hypothetical protein	-2.10	1.84E-06	2.89E-05
Psed_3716	hypothetical protein	-2.11	1.64E-09	1.94E-07
Psed_3725	Extracellular ligand-binding receptor	-2.11	2.35E-06	3.46E-05
Psed_3511	hypothetical protein	-2.11	7.47E-07	1.49E-05
Psed_1730	alpha amylase catalytic region	-2.11	5.21E-08	2.17E-06
Psed_1078	alpha/beta hydrolase fold	-2.14	1.14E-04	7.27E-04
Psed_3732	Monosaccharide-transporting ATPase	-2.15	2.10E-05	1.87E-04
Psed_5026	regulatory protein TetR	-2.18	6.55E-08	2.52E-06
Psed_5473	Linalool 8-monooxygenase	-2.18	3.35E-07	8.26E-06
Psed_0252	amino acid adenylation domain protein	-2.19	8.56E-09	6.26E-07
Psed_6610	pyruvate dehydrogenase (acetyl-transferring) E1 component, alpha subunit	-2.19	4.45E-06	5.46E-05
Psed_3443	hypothetical protein	-2.19	2.31E-06	3.41E-05

Psed_6615	uncharacterized peroxidase-related enzyme	-2.20	2.63E-06	3.76E-05
Psed_6803	hypothetical protein	-2.21	2.29E-06	3.39E-05
Psed_5909	GntR domain protein	-2.21	2.80E-08	1.38E-06
Psed_0251	Xenobiotic-transporting ATPase	-2.23	5.02E-09	4.36E-07
Psed_3728	Pyruvate dehydrogenase (acetyl-transferring)	-2.23	1.25E-06	2.20E-05
Psed_3713	helix-turn-helix domain-containing protein AraC type	-2.23	1.13E-12	6.76E-10
Psed_5235	Butyryl-CoA dehydrogenase	-2.24	1.96E-03	6.87E-03
Psed_5682	hypothetical protein	-2.25	5.25E-06	6.20E-05
Psed_3721	alpha/beta hydrolase fold	-2.26	3.58E-06	4.73E-05
Psed_3733	ABC-type transporter, integral membrane subunit	-2.26	6.45E-05	4.57E-04
Psed_3041	NADH dehydrogenase (ubiquinone) 24 kDa subunit	-2.27	5.49E-07	1.17E-05
Psed_3730	Carboxymuconolactone decarboxylase	-2.29	9.07E-06	9.51E-05
Psed_2777	hypothetical protein	-2.29	9.94E-07	1.84E-05
Psed_3724	3-oxoacyl-[acyl-carrier-protein] reductase	-2.30	3.86E-06	4.95E-05
Psed_6925	ABC-type transporter, integral membrane subunit	-2.30	3.69E-09	3.43E-07
Psed_3719	cyclase family protein	-2.33	1.57E-06	2.59E-05
Psed_5929	beta-lactamase	-2.34	3.10E-11	1.12E-08
Psed_6064	muconate and chloromuconate cycloisomerase	-2.34	1.39E-08	8.63E-07
Psed_3693	oligopeptide/dipeptide ABC transporter, ATPase subunit	-2.35	8.39E-10	1.30E-07
Psed_3510	transcription elongation factor GreA	-2.35	1.58E-06	2.59E-05
Psed_5934	o-succinylbenzoate--CoA ligase	-2.36	3.92E-08	1.78E-06
Psed_1555	peptidase S1 and S6 chymotrypsin/Hap	-2.37	1.04E-03	4.15E-03
Psed_3376	hypothetical protein	-2.37	2.28E-05	2.00E-04
Psed_1763	molybdopterin dehydrogenase FAD-binding	-2.37	4.81E-07	1.07E-05
Psed_0090	hypothetical protein	-2.38	5.41E-10	9.03E-08
Psed_3754	[2Fe-2S]-binding domain-containing protein	-2.38	2.06E-04	1.15E-03
Psed_3759	major facilitator superfamily MFS_1	-2.39	1.04E-03	4.16E-03
Psed_3493	ParB domain protein nuclease	-2.39	1.20E-08	7.69E-07
Psed_5266	hypothetical protein	-2.43	8.10E-08	2.93E-06

Appendix 10. Genes differentially expressed on THF versus succinate.

Psed_1183	acyl-CoA dehydrogenase domain-containing protein	-2.44	1.11E-06	2.02E-05
Psed_3474	membrane protein	-2.44	2.09E-08	1.10E-06
Psed_5267	protein of unknown function DUF692	-2.45	4.71E-08	2.00E-06
Psed_2299	hypothetical protein	-2.47	9.32E-07	1.75E-05
Psed_3717	Transcriptional regulator IclR	-2.48	2.92E-08	1.42E-06
Psed_5370	YjeF-related protein	-2.49	2.57E-09	2.74E-07
Psed_3753	alpha/beta hydrolase fold	-2.51	5.07E-04	2.33E-03
Psed_2498	Carbon-monoxide dehydrogenase (acceptor)	-2.51	2.26E-08	1.16E-06
Psed_1790	ferredoxin	-2.53	4.96E-06	5.96E-05
Psed_1922	major facilitator superfamily MFS_1	-2.54	6.65E-10	1.07E-07
Psed_3752	UbiD family decarboxylase	-2.55	2.14E-03	7.35E-03
Psed_6600	acyl-CoA dehydrogenase domain-containing protein	-2.58	1.39E-05	1.35E-04
Psed_1567	GtrA family protein	-2.58	7.16E-05	4.95E-04
Psed_2497	cyclase family protein	-2.59	6.64E-12	2.70E-09
Psed_6067	flavin reductase domain protein FMN-binding	-2.59	5.96E-09	5.00E-07
Psed_1589	hypothetical protein	-2.60	2.28E-05	2.00E-04
Psed_3727	Pyruvate dehydrogenase (acetyl-transferring)	-2.65	1.13E-08	7.35E-07
Psed_1521	Carboxymuconolactone decarboxylase	-2.66	1.93E-07	5.63E-06
Psed_3702	Methionine synthase vitamin-B12 independent	-2.68	2.30E-10	4.56E-08
Psed_6066	4-hydroxyphenylacetate 3-hydroxylase	-2.71	1.19E-09	1.58E-07
Psed_5922	Carboxylesterase	-2.72	2.25E-06	3.35E-05
Psed_0349	ATPase, P-type (transporting), HAD superfamily, subfamily IC	-2.72	4.10E-09	3.71E-07
Psed_3720	amidohydrolase 2	-2.72	6.07E-09	5.00E-07
Psed_2496	protein of unknown function DUF849	-2.73	3.05E-12	1.66E-09
Psed_0489	alpha/beta hydrolase fold-3	-2.73	1.11E-08	7.27E-07
Psed_6175	regulatory protein TetR	-2.73	1.75E-07	5.20E-06
Psed_3700	LmbE family protein	-2.73	2.47E-09	2.72E-07
Psed_3918	FAD linked oxidase domain protein	-2.77	2.65E-04	1.39E-03
Psed_1416	ABC-type transporter, periplasmic subunit	-2.79	4.47E-10	7.87E-08
Psed_3757	carbon monoxide dehydrogenase subunit G	-2.81	1.24E-03	4.75E-03
Psed_1077	hypothetical protein	-2.86	4.17E-06	5.22E-05

Appendix 10. Genes differentially expressed on THF versus succinate.

Psed_3723	oxidoreductase domain protein	-2.89	2.21E-08	1.14E-06
Psed_3701	GntR domain protein	-3.02	6.01E-14	8.72E-11
Psed_3755	Xanthine dehydrogenase	-3.05	9.49E-04	3.89E-03
Psed_3794	Methyltransferase type 11	-3.10	6.22E-09	5.00E-07
Psed_6065	catechol 1,2-dioxygenase	-3.15	4.42E-12	2.22E-09
Psed_3756	molybdopterin dehydrogenase FAD-binding	-3.19	8.26E-04	3.46E-03
Psed_0126	Methionine synthase vitamin-B12 independent	-3.33	4.25E-08	1.88E-06
Psed_1635	regulatory protein LuxR	-3.33	2.20E-13	1.79E-10
Psed_6174	acetyl-CoA acetyltransferase	-3.36	1.62E-06	2.65E-05
Psed_1491	benzoate-CoA ligase family	-3.55	1.11E-09	1.58E-07
Psed_1489	benzoyl-CoA oxygenase, B subunit	-3.65	6.46E-12	2.70E-09
Psed_1539	hypothetical protein	-3.84	1.04E-07	3.56E-06
Psed_1490	benzoyl-CoA-dihydrodiol lyase	-3.88	1.40E-10	3.14E-08
Psed_1564	4-hydroxybenzoate 3-monooxygenase	-4.03	9.80E-07	1.82E-05
Psed_6176	Acyl-CoA oxidase	-4.04	1.56E-06	2.59E-05
Psed_6172	MaoC domain protein dehydratase	-4.19	4.66E-07	1.04E-05
Psed_5025	4-hydroxyacetophenone monooxygenase	-4.20	1.42E-07	4.51E-06
Psed_0088	protein of unknown function DUF485	-4.35	9.52E-13	6.76E-10
Psed_1565	protocatechuate 3,4-dioxygenase, beta subunit	-4.41	9.61E-08	3.35E-06
Psed_6173	3-oxoacyl-[acyl-carrier-protein] reductase	-4.56	1.25E-06	2.20E-05
Psed_1566	protocatechuate 3,4-dioxygenase, alpha subunit	-4.72	3.22E-07	8.17E-06
Psed_0089	SSS sodium solute transporter superfamily	-5.44	2.05E-13	1.79E-10

Appendix 10. Genes differentially expressed on THF versus succinate.

Appendix 10. Genes differentially expressed on THF versus succinate.

Appendix 11

Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate. Differentially expressed genes have an adjusted p-value < 0.01 and have a log_2FC > 1 or log_2FC < -1 in at least one conditions relative to pyruvate and are sorted by adjust p-value determined by the BH method.

Gene	Protein	log_2FC				p-value	adjusted p-value
		Dioxane vs Pyruvate	Glycolate vs. Pyruvate	Succinate vs. Pyruvate	THF vs. Pyruvate		
Psed_3890	glyoxylate carboligase	6.75	6.83	0.50	0.26	4.44E-17	9.63E-14
Psed_4939	lipase	0.15	0.03	-0.18	6.70	1.33E-16	2.16E-13
Psed_3889	2-hydroxy-3-oxopropionate reductase	7.06	7.02	1.45	0.03	2.12E-16	2.30E-13
Psed_3888	Hydroxypyruvate isomerase	6.99	6.89	0.86	-0.06	1.93E-16	2.30E-13
Psed_3891	glycerate kinase	5.64	5.81	0.62	0.58	1.55E-15	1.26E-12
Psed_4788	D-lactate dehydrogenase (cytochrome)	5.50	5.46	1.10	0.24	1.81E-15	1.31E-12
Psed_4789	FAD linked oxidase domain protein	4.74	4.61	1.20	-0.11	2.17E-15	1.41E-12
Psed_0089	SSS sodium solute transporter superfamily	0.61	6.87	6.25	0.81	2.77E-15	1.64E-12
Psed_0088	protein of unknown function DUF485	0.80	6.41	5.57	1.22	3.34E-15	1.81E-12
Psed_3475	hypothetical protein	0.16	0.37	3.64	2.63	4.59E-15	2.30E-12
Psed_3497	transport-associated	0.11	2.29	5.46	4.36	1.79E-14	8.34E-12
Psed_3477	Erythromycin esterase	0.19	0.74	4.89	3.72	2.16E-14	8.81E-12
Psed_3446	Ferritin Dps family protein	-0.48	2.03	5.42	4.47	3.30E-14	1.26E-11
Psed_3701	GntR domain protein	0.03	0.04	3.24	0.22	3.88E-14	1.36E-11
Psed_1635	regulatory protein LuxR	-0.91	-0.87	3.21	-0.11	3.96E-14	1.36E-11
Psed_3462	1-deoxy-D-xylulose-5-phosphate synthase	0.26	0.68	4.81	3.32	5.14E-14	1.67E-11
Psed_5791	hypothetical protein	-2.10	2.29	2.53	2.90	1.23E-13	3.65E-11
Psed_3470	MscS Mechanosensitive ion channel	0.44	0.60	3.79	2.47	1.22E-13	3.65E-11
Psed_5248	nitroreductase	0.19	0.06	-0.04	4.37	1.44E-13	4.07E-11

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_3472	protein of unknown function (DUF461)	-0.09	1.23	4.45	3.26	1.65E-13	4.48E-11
Psed_3483	chaperone DnaJ domain protein	-0.14	1.91	4.75	4.19	2.32E-13	6.05E-11
Psed_3460	formaldehyde dehydrogenase, glutathione-independent	-0.07	2.24	5.43	4.25	2.52E-13	6.31E-11
Psed_3508	intracellular protease, PfpI family	-0.22	2.13	5.48	4.39	3.11E-13	7.49E-11
Psed_3484	Protein grpE	0.09	2.10	5.82	4.47	3.94E-13	8.84E-11
Psed_3485	Chaperone protein dnaK	-0.83	2.42	5.47	4.60	4.42E-13	9.60E-11
Psed_6745	ATP-binding protein	1.91	-0.15	-0.47	0.32	4.62E-13	9.71E-11
Psed_3496	hypothetical protein	0.29	2.27	5.31	4.78	5.14E-13	9.93E-11
Psed_3448	hypothetical protein	0.68	1.19	4.18	2.59	5.19E-13	9.93E-11
Psed_3444	hypothetical protein	0.35	0.49	4.18	2.59	5.05E-13	9.93E-11
Psed_3490	major facilitator superfamily MFS_1	0.28	0.62	5.10	3.56	6.38E-13	1.15E-10
Psed_3713	helix-turn-helix domain-containing protein AraC type	-1.29	-1.48	0.93	-1.31	6.62E-13	1.16E-10
Psed_0078	Methyltransferase type 12	-2.64	-3.67	-4.21	-1.77	7.79E-13	1.30E-10
Psed_0090	hypothetical protein	0.34	3.93	2.53	0.15	7.71E-13	1.30E-10
Psed_3489	Protein of unknown function DUF2267	0.13	0.12	3.09	1.38	8.39E-13	1.37E-10
Psed_3473	Pyruvate dehydrogenase (cytochrome)	0.22	1.22	5.91	4.07	9.85E-13	1.56E-10
Psed_3480	thioredoxin	0.03	0.96	5.51	3.66	1.03E-12	1.60E-10
Psed_3487	heat shock protein Hsp20	-0.83	2.47	5.55	4.19	1.16E-12	1.75E-10
Psed_3463	hypothetical protein	0.30	1.96	5.84	3.89	1.32E-12	1.96E-10
Psed_6065	catechol 1,2-dioxygenase	-0.48	-0.32	3.26	0.11	1.41E-12	2.04E-10
Psed_3500	glucose-6-phosphate 1-dehydrogenase	0.42	1.62	5.43	4.90	1.65E-12	2.26E-10
Psed_3451	Domain of unknown function DUF1931	0.05	2.29	5.07	4.47	1.73E-12	2.26E-10
Psed_6878	hypothetical protein	0.39	0.14	3.05	1.16	1.65E-12	2.26E-10
Psed_3502	hypothetical protein	0.19	0.47	3.00	1.35	1.83E-12	2.34E-10

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_3493	ParB domain protein nuclease	0.29	0.30	4.89	2.50	1.89E-12	2.36E-10
Psed_3486	hypothetical protein	-0.40	2.79	5.97	5.04	2.24E-12	2.74E-10
Psed_3456	Thioredoxin-disulfide reductase	0.11	1.41	4.20	2.91	2.32E-12	2.74E-10
Psed_4478	hypothetical protein	0.08	0.16	3.41	1.94	2.31E-12	2.74E-10
Psed_4940	fumarylacetoacetate (FAA) hydrolase	0.45	0.23	-0.01	5.21	2.48E-12	2.88E-10
Psed_5799	hypothetical protein	-0.83	2.76	2.91	3.86	2.89E-12	3.30E-10
Psed_5787	hypothetical protein	-0.79	2.64	3.16	3.87	2.99E-12	3.36E-10
Psed_6779	Integrase catalytic region	2.90	2.44	0.11	0.15	4.16E-12	4.51E-10
Psed_1489	benzoyl-CoA oxygenase, B subunit	0.06	-0.21	3.84	0.19	4.35E-12	4.65E-10
Psed_6066	4-hydroxyphenylacetate 3-hydroxylase	-0.65	-0.28	3.87	1.16	4.98E-12	5.15E-10
Psed_5794	hypothetical protein	-2.08	2.50	2.56	2.87	5.08E-12	5.17E-10
Psed_3478	RNA polymerase sigma-70 factor, sigma-B/F/G subfamily	0.24	0.44	4.69	2.75	5.20E-12	5.21E-10
Psed_6780	hypothetical protein	3.18	3.12	-0.10	1.57	5.81E-12	5.73E-10
Psed_2496	protein of unknown function DUF849	-0.89	-0.59	1.94	-0.79	6.13E-12	5.96E-10
Psed_5788	peptidase S14 ClpP	-1.89	2.34	2.36	3.39	6.30E-12	6.03E-10
Psed_2204	50S ribosomal protein L33	-0.09	-5.44	-3.54	-0.53	6.63E-12	6.17E-10
Psed_3514	glycosyl transferase group 1	0.25	0.62	4.07	2.42	6.58E-12	6.17E-10
Psed_3445	hypothetical protein	0.14	0.13	2.69	1.28	9.20E-12	8.43E-10
Psed_5795	hypothetical protein	-2.03	2.92	2.90	3.83	9.96E-12	8.88E-10
Psed_2497	cyclase family protein	-0.81	-0.62	1.93	-0.66	9.90E-12	8.88E-10
Psed_1422	Ferritin Dps family protein	-1.71	0.29	-4.23	-0.69	1.06E-11	9.29E-10
Psed_3453	hypothetical protein	0.09	2.03	4.84	3.42	1.08E-11	9.29E-10
Psed_3458	hypothetical protein	0.27	0.71	4.14	2.11	1.08E-11	9.29E-10
Psed_3515	hypothetical protein	0.13	0.33	3.49	1.95	1.16E-11	9.79E-10
Psed_3461	transport-associated	0.57	1.17	4.94	3.89	1.21E-11	1.01E-09

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_3481	ATP-dependent chaperone ClpB	0.24	1.63	5.44	4.62	1.28E-11	1.05E-09
Psed_5929	beta-lactamase	-0.07	-0.25	2.46	0.12	1.44E-11	1.17E-09
Psed_4790	protein of unknown function DUF224 cysteine-rich region domain protein	4.84	4.88	0.45	0.15	2.13E-11	1.71E-09
Psed_3476	dienelactone hydrolase	0.17	0.45	4.20	2.68	2.36E-11	1.88E-09
Psed_0079	ABC-type transporter, periplasmic subunit	-2.74	-3.87	-3.80	-0.39	2.42E-11	1.90E-09
Psed_2680	Cysteine desulfurase	0.33	3.92	0.16	0.66	2.66E-11	2.06E-09
Psed_4782	Malate synthase	3.38	3.51	0.38	0.87	2.74E-11	2.09E-09
Psed_3443	hypothetical protein	0.06	0.86	5.53	3.34	2.75E-11	2.09E-09
Psed_6975	Betaine-aldehyde dehydrogenase	1.47	-0.41	0.73	1.63	3.23E-11	2.42E-09
Psed_6067	flavin reductase domain protein FMN-binding	-0.46	-0.41	3.66	1.07	3.46E-11	2.56E-09
Psed_5912	Acetone carboxylase gamma subunit	-0.66	-1.04	1.34	-0.50	3.63E-11	2.65E-09
Psed_3474	membrane protein	0.37	0.45	4.35	1.91	3.82E-11	2.77E-09
Psed_0276	major facilitator superfamily MFS_1	-2.83	-3.00	-3.20	-3.12	4.06E-11	2.91E-09
Psed_3495	protein of unknown function DUF308 membrane	0.20	0.98	4.61	3.40	4.35E-11	3.08E-09
Psed_3449	NADH dehydrogenase (ubiquinone)	0.40	0.59	2.77	1.31	4.58E-11	3.20E-09
Psed_6980	hypothetical protein	2.60	-0.61	1.18	2.93	5.01E-11	3.47E-09
Psed_6787	Formyl-CoA transferase	3.89	3.68	0.76	0.22	5.60E-11	3.76E-09
Psed_6981	Aldehyde Dehydrogenase	3.38	-0.33	0.88	3.35	5.49E-11	3.76E-09
Psed_5800	hypothetical protein	0.05	1.99	2.67	3.80	5.58E-11	3.76E-09
Psed_0124	Transcriptional regulator IclR	-0.03	-0.00	1.38	0.01	5.76E-11	3.83E-09
Psed_1655	hypothetical protein	-0.65	-1.89	-2.50	-0.26	6.31E-11	4.15E-09
Psed_6977	Ferredoxin--NAD(+) reductase	2.26	-0.44	0.70	2.48	7.66E-11	4.99E-09
Psed_6599	N-acetylmuramoyl-L-alanine amidase family 2	-1.24	-2.93	1.02	-0.73	7.97E-11	5.13E-09
Psed_0011	hypothetical protein	0.04	-3.15	-1.19	-2.24	8.11E-11	5.18E-09

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_4322	Dihydrolipoyl dehydrogenase	-0.39	-0.44	-0.42	2.21	8.46E-11	5.35E-09
Psed_6978	methane/phenol/toluene hydroxylase	1.60	-0.29	0.54	1.59	9.06E-11	5.67E-09
Psed_4477	transcription activator effector binding protein	-0.00	0.01	1.71	0.27	1.03E-10	6.36E-09
Psed_6746	hypothetical protein	2.60	-0.78	-0.84	0.20	1.11E-10	6.79E-09
Psed_3513	Helix-turn-helix, AraC domain	0.22	1.30	4.61	3.36	1.12E-10	6.79E-09
Psed_0023	regulatory protein GntR HTH	-0.32	-1.45	-0.33	-1.25	1.20E-10	7.15E-09
Psed_0251	Xenobiotic-transporting ATPase	0.31	-2.48	0.51	-1.72	1.20E-10	7.15E-09
Psed_2008	degV family protein	0.03	0.07	1.24	-0.38	1.21E-10	7.15E-09
Psed_1656	hypothetical protein	-1.28	-3.18	-2.19	-0.55	1.24E-10	7.19E-09
Psed_3482	hypothetical protein	0.31	1.26	4.55	3.61	1.30E-10	7.46E-09
Psed_6979	monooxygenase component MmoB/DmpM	1.98	-0.38	0.68	2.23	1.35E-10	7.71E-09
Psed_2554	Xenobiotic-transporting ATPase	0.17	3.78	-0.49	0.11	1.39E-10	7.83E-09
Psed_6196	protein of unknown function DUF151	0.14	0.00	2.47	0.67	1.39E-10	7.83E-09
Psed_1849	asparagine synthase (glutamine-hydrolyzing)	-1.46	-1.35	1.22	-0.53	1.42E-10	7.90E-09
Psed_6974	Ethyl tert-butyl ether degradation EthD	1.85	-0.47	0.92	1.82	1.46E-10	7.90E-09
Psed_0922	hypothetical protein	0.25	0.14	2.22	2.74	1.44E-10	7.90E-09
Psed_1490	benzoyl-CoA-dihydrodiol lyase	0.35	0.21	4.18	0.30	1.45E-10	7.90E-09
Psed_5784	hypothetical protein	0.06	2.42	2.19	3.79	1.53E-10	8.26E-09
Psed_3718	3-oxoacyl-[acyl-carrier-protein] reductase	-2.16	-2.89	1.13	-0.64	1.56E-10	8.30E-09
Psed_5913	5-oxoprolinase (ATP-hydrolyzing)	-1.08	-1.38	1.24	-0.59	1.64E-10	8.66E-09
Psed_2011	threonine synthase	0.98	2.47	0.51	0.73	1.76E-10	9.26E-09
Psed_6791	IstB domain protein ATP-binding protein	1.79	1.24	0.28	0.46	1.85E-10	9.65E-09
Psed_1468	Cupin 2 conserved barrel domain protein	-1.61	-1.62	0.42	-0.75	1.97E-10	1.02E-08
Psed_3457	polyphosphate:nucleotide phosphotransferase, PPK2 family	0.56	0.83	3.10	1.95	2.21E-10	1.12E-08
Psed_5249	NADPH:quinone reductase	0.42	0.11	-0.25	3.45	2.21E-10	1.12E-08

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_5796	hypothetical protein	-1.20	2.72	3.18	3.57	2.23E-10	1.12E-08
Psed_6784	Alkylglycerone-phosphate synthase	1.98	1.96	0.19	-0.72	2.27E-10	1.13E-08
Psed_6922	oligopeptide/dipeptide ABC transporter, ATPase subunit	-0.16	-0.27	1.64	-0.31	2.35E-10	1.16E-08
Psed_1584	6-phosphofructokinase	1.76	1.85	-0.49	1.05	3.00E-10	1.47E-08
Psed_3447	hypothetical protein	0.10	0.56	3.54	3.32	3.06E-10	1.49E-08
Psed_1416	ABC-type transporter, periplasmic subunit	-0.42	-0.59	2.58	-0.21	3.14E-10	1.52E-08
Psed_0248	ABC-type transporter, periplasmic subunit	0.37	-2.06	0.45	-0.85	3.44E-10	1.65E-08
Psed_5804	hypothetical protein	-0.98	2.77	2.98	3.66	3.61E-10	1.72E-08
Psed_3723	oxidoreductase domain protein	-1.95	-2.96	1.67	-1.22	4.01E-10	1.89E-08
Psed_3720	amidohydrolase 2	-1.75	-2.67	1.18	-1.55	4.06E-10	1.90E-08
Psed_6742	NLP/P60 protein	1.50	0.08	-0.02	0.01	4.09E-10	1.90E-08
Psed_5790	hypothetical protein	-2.57	2.10	2.06	2.58	4.18E-10	1.93E-08
Psed_3722	hypothetical protein	-2.26	-3.38	1.36	-0.75	4.25E-10	1.94E-08
Psed_5683	hypothetical protein	-0.47	-0.82	1.35	0.36	4.26E-10	1.94E-08
Psed_5811	hypothetical protein	-0.34	2.38	2.58	3.75	4.51E-10	2.03E-08
Psed_3702	Methionine synthase vitamin-B12 independent	-0.59	-0.52	2.12	-0.55	4.51E-10	2.03E-08
Psed_5793	hypothetical protein	-1.04	2.27	2.54	2.59	4.69E-10	2.08E-08
Psed_0252	amino acid adenylation domain protein	0.45	-2.09	0.74	-1.45	4.70E-10	2.08E-08
Psed_3658	RNA polymerase sigma-70 factor	-1.18	-1.83	-1.22	-1.30	4.78E-10	2.10E-08
Psed_5798	hypothetical protein	-0.82	2.70	2.88	3.24	4.83E-10	2.11E-08
Psed_3780	conserved hypothetical protein	0.31	1.07	2.69	3.28	5.00E-10	2.17E-08
Psed_0038	regulatory protein ArsR	1.22	-1.31	0.73	-1.04	5.28E-10	2.28E-08
Psed_2553	Glycine dehydrogenase [decarboxylating]	0.18	1.46	-0.34	-0.16	5.44E-10	2.31E-08
Psed_5681	Radical SAM domain protein	-0.45	-0.94	2.31	1.39	5.44E-10	2.31E-08
Psed_4688	polysaccharide deacetylase	-1.86	-2.97	-1.40	-2.14	5.95E-10	2.50E-08

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_3725	Extracellular ligand-binding receptor	-1.94	-3.38	1.50	-0.61	6.34E-10	2.65E-08
Psed_3510	transcription elongation factor GreA	0.18	0.57	4.69	2.33	6.97E-10	2.89E-08
Psed_6747	hypothetical protein	1.92	-0.54	-0.56	-0.16	7.10E-10	2.93E-08
Psed_7003	hypothetical protein	1.48	-0.10	-0.49	0.25	7.68E-10	3.15E-08
Psed_1657	Cobalt transporter subunit CbtB putative	-1.29	-3.11	-2.34	-0.22	7.76E-10	3.16E-08
Psed_3243	nitrile hydratase, alpha subunit	0.34	0.48	1.53	0.47	7.87E-10	3.18E-08
Psed_3504	dienelactone hydrolase	0.24	0.72	3.80	2.48	8.28E-10	3.31E-08
Psed_5801	hypothetical protein	-0.13	2.84	2.98	3.68	8.49E-10	3.37E-08
Psed_0473	hypothetical protein	0.31	1.24	1.34	1.28	9.03E-10	3.56E-08
Psed_1848	nucleotide sugar dehydrogenase	-1.66	-1.44	0.99	-0.34	9.15E-10	3.59E-08
Psed_1844	exopolysaccharide biosynthesis polyprenyl glycosylphosphotransferase	-1.20	-0.82	1.90	0.29	9.46E-10	3.67E-08
Psed_5471	hypothetical protein	-0.93	-3.22	-4.00	-1.41	9.45E-10	3.67E-08
Psed_0022	hypothetical protein	-0.00	-1.09	-0.84	-0.80	9.56E-10	3.68E-08
Psed_3794	Methyltransferase type 11	0.63	0.68	4.14	1.04	1.05E-09	3.99E-08
Psed_1457	alpha/beta hydrolase fold	-1.03	-1.42	0.54	-1.14	1.12E-09	4.22E-08
Psed_1846	lipopolysaccharide biosynthesis protein	-2.25	-1.88	0.88	-0.36	1.12E-09	4.22E-08
Psed_3717	Transcriptional regulator IclR	-1.76	-2.29	1.33	-1.15	1.14E-09	4.25E-08
Psed_6426	Copper amine oxidase domain-containing protein	-0.34	0.87	2.25	1.47	1.14E-09	4.25E-08
Psed_1726	hypothetical protein	-0.24	-0.49	-1.02	-2.40	1.28E-09	4.72E-08
Psed_5484	peptidase S15	-1.35	-0.36	1.38	-0.60	1.32E-09	4.87E-08
Psed_6743	hypothetical protein	2.52	-0.67	-0.02	0.25	1.35E-09	4.93E-08
Psed_6744	hypothetical protein	1.97	-0.24	-0.29	0.33	1.42E-09	5.16E-08
Psed_1491	benzoate-CoA ligase family	0.34	0.27	3.78	0.24	1.43E-09	5.18E-08
Psed_4992	hypothetical protein	-0.44	-0.51	0.57	-1.40	1.44E-09	5.20E-08

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_3727	Pyruvate dehydrogenase (acetyl-transferring)	-1.50	-2.21	1.39	-1.27	1.48E-09	5.28E-08
Psed_2026	glutamate decarboxylase	-0.12	-0.49	1.30	-0.62	1.58E-09	5.63E-08
Psed_6748	hypothetical protein	2.28	-0.71	-0.38	0.28	1.59E-09	5.63E-08
Psed_5782	Collagen triple helix repeat-containing protein	-0.55	3.09	3.22	3.39	1.62E-09	5.72E-08
Psed_3193	Cupin 2 conserved barrel domain protein	-0.74	-0.59	1.15	-0.27	1.77E-09	6.20E-08
Psed_2973	dihydroxyacetone kinase, L subunit	-1.36	0.48	-0.26	-0.92	1.78E-09	6.21E-08
Psed_4700	Cyclopropane-fatty-acyl-phospholipid synthase	-0.05	-0.01	1.02	4.86	1.89E-09	6.44E-08
Psed_0895	Tetratricopeptide TPR_1 repeat-containing protein	-0.47	-0.44	1.09	-0.71	1.87E-09	6.44E-08
Psed_4142	hypothetical protein	0.18	0.07	1.23	0.49	1.88E-09	6.44E-08
Psed_6064	muconate and chloromuconate cycloisomerase	-0.33	-0.19	2.69	0.34	1.88E-09	6.44E-08
Psed_3731	Phosphonate-transporting ATPase	-2.12	-2.39	1.91	-0.10	1.96E-09	6.64E-08
Psed_3719	cyclase family protein	-2.37	-3.37	1.16	-1.17	2.12E-09	7.16E-08
Psed_6982	Mn2+/Fe2+ transporter, NRAMP family	3.87	-0.08	0.25	3.46	2.16E-09	7.18E-08
Psed_3812	Tyrosine recombinase xerC	-0.73	-1.29	-0.26	-1.58	2.15E-09	7.18E-08
Psed_4077	hypothetical protein	-0.11	0.07	1.15	-0.02	2.16E-09	7.18E-08
Psed_5209	Peroxiredoxin	-0.80	-1.37	-1.56	-1.77	2.19E-09	7.22E-08
Psed_6427	amino acid permease-associated region	-0.28	0.97	2.81	1.32	2.46E-09	8.08E-08
Psed_1116	transcription factor WhiB	-1.38	-0.33	-2.06	-2.12	2.51E-09	8.22E-08
Psed_0013	hypothetical protein	-0.09	-2.06	-1.35	-1.85	2.69E-09	8.76E-08
Psed_2640	hypothetical protein	-1.83	-1.00	0.87	-0.36	2.79E-09	9.01E-08
Psed_0349	ATPase, P-type (transporting), HAD superfamily, subfamily IC	0.49	0.71	3.25	0.54	2.79E-09	9.01E-08
Psed_6750	hypothetical protein	2.09	-0.25	-0.32	0.28	2.85E-09	9.13E-08
Psed_3023	cobaltochelatase, CobN subunit	-0.92	-1.25	-1.70	-0.14	2.89E-09	9.17E-08
Psed_3716	hypothetical protein	-0.56	-0.88	1.40	-0.71	2.91E-09	9.21E-08

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_3728	Pyruvate dehydrogenase (acetyl-transferring)	-2.12	-3.19	0.97	-1.26	3.02E-09	9.49E-08
Psed_1320	hypothetical protein	-1.12	-1.03	-0.35	-1.11	3.15E-09	9.87E-08
Psed_1303	Heavy metal transport/detoxification protein	1.43	2.95	-0.72	1.05	3.21E-09	9.97E-08
Psed_1594	protein of unknown function UPF0016	1.19	0.24	0.10	0.32	3.24E-09	9.97E-08
Psed_6751	Transglycosylase-like domain protein	2.33	-0.66	-0.81	0.97	3.25E-09	9.97E-08
Psed_6968	transposase mutator type	-0.01	-1.95	-0.31	-0.43	3.22E-09	9.97E-08
Psed_0280	Alanine--tRNA ligase	-0.58	-0.48	1.24	-0.37	3.37E-09	1.03E-07
Psed_3128	HpcH/HpaI aldolase	-0.01	-0.30	1.20	-0.19	3.42E-09	1.04E-07
Psed_2550	Glycine hydroxymethyltransferase	0.22	1.39	-0.37	-0.17	3.49E-09	1.06E-07
Psed_1967	major facilitator superfamily MFS_1	0.33	0.35	0.09	1.85	3.58E-09	1.08E-07
Psed_3501	hypothetical protein	0.81	1.24	2.88	2.30	3.89E-09	1.16E-07
Psed_0934	YCII-related	-0.30	-0.51	-0.16	2.58	3.88E-09	1.16E-07
Psed_5370	YjeF-related protein	-0.05	-0.25	2.37	-0.12	3.95E-09	1.17E-07
Psed_6749	hypothetical protein	3.05	-0.73	-0.45	0.22	4.23E-09	1.24E-07
Psed_5779	hypothetical protein	0.14	0.22	0.08	2.01	4.44E-09	1.30E-07
Psed_3675	PucR family transcriptional regulator	-0.99	0.58	-1.63	-2.49	4.47E-09	1.31E-07
Psed_6754	hypothetical protein	1.86	-0.38	-0.80	0.42	4.53E-09	1.31E-07
Psed_5910	Phenylacetate--CoA ligase	-0.27	-0.47	1.47	0.04	4.54E-09	1.31E-07
Psed_5789	hypothetical protein	-1.73	2.19	2.10	2.79	4.59E-09	1.32E-07
Psed_5797	hypothetical protein	-0.07	2.28	2.59	3.12	4.83E-09	1.37E-07
Psed_3903	hypothetical protein	0.09	-3.23	-2.29	-1.21	4.86E-09	1.37E-07
Psed_0250	Xenobiotic-transporting ATPase	0.36	-2.64	-0.05	-1.26	4.84E-09	1.37E-07
Psed_0312	Sulfate transporter/antisigma-factor antagonist STAS	-2.81	0.15	-0.24	-1.35	4.89E-09	1.38E-07
Psed_3492	transcription factor WhiB	0.34	0.27	1.61	0.65	5.11E-09	1.43E-07
Psed_1421	ferric-uptake regulator	-1.93	-0.09	-3.56	-2.00	5.16E-09	1.44E-07

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_2499	Carbon-monoxide dehydrogenase (acceptor)	-1.22	-0.83	1.02	-1.01	5.19E-09	1.44E-07
Psed_5792	hypothetical protein	-1.02	2.33	2.61	3.45	5.46E-09	1.51E-07
Psed_5632	pyrroline-5-carboxylate reductase	-0.54	1.12	-0.34	-0.87	5.58E-09	1.53E-07
Psed_3697	ABC-type transporter, periplasmic subunit	-0.55	-0.58	0.87	-1.19	6.08E-09	1.65E-07
Psed_2429	hypothetical protein	-0.70	-0.60	0.43	-1.04	6.16E-09	1.67E-07
Psed_2498	Carbon-monoxide dehydrogenase (acceptor)	-1.66	-1.12	1.49	-1.02	6.23E-09	1.68E-07
Psed_1968	hypothetical protein	0.67	0.47	0.27	2.11	6.36E-09	1.71E-07
Psed_5869	cytochrome c oxidase, subunit I	-0.99	-0.60	-1.12	-0.89	6.39E-09	1.71E-07
Psed_6094	ABC-type metal ion transporter, periplasmic subunit	-0.57	-3.67	-3.31	-0.52	6.43E-09	1.72E-07
Psed_3038	Sulfate-transporting ATPase	-1.15	-1.61	-1.16	0.70	6.48E-09	1.72E-07
Psed_5826	L-2,4-diaminobutyric acid acetyltransferase	-1.19	0.10	-1.97	-1.81	6.48E-09	1.72E-07
Psed_0425	Threonine aldolase	-0.29	0.75	1.06	0.26	6.89E-09	1.82E-07
Psed_3043	formate dehydrogenase, alpha subunit	0.40	-0.77	2.70	1.11	7.12E-09	1.87E-07
Psed_3042	NADH dehydrogenase (quinone)	0.87	-0.24	3.31	1.27	7.21E-09	1.88E-07
Psed_6006	hypothetical protein	0.03	0.59	1.85	1.05	7.33E-09	1.91E-07
Psed_5082	YVTN beta-propeller repeat-containing protein	0.03	0.01	0.23	1.20	7.35E-09	1.91E-07
Psed_4524	Barstar (barnase inhibitor)	0.07	0.04	1.61	0.07	7.40E-09	1.91E-07
Psed_6782	Hydroxyacid-oxoacid transhydrogenase	2.33	2.24	0.60	-0.35	7.53E-09	1.92E-07
Psed_5273	Dihydrolipoyl dehydrogenase	-1.00	-0.29	1.05	-1.00	7.51E-09	1.92E-07
Psed_5346	GMP synthase [glutamine-hydrolyzing]	-0.63	-0.07	-0.63	-1.46	7.48E-09	1.92E-07
Psed_1857	glycosyl transferase family 2	-1.53	-1.34	1.61	0.17	7.93E-09	2.02E-07
Psed_6853	protein of unknown function DUF302	0.04	1.30	-0.01	0.48	8.50E-09	2.15E-07
Psed_3693	oligopeptide/dipeptide ABC transporter, ATPase subunit	-0.98	-0.70	0.47	-1.89	8.51E-09	2.15E-07
Psed_2789	hypothetical protein	-1.02	-1.11	-2.22	0.29	8.67E-09	2.18E-07

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_1852	lipoprotein	-2.32	-1.47	0.93	-0.13	8.75E-09	2.19E-07
Psed_5909	GntR domain protein	-0.41	-0.35	2.25	0.04	8.80E-09	2.19E-07
Psed_5786	phage terminase	-0.18	2.20	2.28	3.43	8.89E-09	2.20E-07
Psed_3729	3-oxoacyl-[acyl-carrier-protein] reductase	-2.03	-3.24	0.87	-0.89	9.16E-09	2.26E-07
Psed_6161	Siderophore-interacting protein	0.35	-2.49	0.21	-0.39	9.34E-09	2.29E-07
Psed_6547	ATP-dependent chaperone ClpB	-0.16	1.97	-0.69	0.12	9.60E-09	2.35E-07
Psed_3291	hypothetical protein	0.01	-0.30	1.83	0.80	9.77E-09	2.38E-07
Psed_3667	SSS sodium solute transporter superfamily	-1.08	-3.54	-0.41	-1.52	9.96E-09	2.41E-07
Psed_2123	Peptidoglycan-binding lysin domain	-1.01	-1.76	-2.03	-0.66	1.00E-08	2.42E-07
Psed_1922	major facilitator superfamily MFS_1	0.69	0.35	1.29	-1.25	1.02E-08	2.46E-07
Psed_5815	hypothetical protein	-0.32	2.52	2.85	2.88	1.04E-08	2.50E-07
Psed_3904	CO dehydrogenase maturation factor-like protein	0.11	-3.20	-2.75	-1.02	1.06E-08	2.52E-07
Psed_0094	YCII-related	-0.38	-0.64	0.77	2.46	1.07E-08	2.55E-07
Psed_4984	isochorismatase hydrolase	-2.04	-0.08	0.71	-0.72	1.09E-08	2.58E-07
Psed_1117	hypothetical protein	-1.66	0.58	-1.84	-1.45	1.11E-08	2.61E-07
Psed_0355	hypothetical protein	-0.12	1.08	-0.41	1.26	1.14E-08	2.67E-07
Psed_3732	Monosaccharide-transporting ATPase	-2.80	-3.05	1.21	-0.94	1.18E-08	2.76E-07
Psed_1654	hypothetical protein	-1.19	-2.56	-1.93	-0.92	1.19E-08	2.77E-07
Psed_5783	hypothetical protein	-0.02	2.45	1.65	4.12	1.22E-08	2.84E-07
Psed_5081	DoxX family protein	-0.86	-1.57	1.83	1.28	1.24E-08	2.88E-07
Psed_3589	stress protein	-2.04	-1.22	-2.78	-1.78	1.32E-08	3.00E-07
Psed_3575	Histidyl-tRNA synthetase	-0.37	-0.15	0.18	-1.45	1.32E-08	3.00E-07
Psed_4424	hypothetical protein	1.42	0.44	0.13	-0.19	1.38E-08	3.14E-07
Psed_3621	hypothetical protein	-1.42	-1.21	-0.29	-1.17	1.43E-08	3.21E-07
Psed_5809	hypothetical protein	-0.01	1.77	1.95	3.02	1.42E-08	3.21E-07

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_0282	esterase	0.02	-1.49	-1.31	-1.26	1.43E-08	3.21E-07
Psed_3700	LmbE family protein	-0.51	-0.36	1.96	-0.77	1.42E-08	3.21E-07
Psed_0489	alpha/beta hydrolase fold-3	0.18	0.06	2.85	0.12	1.46E-08	3.25E-07
Psed_3242	Nitrile hydratase	0.22	0.79	1.66	0.45	1.48E-08	3.29E-07
Psed_5911	Hydantoinase/oxoprolinase	-0.51	-1.02	1.36	-0.14	1.49E-08	3.30E-07
Psed_3037	ABC-type metal ion transporter, periplasmic subunit	-1.69	-2.25	-1.99	-0.03	1.52E-08	3.34E-07
Psed_6914	amidohydrolase	-0.57	-2.21	-1.59	-1.33	1.53E-08	3.34E-07
Psed_1854	glycosyl transferase group 1	-0.35	-0.34	1.28	-0.13	1.52E-08	3.34E-07
Psed_2203	cobalamin synthesis CobW domain protein	-0.17	-2.21	-2.89	-0.43	1.54E-08	3.36E-07
Psed_4569	hypothetical protein	-0.42	-0.44	1.30	-0.38	1.57E-08	3.42E-07
Psed_7008	hypothetical protein	1.20	-0.09	0.23	0.16	1.59E-08	3.44E-07
Psed_6477	peptidase M10A and M12B matrixin and adamalysin	-0.84	-0.79	1.37	0.76	1.61E-08	3.47E-07
Psed_3680	squalene-hopene cyclase	0.13	-1.39	-0.62	-1.68	1.67E-08	3.60E-07
Psed_5195	alanine dehydrogenase	-3.89	-2.39	-3.92	-3.56	1.68E-08	3.60E-07
Psed_6892	hypothetical protein	-0.51	-1.72	-1.59	-1.04	1.70E-08	3.63E-07
Psed_1539	hypothetical protein	0.56	0.18	4.95	1.11	1.73E-08	3.68E-07
Psed_3995	ABC-type transporter, periplasmic subunit	-1.43	-2.64	-1.75	-1.98	1.88E-08	3.97E-07
Psed_6752	hypothetical protein	2.15	-0.29	-0.29	0.25	1.88E-08	3.97E-07
Psed_3205	ABC-type transporter, integral membrane subunit	0.30	-0.19	1.81	0.25	1.88E-08	3.97E-07
Psed_3522	response regulator receiver	1.74	1.93	3.98	2.11	1.89E-08	3.98E-07
Psed_1951	hypothetical protein	-0.69	-1.11	0.27	-0.49	2.04E-08	4.27E-07
Psed_0091	regulatory protein MerR	0.29	1.93	0.91	0.36	2.10E-08	4.37E-07
Psed_5324	hypothetical protein	-0.51	-0.95	-1.52	-0.06	2.09E-08	4.37E-07

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_3676	squalene synthase HpnC	-0.79	-2.20	-0.98	-1.90	2.12E-08	4.39E-07
Psed_5316	hypothetical protein	-1.03	-0.62	1.49	1.01	2.17E-08	4.47E-07
Psed_1892	C-methyltransferase	-0.12	-0.02	1.11	-0.05	2.16E-08	4.47E-07
Psed_0531	G3E family GTPase	-0.21	-0.27	1.92	2.54	2.19E-08	4.50E-07
Psed_5747	hypothetical protein	0.22	1.36	0.53	1.83	2.22E-08	4.55E-07
Psed_6755	hypothetical protein	1.83	-0.13	-0.71	0.44	2.26E-08	4.61E-07
Psed_0082	Monosaccharide-transporting ATPase	-0.82	-1.13	-1.31	0.22	2.28E-08	4.64E-07
Psed_1858	polysaccharide biosynthesis protein	-1.30	-1.18	1.53	0.15	2.31E-08	4.69E-07
Psed_2630	Xenobiotic-transporting ATPase	-0.33	1.58	-0.36	-0.50	2.34E-08	4.73E-07
Psed_3182	hypothetical protein	-0.44	-0.21	1.54	-0.26	2.42E-08	4.87E-07
Psed_0080	ABC-type transporter, integral membrane subunit	-1.04	-1.36	-1.49	-0.31	2.45E-08	4.89E-07
Psed_0334	hypothetical protein	-0.02	0.11	1.23	0.45	2.44E-08	4.89E-07
Psed_1179	protein tyrosine/serine phosphatase	-0.13	-0.11	1.29	-0.11	2.49E-08	4.95E-07
Psed_0350	Bile acid:sodium symporter	1.60	3.74	1.17	0.35	2.52E-08	4.99E-07
Psed_2679	NADH dehydrogenase (ubiquinone)	-0.10	2.25	0.16	0.22	2.54E-08	5.02E-07
Psed_6971	Hydroxyacid-oxoacid transhydrogenase	2.47	-0.55	0.44	2.17	2.61E-08	5.14E-07
Psed_6506	major facilitator superfamily MFS_1	-0.01	1.44	-0.42	-0.05	2.63E-08	5.18E-07
Psed_3887	Transcriptional regulator IclR	0.37	0.87	-0.07	-1.11	2.80E-08	5.49E-07
Psed_0227	hypothetical protein	0.17	-0.03	1.45	0.39	2.86E-08	5.58E-07
Psed_1023	hypothetical protein	-2.54	-2.05	-2.27	-1.70	2.89E-08	5.59E-07
Psed_2641	ATPase associated with various cellular activities AAA_5	-1.18	-0.94	0.95	-0.64	2.88E-08	5.59E-07
Psed_7002	transcription factor WhiB	1.51	-0.58	-0.80	-0.32	2.89E-08	5.59E-07
Psed_1927	hypothetical protein	-0.34	1.88	-0.58	-0.52	2.90E-08	5.59E-07

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_3726	Xylose isomerase domain-containing protein TIM barrel	-2.16	-3.73	1.11	-0.73	2.96E-08	5.69E-07
Psed_6925	ABC-type transporter, integral membrane subunit	-0.08	-0.70	1.45	-0.85	3.00E-08	5.74E-07
Psed_5358	transcription factor WhiB	-0.34	2.13	1.90	1.05	3.03E-08	5.79E-07
Psed_0288	S-(hydroxymethyl)glutathione dehydrogenase	0.02	-0.01	0.18	1.76	3.09E-08	5.86E-07
Psed_1178	hypothetical protein	0.96	0.74	3.71	1.82	3.12E-08	5.87E-07
Psed_6539	hypothetical protein	-0.54	-0.07	1.88	0.76	3.18E-08	5.96E-07
Psed_3041	NADH dehydrogenase (ubiquinone) 24 kDa subunit	1.07	0.08	3.30	1.03	3.24E-08	6.07E-07
Psed_3172	Serine/threonine protein kinase-related	-0.81	-0.92	-0.67	-1.79	3.28E-08	6.13E-07
Psed_0164	hypothetical protein	0.60	-2.62	-0.43	-0.64	3.37E-08	6.26E-07
Psed_2780	transcriptional regulatory protein	-0.03	0.37	1.23	-0.17	3.44E-08	6.39E-07
Psed_3669	hypothetical protein	0.55	2.61	0.66	2.28	3.52E-08	6.51E-07
Psed_6921	Xylose isomerase domain-containing protein TIM barrel	-0.47	-0.43	1.99	-0.07	3.71E-08	6.83E-07
Psed_5211	Mannose-1-phosphate guanylyltransferase	-0.90	-0.69	-0.38	-1.45	3.70E-08	6.83E-07
Psed_6550	pyridoxamine 5'-phosphate oxidase-related FMN-binding	-0.87	-0.18	1.10	-0.52	3.83E-08	7.03E-07
Psed_2650	hypothetical protein	-1.22	-0.81	-1.20	0.16	3.88E-08	7.08E-07
Psed_3174	hypothetical protein	-0.92	-2.71	-1.55	-1.33	3.93E-08	7.12E-07
Psed_2660	regulatory protein TetR	0.31	1.59	0.27	-0.20	3.93E-08	7.12E-07
Psed_6525	hypothetical protein	-0.78	-0.57	0.12	-1.20	3.97E-08	7.19E-07
Psed_0249	L-lysine 6-monooxygenase (NADPH)	0.64	-2.84	-0.04	-0.13	4.17E-08	7.51E-07
Psed_1730	alpha amylase catalytic region	-1.42	-1.75	-0.08	-2.19	4.24E-08	7.62E-07
Psed_0021	isochorismatase hydrolase	0.16	-1.75	-0.59	-1.08	4.28E-08	7.67E-07
Psed_2995	monosaccharide-transporting ATPase	-1.23	-2.51	-0.18	-1.65	4.36E-08	7.80E-07

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_0253	Pentachlorophenol monooxygenase	0.63	-0.34	1.56	0.22	4.41E-08	7.87E-07
Psed_3734	ABC-type transporter, integral membrane sub-unit	-3.18	-3.71	1.20	-0.51	4.50E-08	8.01E-07
Psed_6619	Phosphoenolpyruvate carboxykinase [GTP]	-1.41	-1.88	-0.95	-0.77	4.55E-08	8.07E-07
Psed_1847	lipopolysaccharide biosynthesis protein	-1.54	-1.32	0.63	-0.29	4.93E-08	8.72E-07
Psed_0930	hypothetical protein	0.24	0.33	1.11	-0.29	4.97E-08	8.76E-07
Psed_3721	alpha/beta hydrolase fold	-2.02	-2.57	1.05	-1.21	4.99E-08	8.79E-07
Psed_2655	hypothetical protein	-1.13	-0.87	-0.19	-0.41	5.06E-08	8.88E-07
Psed_1866	ATP-grasp fold domain protein, DUF201-type	0.06	0.18	1.47	0.08	5.12E-08	8.96E-07
Psed_3882	hypothetical protein	0.07	0.06	1.09	0.85	5.20E-08	9.07E-07
Psed_3894	ErfK/YbiS/YcfS/YnhG family protein	-0.28	-1.75	-0.43	-1.21	5.34E-08	9.27E-07
Psed_0914	Luciferase-like, subgroup	-1.46	-1.01	1.65	-0.02	5.43E-08	9.37E-07
Psed_1675	glycosyl transferase family 4	-1.03	-0.32	-0.09	-1.26	5.54E-08	9.54E-07
Psed_3715	Phosphoglycerate dehydrogenase	-0.72	-0.77	1.24	-0.31	5.58E-08	9.59E-07
Psed_6970	D-lactate dehydrogenase (cytochrome)	2.80	-0.60	-0.20	2.71	5.71E-08	9.78E-07
Psed_5814	hypothetical protein	-0.15	2.11	2.26	3.71	5.73E-08	9.78E-07
Psed_1671	Sua5/YciO/YrdC/YwlC family protein	-0.51	-0.03	-0.37	-1.50	5.77E-08	9.83E-07
Psed_5844	hypothetical protein	-1.08	-3.85	-2.68	-1.19	5.87E-08	9.96E-07
Psed_4770	hypothetical protein	-0.84	0.02	0.51	-1.16	5.88E-08	9.96E-07
Psed_6961	hypothetical protein	-0.43	-1.51	-0.35	-1.06	5.96E-08	1.01E-06
Psed_5100	hypothetical protein	-0.39	-0.14	1.36	-0.18	6.07E-08	1.02E-06
Psed_0902	NADH dehydrogenase (ubiquinone)	-2.26	-1.48	-1.98	-1.09	6.16E-08	1.04E-06
Psed_6855	beta-lactamase domain protein	-0.07	1.79	-0.46	0.79	6.22E-08	1.04E-06
Psed_5816	hypothetical protein	-0.65	-0.11	1.61	1.02	6.28E-08	1.05E-06
Psed_2972	dihydroxyacetone kinase, DhaK subunit	-1.78	0.27	-0.21	-1.22	6.40E-08	1.07E-06
Psed_2552	Aminomethyltransferase	0.23	1.21	-0.20	-0.08	6.59E-08	1.10E-06

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_3724	3-oxoacyl-[acyl-carrier-protein] reductase	-1.72	-2.33	1.43	-0.87	6.65E-08	1.10E-06
Psed_3696	4Fe-4S ferredoxin iron-sulfur binding domain-containing protein	-0.55	-0.44	1.10	-0.64	6.64E-08	1.10E-06
Psed_3571	Radical SAM domain protein	-0.77	-0.97	-2.43	-0.06	6.79E-08	1.12E-06
Psed_0095	RNA polymerase sigma factor, sigma-70 family	0.05	0.04	0.21	1.52	6.91E-08	1.14E-06
Psed_5803	hypothetical protein	-0.09	1.71	1.83	2.01	7.02E-08	1.15E-06
Psed_5934	o-succinylbenzoate--CoA ligase	0.31	0.12	2.45	0.10	7.20E-08	1.18E-06
Psed_6211	NLP/P60 protein	-0.34	-0.19	-0.03	-1.19	7.30E-08	1.19E-06
Psed_5250	major facilitator superfamily MFS_1	0.10	0.07	0.10	1.04	7.62E-08	1.23E-06
Psed_3964	Lysine exporter protein (LYSE/YGGA)	-0.25	1.43	-0.78	-1.03	7.72E-08	1.25E-06
Psed_2723	Magnesium chelatase	-0.78	-0.70	-0.92	-1.34	7.92E-08	1.28E-06
Psed_5743	thiamine biosynthesis protein ThiS	-0.47	0.06	-0.01	-1.02	7.98E-08	1.28E-06
Psed_1845	Glutamate-1-semialdehyde 2,1-aminomutase	-0.65	-0.47	1.33	0.22	8.23E-08	1.32E-06
Psed_4941	Formyl-CoA transferase	0.10	0.06	0.02	2.78	8.24E-08	1.32E-06
Psed_3503	PDZ/DHR/GLGF domain-containing protein	0.31	0.94	2.43	2.84	8.28E-08	1.32E-06
Psed_5768	hypothetical protein	0.43	0.87	0.56	1.43	8.28E-08	1.32E-06
Psed_3898	3-oxoacyl-[acyl-carrier-protein] reductase	-0.92	-1.38	0.18	-0.92	8.50E-08	1.35E-06
Psed_7007	hypothetical protein	1.40	0.18	0.19	0.34	8.58E-08	1.36E-06
Psed_5802	hypothetical protein	0.24	1.49	1.74	2.79	8.68E-08	1.37E-06
Psed_4306	hypothetical protein	1.05	1.00	2.47	0.77	9.09E-08	1.43E-06
Psed_0549	cysteine dioxygenase type I	-1.00	-1.33	-0.65	-0.30	9.49E-08	1.49E-06
Psed_1709	Aldehyde Dehydrogenase	0.04	0.49	1.22	-0.16	9.52E-08	1.49E-06
Psed_5647	hypothetical protein	-0.63	-0.26	0.08	-1.35	9.78E-08	1.52E-06
Psed_6924	ABC-type transporter, periplasmic subunit	-0.26	-0.78	1.98	0.28	1.04E-07	1.61E-06
Psed_4938	regulatory protein MarR	0.46	0.23	-0.16	2.83	1.06E-07	1.63E-06
Psed_6948	transposase mutator type	-0.61	-2.12	-1.06	-0.84	1.08E-07	1.67E-06

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_0247	ABC-type transporter, integral membrane subunit	0.78	-1.79	1.10	-0.91	1.15E-07	1.76E-06
Psed_5026	regulatory protein TetR	1.19	1.41	2.41	0.23	1.16E-07	1.78E-06
Psed_5472	regulatory protein LuxR	-0.68	-0.52	-1.67	-1.25	1.20E-07	1.83E-06
Psed_0126	Methionine synthase vitamin-B12 independent	-0.41	-0.16	2.88	-0.44	1.20E-07	1.83E-06
Psed_6640	extracellular solute-binding protein family 1	-1.27	-1.09	-3.81	-1.54	1.22E-07	1.85E-06
Psed_5748	hypothetical protein	0.19	1.31	0.58	1.10	1.22E-07	1.85E-06
Psed_3241	Amidase	0.50	0.75	1.52	-0.01	1.27E-07	1.91E-06
Psed_1187	protein of unknown function DUF214	-0.44	-0.60	0.29	-1.28	1.28E-07	1.93E-06
Psed_6729	hypothetical protein	1.91	0.03	-0.04	0.83	1.30E-07	1.95E-06
Psed_2501	GntR domain protein	-0.31	-0.13	1.40	-0.46	1.33E-07	2.00E-06
Psed_1166	hypothetical protein	-1.24	-1.28	0.19	-1.05	1.37E-07	2.05E-06
Psed_5872	hypothetical protein	-2.08	-0.39	-2.33	-1.99	1.38E-07	2.06E-06
Psed_2763	hypothetical protein	-1.18	-2.23	-1.65	-1.78	1.39E-07	2.06E-06
Psed_6923	oligopeptide/dipeptide ABC transporter, ATPase subunit	-0.41	-0.72	1.41	-0.44	1.38E-07	2.06E-06
Psed_5092	NADP-dependent oxidoreductase domain	-0.53	-0.51	-0.37	-1.11	1.39E-07	2.06E-06
Psed_5916	Acetyl-CoA C-acetyltransferase	-0.19	-0.29	1.20	0.26	1.43E-07	2.11E-06
Psed_1086	Conserved hypothetical protein CHP02569	-0.66	-1.13	0.06	-1.03	1.47E-07	2.17E-06
Psed_3906	Luciferase-like, subgroup	-0.89	-1.07	0.90	-0.71	1.57E-07	2.32E-06
Psed_5874	ErfK/YbiS/YcfS/YnhG family protein	-0.60	-1.16	-1.28	-1.40	1.65E-07	2.41E-06
Psed_1652	protein of unknown function DUF477	-0.67	-0.70	-0.77	-1.01	1.67E-07	2.44E-06
Psed_6162	MbtH domain protein	0.08	-2.28	-0.34	-0.08	1.72E-07	2.49E-06
Psed_3795	bifunctional deaminase-reductase domain protein	0.22	0.11	1.77	0.32	1.72E-07	2.49E-06
Psed_1653	hypothetical protein	-0.58	-1.39	-1.84	-0.36	1.76E-07	2.54E-06

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_4052	(S)-2-hydroxy-acid oxidase	-0.79	-0.84	2.00	0.31	1.76E-07	2.54E-06
Psed_0246	ABC-type transporter, integral membrane subunit	0.66	-1.16	1.20	-0.36	1.79E-07	2.58E-06
Psed_3590	Tellurium resistance	-2.37	-1.18	-3.16	-2.49	1.83E-07	2.63E-06
Psed_3934	major facilitator superfamily MFS_1	1.22	0.55	-0.81	-0.01	1.86E-07	2.66E-06
Psed_6290	dienelactone hydrolase	-0.94	-1.30	-0.46	-0.49	1.86E-07	2.66E-06
Psed_3858	protein of unknown function DUF6 transmembrane	0.38	1.96	0.02	-0.24	1.87E-07	2.66E-06
Psed_6175	regulatory protein TetR	1.73	1.98	3.17	0.44	1.88E-07	2.67E-06
Psed_3591	hypothetical protein	-1.74	-0.80	-2.10	-1.49	1.89E-07	2.67E-06
Psed_6888	C-5 cytosine-specific DNA methylase	1.17	-0.22	-0.57	0.12	1.88E-07	2.67E-06
Psed_2978	RNA polymerase sigma factor, sigma-70 family	-1.05	1.31	-0.56	-0.02	1.90E-07	2.67E-06
Psed_1896	C-methyltransferase	-0.66	-0.49	1.25	0.24	1.92E-07	2.67E-06
Psed_4571	Lipoyl synthase	-0.88	-0.45	-0.47	-1.27	1.91E-07	2.67E-06
Psed_4549	Stearoyl-CoA 9-desaturase	0.00	1.17	0.07	0.21	1.93E-07	2.68E-06
Psed_6757	hypothetical protein	1.03	-0.64	-0.44	0.14	1.98E-07	2.74E-06
Psed_5539	hypothetical protein	0.75	0.95	4.63	2.67	1.97E-07	2.74E-06
Psed_3733	ABC-type transporter, integral membrane subunit	-2.48	-2.78	1.35	-0.91	2.01E-07	2.78E-06
Psed_3280	PspC domain protein	0.24	0.12	1.26	-0.25	2.07E-07	2.83E-06
Psed_4656	hypothetical protein	-2.21	-2.03	-1.18	-2.39	2.10E-07	2.87E-06
Psed_1060	magnesium and cobalt transport protein CorA	-1.47	-1.47	-1.70	-1.44	2.16E-07	2.94E-06
Psed_6511	NUDIX hydrolase	-0.39	-0.03	-0.14	-1.41	2.16E-07	2.94E-06
Psed_4689	RIO-like kinase	0.23	1.35	0.07	0.20	2.19E-07	2.96E-06
Psed_3682	hopanoid biosynthesis associated radical SAM protein HpnH	-0.23	-1.91	-0.47	-1.70	2.27E-07	3.05E-06

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_5637	Ppx/GppA phosphatase	0.08	-0.49	-1.09	-0.10	2.28E-07	3.06E-06
Psed_6096	ABC-type transporter, integral membrane sub-unit	-0.24	-2.18	-1.89	-0.07	2.29E-07	3.07E-06
Psed_6665	NLP/P60 protein	0.68	3.45	0.27	2.19	2.30E-07	3.08E-06
Psed_1261	hypothetical protein	-0.89	-1.29	0.06	0.41	2.35E-07	3.13E-06
Psed_5812	hypothetical protein	-0.25	1.60	2.12	2.62	2.46E-07	3.27E-06
Psed_5466	hypothetical protein	-0.76	-1.57	0.08	-1.09	2.49E-07	3.29E-06
Psed_3440	SH3 type 3 domain protein	-0.26	-2.06	-2.66	-0.76	2.53E-07	3.35E-06
Psed_3960	Phosphonate-transporting ATPase	0.06	-0.46	1.03	-1.07	2.55E-07	3.36E-06
Psed_5758	hypothetical protein	-0.16	1.29	0.60	2.27	2.60E-07	3.42E-06
Psed_3972	nitroreductase	0.32	-1.02	0.80	-0.15	2.62E-07	3.45E-06
Psed_3511	hypothetical protein	0.19	0.26	2.59	0.48	2.68E-07	3.51E-06
Psed_6804	hypothetical protein	0.09	-0.60	-0.02	-1.22	2.68E-07	3.51E-06
Psed_2271	GCN5-related N-acetyltransferase	0.01	1.86	-0.27	-0.12	2.75E-07	3.58E-06
Psed_6758	hypothetical protein	1.17	-0.00	-0.40	0.11	2.83E-07	3.66E-06
Psed_5843	hypothetical protein	-1.05	-3.27	-2.24	-1.98	2.85E-07	3.67E-06
Psed_1763	molybdopterin dehydrogenase FAD-binding	-1.89	-1.74	0.34	-2.03	2.86E-07	3.67E-06
Psed_0131	Alcohol dehydrogenase GroES domain protein	-0.01	-0.60	0.60	1.99	2.84E-07	3.67E-06
Psed_0172	protein of unknown function DUF350	-0.23	-0.41	0.39	-1.04	2.86E-07	3.67E-06
Psed_5720	PspC domain protein	0.16	0.35	1.65	0.52	2.89E-07	3.71E-06
Psed_2766	Long-chain-fatty-acid--CoA ligase	-0.77	-0.94	-0.09	-1.29	2.90E-07	3.71E-06
Psed_1304	heavy metal translocating P-type ATPase	1.38	3.23	-0.49	0.72	3.07E-07	3.91E-06
Psed_5755	hypothetical protein	0.05	0.50	0.66	1.21	3.10E-07	3.94E-06
Psed_5134	diguanylate cyclase	-0.47	-1.43	-0.89	-0.86	3.11E-07	3.95E-06
Psed_6889	hypothetical protein	0.02	-1.65	-0.85	-0.56	3.11E-07	3.95E-06
Psed_5192	Uncharacterised protein family UPF0324	0.67	2.34	-0.33	-0.11	3.16E-07	4.00E-06

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_0550	Rhodanese-like protein	-0.88	-1.16	-0.64	-1.33	3.18E-07	4.01E-06
Psed_1302	protein of unknown function DUF156	1.85	3.53	-0.51	1.20	3.19E-07	4.01E-06
Psed_5458	hypothetical protein	0.05	1.96	-0.11	0.25	3.22E-07	4.03E-06
Psed_4799	Prolyl oligopeptidase	-0.24	-0.01	-0.15	-1.13	3.26E-07	4.08E-06
Psed_4100	hypothetical protein	-0.61	-0.46	-0.10	-1.58	3.34E-07	4.17E-06
Psed_5267	protein of unknown function DUF692	-0.26	-1.13	1.09	-1.37	3.38E-07	4.22E-06
Psed_0527	hypothetical protein	0.06	0.15	1.20	0.43	3.39E-07	4.22E-06
Psed_0967	RmuC-domain protein	-1.15	-1.53	-1.64	-1.22	3.43E-07	4.26E-06
Psed_5266	hypothetical protein	0.12	-0.66	1.85	-0.58	3.44E-07	4.26E-06
Psed_2281	ABC transporter related	-2.25	-0.14	-0.85	-1.72	3.45E-07	4.27E-06
Psed_4548	GCN5-related N-acetyltransferase	-0.83	1.54	-1.27	-1.22	3.46E-07	4.27E-06
Psed_4755	ammonium transporter	1.14	3.29	1.70	1.85	3.50E-07	4.32E-06
Psed_2887	diguanylate cyclase	-0.66	-1.83	-1.27	-1.64	3.55E-07	4.36E-06
Psed_5204	transcription factor WhiB	-0.64	-1.31	0.09	-0.81	3.55E-07	4.36E-06
Psed_1555	peptidase S1 and S6 chymotrypsin/Hap	1.51	1.27	6.08	3.71	3.59E-07	4.40E-06
Psed_3450	hypothetical protein	-0.05	0.25	2.36	2.39	3.65E-07	4.47E-06
Psed_3614	hypothetical protein	-1.10	-0.23	0.50	-1.08	3.68E-07	4.49E-06
Psed_1770	hypothetical protein	-1.68	-1.57	-0.05	-0.53	3.69E-07	4.49E-06
Psed_5785	hypothetical protein	0.72	1.60	1.66	2.24	3.70E-07	4.49E-06
Psed_2654	Carbon-monoxide dehydrogenase (acceptor)	-1.12	-0.95	-0.51	-0.28	3.72E-07	4.50E-06
Psed_2849	Histidinol-phosphate aminotransferase	-0.25	0.33	-0.02	-1.12	3.71E-07	4.50E-06
Psed_3358	hypothetical protein	-0.45	-0.61	-0.24	-1.11	3.72E-07	4.50E-06
Psed_6486	Conserved hypothetical protein CHP02680	-0.58	-1.39	-0.25	-0.84	3.79E-07	4.57E-06
Psed_1002	ABC-type transporter, integral membrane sub-unit	-0.04	0.04	0.28	-1.36	3.80E-07	4.57E-06

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_4030	protein of unknown function DUF224 cysteine-rich region domain protein	0.52	1.12	-0.86	-0.23	3.85E-07	4.61E-06
Psed_3730	Carboxymuconolactone decarboxylase	-1.87	-2.54	0.85	-1.44	3.86E-07	4.61E-06
Psed_1260	DEAD/DEAH box helicase domain protein	-0.72	-1.55	0.27	-0.64	3.96E-07	4.72E-06
Psed_5808	hypothetical protein	-0.14	2.04	2.15	2.40	4.00E-07	4.76E-06
Psed_3158	OsmC family protein	-0.94	-0.55	-0.22	-1.02	4.10E-07	4.87E-06
Psed_3695	ABC-type transporter, integral membrane subunit	-0.73	-0.64	0.26	-1.15	4.17E-07	4.94E-06
Psed_1855	hypothetical protein	-0.44	-0.45	1.32	0.19	4.21E-07	4.98E-06
Psed_5343	PspC domain protein	0.05	0.06	1.37	-0.24	4.27E-07	5.03E-06
Psed_2812	Cell division protein sepF	-1.35	-2.89	-0.76	-1.44	4.36E-07	5.11E-06
Psed_1370	regulatory protein LacI	-0.39	-0.59	-0.35	-1.40	4.46E-07	5.19E-06
Psed_0338	hypothetical protein	0.82	1.75	0.15	-0.64	4.48E-07	5.21E-06
Psed_5025	4-hydroxyacetophenone monooxygenase	2.42	1.82	4.26	0.06	4.57E-07	5.29E-06
Psed_5201	phosphoglucomutase/phosphomannomutase alpha/beta/alpha domain II	-0.38	0.11	-0.08	-1.06	4.58E-07	5.29E-06
Psed_5609	cytochrome c biogenesis protein transmembrane region	-0.88	-0.26	-0.98	-2.46	4.60E-07	5.31E-06
Psed_3962	polar amino acid ABC transporter, inner membrane subunit	-0.28	-1.08	0.52	-1.32	4.66E-07	5.36E-06
Psed_3256	hypothetical protein	0.12	-0.12	0.99	3.34	4.69E-07	5.39E-06
Psed_5161	3-phosphoshikimate 1-carboxyvinyltransferase	-0.36	0.04	-0.54	-1.11	4.69E-07	5.39E-06
Psed_4051	hypothetical protein	0.04	1.17	0.13	-0.17	4.75E-07	5.44E-06
Psed_3935	GntR domain protein	1.46	0.22	-0.83	-0.92	4.77E-07	5.46E-06
Psed_3681	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	-0.03	-1.58	-0.76	-1.48	4.80E-07	5.49E-06
Psed_5810	hypothetical protein	-0.22	1.68	1.91	2.20	4.85E-07	5.52E-06

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_0183	hypothetical protein	-0.23	0.10	-0.26	-1.09	4.85E-07	5.52E-06
Psed_1708	Alcohol dehydrogenase zinc-binding domain protein	-0.32	0.37	1.70	0.29	4.90E-07	5.57E-06
Psed_5753	hypothetical protein	0.21	0.70	0.29	1.73	4.95E-07	5.62E-06
Psed_2299	hypothetical protein	-2.01	-0.56	-0.28	-2.75	5.04E-07	5.70E-06
Psed_1860	Glucose-1-phosphate cytidylyltransferase	-1.56	-0.97	0.84	-0.15	5.10E-07	5.76E-06
Psed_5101	Phosphoglycerate mutase	-0.11	0.34	1.06	0.19	5.12E-07	5.78E-06
Psed_6289	hypothetical protein	-1.13	-1.42	-0.51	-0.57	5.15E-07	5.79E-06
Psed_0881	Protein of unknown function DUF2277	-1.01	-0.49	0.23	-0.18	5.15E-07	5.79E-06
Psed_5268	hypothetical protein	-0.10	-0.64	1.07	-0.82	5.20E-07	5.82E-06
Psed_6662	hypothetical protein	-0.42	-0.04	-0.26	-1.09	5.19E-07	5.82E-06
Psed_0175	UbiA prenyltransferase	-0.59	-0.31	-0.21	-1.20	5.20E-07	5.82E-06
Psed_0545	Cna B domain protein	-0.68	-1.13	-0.41	-1.12	5.28E-07	5.89E-06
Psed_1113	glutaredoxin-like protein	0.61	1.95	0.53	0.78	5.28E-07	5.89E-06
Psed_3585	Crossover junction endodeoxyribonuclease ruvC	-0.36	-0.86	-1.09	-1.45	5.40E-07	6.01E-06
Psed_5540	hypothetical protein	0.08	0.13	1.44	-0.05	5.53E-07	6.14E-06
Psed_5761	hypothetical protein	0.02	0.72	0.61	2.02	5.63E-07	6.22E-06
Psed_1540	Phosphonate-transporting ATPase	-0.59	-0.50	1.03	-0.02	5.74E-07	6.33E-06
Psed_5745	Thiamine-phosphate pyrophosphorylase	-0.34	-0.02	-0.11	-1.05	5.80E-07	6.39E-06
Psed_2843	peptidase S16 lon domain protein	-0.24	0.73	1.02	1.42	5.82E-07	6.40E-06
Psed_5371	hypothetical protein	3.34	3.66	5.99	4.37	5.91E-07	6.48E-06
Psed_3961	ABC-type transporter, periplasmic subunit family 3	-0.42	-1.10	0.41	-1.58	5.92E-07	6.48E-06
Psed_1712	DivIVA domain	-1.06	-0.63	-0.08	-1.16	6.00E-07	6.55E-06
Psed_0546	alpha/beta hydrolase fold	-0.37	-1.09	-0.01	-0.99	5.99E-07	6.55E-06

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_6999	hypothetical protein	-0.28	-1.02	-0.84	-1.71	6.15E-07	6.68E-06
Psed_5762	hypothetical protein	0.38	1.05	0.33	1.63	6.24E-07	6.77E-06
Psed_5207	F420-0:gamma-glutamyl ligase	-0.65	-0.57	-0.73	-1.42	6.27E-07	6.80E-06
Psed_4655	protein of unknown function DUF1778	-1.71	-1.75	-1.08	-1.98	6.33E-07	6.85E-06
Psed_6538	AAA ATPase central domain protein	-0.68	-0.18	1.38	0.53	6.41E-07	6.92E-06
Psed_0137	copper resistance D domain protein	-1.52	-1.55	-2.18	-0.60	6.74E-07	7.24E-06
Psed_5813	hypothetical protein	-0.28	1.74	1.51	2.18	6.77E-07	7.26E-06
Psed_1565	protocatechuate 3,4-dioxygenase, beta subunit	0.11	-0.24	3.63	-0.79	6.95E-07	7.44E-06
Psed_3876	ABC-type transporter, integral membrane subunit	-0.03	-0.47	-0.25	-1.63	6.96E-07	7.44E-06
Psed_6890	hypothetical protein	0.05	-1.59	-1.18	-0.78	7.04E-07	7.51E-06
Psed_3327	protein of unknown function DUF75	-0.50	-0.36	0.17	-1.19	7.15E-07	7.58E-06
Psed_5257	Acyl-CoA dehydrogenase	-0.31	-0.29	1.68	0.54	7.17E-07	7.59E-06
Psed_4976	NADP oxidoreductase coenzyme F420-dependent	-1.92	-2.04	-0.83	-1.46	7.19E-07	7.60E-06
Psed_2549	L-serine dehydratase 1	0.49	1.17	-0.74	0.27	7.27E-07	7.67E-06
Psed_5320	thioredoxin	0.32	1.03	-0.81	0.69	7.41E-07	7.76E-06
Psed_5601	SH3 type 3 domain protein	-0.68	-1.24	0.20	-0.61	7.63E-07	7.95E-06
Psed_3953	(Dimethylallyl)adenosine tRNA methylthiotransferase miaB	-1.00	-0.41	-0.35	-1.48	7.63E-07	7.95E-06
Psed_3928	major facilitator superfamily MFS_1	-0.83	-1.38	-1.48	-1.08	7.70E-07	7.98E-06
Psed_4332	hypothetical protein	-0.77	1.31	1.95	0.93	7.69E-07	7.98E-06
Psed_6062	Phenol 2-monooxygenase	-0.09	-0.08	2.81	0.83	7.71E-07	7.98E-06
Psed_5767	hypothetical protein	0.33	0.95	0.14	1.50	7.67E-07	7.98E-06
Psed_4598	hypothetical protein	-0.46	-0.11	0.01	-1.04	7.78E-07	8.04E-06
Psed_5247	hypothetical protein	0.18	0.22	-0.13	2.10	8.00E-07	8.24E-06

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_7017	hypothetical protein	-0.83	-1.10	-1.29	-1.00	8.18E-07	8.40E-06
Psed_1183	acyl-CoA dehydrogenase domain-containing protein	0.17	0.58	2.84	0.40	8.22E-07	8.43E-06
Psed_3357	hypothetical protein	-0.66	-1.47	0.04	-0.92	8.28E-07	8.48E-06
Psed_3338	Penicillinase repressor	-0.19	-0.14	-0.49	-1.83	8.45E-07	8.61E-06
Psed_4459	protein of unknown function DUF182	-0.70	-0.50	-0.02	-1.13	8.64E-07	8.78E-06
Psed_3975	hypothetical protein	-0.18	0.20	1.56	0.84	8.73E-07	8.84E-06
Psed_3424	hypothetical protein	-0.14	-0.46	-1.07	-0.18	8.75E-07	8.85E-06
Psed_4603	DEAD/H associated domain protein	-0.70	-1.24	-0.65	-0.79	8.78E-07	8.85E-06
Psed_5317	Error-prone DNA polymerase	-0.47	-0.65	-1.39	0.46	8.78E-07	8.85E-06
Psed_6672	tRNA adenylyltransferase	0.34	1.20	-0.09	-0.56	8.80E-07	8.86E-06
Psed_6645	hypothetical protein	-1.01	-1.60	-1.99	-0.85	8.85E-07	8.89E-06
Psed_1447	Phosphoribosylaminoimidazolecarboxamide formyltransferase	-1.16	-1.16	-1.12	-1.27	9.09E-07	9.11E-06
Psed_6521	phospholipid/glycerol acyltransferase	-0.96	-0.61	-0.50	-1.48	9.17E-07	9.15E-06
Psed_6063	muconolactone delta-isomerase	-0.22	-0.08	2.55	0.71	9.23E-07	9.20E-06
Psed_1566	protocatechuate 3,4-dioxygenase, alpha subunit	0.31	-0.24	4.46	-0.27	9.36E-07	9.29E-06
Psed_5780	hypothetical protein	-0.75	0.37	0.78	1.37	9.53E-07	9.41E-06
Psed_4576	regulatory protein TetR	0.82	1.61	2.27	0.65	9.65E-07	9.50E-06
Psed_2831	DNA polymerase III, alpha subunit	-1.08	-1.54	-0.01	-1.40	9.72E-07	9.56E-06
Psed_1049	extracellular solute-binding protein family 1	-1.66	-1.70	0.44	-0.31	9.78E-07	9.56E-06
Psed_2291	pyridoxamine 5'-phosphate oxidase-related FMN-binding protein	-1.67	-0.05	-0.73	-1.54	9.76E-07	9.56E-06
Psed_6263	Extracellular ligand-binding receptor	1.21	-0.17	-0.46	0.34	9.89E-07	9.65E-06
Psed_2914	Iron-chelate-transporting ATPase	0.06	-1.31	0.76	0.08	9.98E-07	9.73E-06
Psed_6812	hypothetical protein	0.46	1.49	0.92	1.37	1.01E-06	9.77E-06

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_1828	monooxygenase FAD-binding	-1.23	-1.22	-1.24	-1.31	1.01E-06	9.78E-06
Psed_6856	Rhodanese-like protein	-0.28	1.08	-0.73	0.53	1.03E-06	9.91E-06
Psed_3401	Fe(3+)-transporting ATPase	-0.01	0.10	-0.45	-1.14	1.05E-06	1.00E-05
Psed_0357	helix-turn-helix domain protein	0.29	0.26	0.06	1.08	1.05E-06	1.00E-05
Psed_5922	Carboxylesterase	-1.95	-1.97	0.91	-1.81	1.07E-06	1.02E-05
Psed_3387	transcriptional regulator	-0.48	-1.17	0.24	-0.12	1.08E-06	1.03E-05
Psed_4080	protein of unknown function DUF574	-1.56	-1.90	-0.03	-1.03	1.08E-06	1.03E-05
Psed_6972	GntR domain protein	1.93	-0.70	0.31	1.66	1.09E-06	1.04E-05
Psed_2893	hypothetical protein	-0.15	-1.24	-0.04	-0.40	1.12E-06	1.06E-05
Psed_1615	Conserved hypothetical protein CHP00268	-0.90	-0.79	-0.30	-1.10	1.12E-06	1.06E-05
Psed_1589	hypothetical protein	0.79	0.59	4.08	1.48	1.15E-06	1.08E-05
Psed_1851	Heparinase II/III family protein	-1.13	-0.70	0.16	-0.23	1.15E-06	1.08E-05
Psed_5215	cell envelope-related function transcriptional attenuator, LytR/CpsA family	-1.08	-2.14	-0.93	-0.91	1.16E-06	1.08E-05
Psed_6553	Chaperone protein dnaJ	0.00	1.26	-0.98	-0.68	1.16E-06	1.09E-05
Psed_2386	cyclase/dehydrase	0.50	2.18	0.25	0.32	1.18E-06	1.10E-05
Psed_3204	extracellular solute-binding protein family 1	0.45	-0.06	1.99	0.40	1.19E-06	1.11E-05
Psed_6524	RNA polymerase sigma-70 factor, sigma-E family	-0.66	-0.24	-0.52	-1.09	1.20E-06	1.11E-05
Psed_0311	regulatory protein TetR	-0.22	1.40	0.42	-0.67	1.20E-06	1.11E-05
Psed_6913	hypothetical protein	1.27	-0.01	-0.12	0.56	1.21E-06	1.12E-05
Psed_1456	RNA polymerase sigma factor, sigma-70 family	-0.24	-0.38	1.00	-0.14	1.22E-06	1.12E-05
Psed_1881	glycosyl transferase group 1	-0.84	-0.58	-0.82	-1.71	1.22E-06	1.12E-05
Psed_2805	cell division protein FtsW	-0.55	-0.67	-0.58	-1.03	1.22E-06	1.12E-05
Psed_5682	hypothetical protein	-0.69	-0.99	2.03	-0.22	1.23E-06	1.13E-05
Psed_5548	Enoyl-CoA hydratase/isomerase	-0.81	-0.51	-0.53	-1.60	1.23E-06	1.13E-05

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_2784	Geranyltranstransferase	-0.33	0.54	1.10	0.25	1.24E-06	1.14E-05
Psed_4725	phosphatidate cytidylyltransferase	-0.26	0.28	0.65	-1.09	1.25E-06	1.14E-05
Psed_3271	BFD domain protein [2Fe-2S]-binding domain protein	0.77	-0.39	1.45	0.49	1.25E-06	1.14E-05
Psed_5362	Aryl-alcohol dehydrogenase (NADP(+))	-0.57	-0.49	-0.63	-1.86	1.28E-06	1.16E-05
Psed_5769	hypothetical protein	0.12	0.35	0.19	1.55	1.28E-06	1.17E-05
Psed_0488	Cyclohexanone monooxygenase	0.12	0.10	1.26	0.14	1.29E-06	1.17E-05
Psed_5355	regulatory protein LuxR	-0.32	-1.68	-1.67	-1.10	1.30E-06	1.17E-05
Psed_4333	UBA/THIF-type NAD/FAD binding protein	-0.30	1.47	2.60	0.91	1.31E-06	1.18E-05
Psed_5240	GtrA family protein	-0.51	-0.38	-0.21	-1.58	1.32E-06	1.19E-05
Psed_1978	Polyamine-transporting ATPase	-0.20	0.35	0.01	-1.38	1.33E-06	1.19E-05
Psed_6600	acyl-CoA dehydrogenase domain-containing protein	2.00	1.70	4.20	1.61	1.33E-06	1.19E-05
Psed_0951	hypothetical protein	-0.39	-1.74	-1.65	-0.30	1.34E-06	1.19E-05
Psed_2430	3-oxoacyl-[acyl-carrier-protein] reductase	-1.02	-0.91	-0.23	-1.05	1.37E-06	1.22E-05
Psed_4983	Aminocarboxymuconate-semialdehyde decarboxylase	-1.26	-0.60	-0.02	-1.45	1.38E-06	1.22E-05
Psed_5766	hypothetical protein	0.11	0.80	0.53	1.43	1.38E-06	1.23E-05
Psed_3967	GCN5-related N-acetyltransferase	-0.45	1.05	-0.29	-0.83	1.39E-06	1.23E-05
Psed_4754	nitrogen regulatory protein P-II	0.60	2.17	0.41	1.34	1.41E-06	1.25E-05
Psed_1420	hypothetical protein	-1.03	0.17	0.31	-1.05	1.47E-06	1.30E-05
Psed_3541	aminodeoxychorismate lyase	-0.28	0.15	0.02	-1.02	1.48E-06	1.30E-05
Psed_4003	hypothetical protein	-0.88	-1.62	-1.26	-0.97	1.49E-06	1.30E-05
Psed_7015	hypothetical protein	-0.56	-1.12	-0.48	-0.02	1.49E-06	1.30E-05
Psed_1727	RNA polymerase sigma factor, sigma-70 family	-0.64	-0.88	-1.07	-2.50	1.49E-06	1.30E-05
Psed_5289	lipolytic protein G-D-S-L family	-0.27	0.04	1.07	0.02	1.49E-06	1.30E-05

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_0327	Ku protein	-0.24	-0.19	1.22	-0.20	1.49E-06	1.30E-05
Psed_4958	hypothetical protein	0.22	0.33	1.71	2.13	1.50E-06	1.31E-05
Psed_6609	Pyruvate dehydrogenase (acetyl-transferring)	-1.00	0.07	0.74	-0.96	1.52E-06	1.32E-05
Psed_1842	HNH endonuclease	-0.88	-0.46	-0.61	-1.33	1.56E-06	1.35E-05
Psed_1912	hypothetical protein	-0.91	-0.39	-0.13	-1.23	1.56E-06	1.35E-05
Psed_2867	Prolipoprotein diacylglyceryl transferase	-0.22	-0.01	0.38	-1.01	1.58E-06	1.37E-05
Psed_1521	Carboxymuconolactone decarboxylase	-0.32	-0.10	1.98	-0.68	1.60E-06	1.38E-05
Psed_6105	hypothetical protein	-0.15	-0.84	-1.24	-0.51	1.61E-06	1.39E-05
Psed_0138	copper resistance protein CopC	-2.67	-2.59	-2.83	-1.36	1.69E-06	1.45E-05
Psed_6362	Cytochrome-c3 hydrogenase	0.37	0.72	1.92	1.02	1.72E-06	1.47E-05
Psed_1052	Glycerol-3-phosphate-transporting ATPase	-1.54	-1.90	0.41	-0.24	1.75E-06	1.49E-05
Psed_4532	band 7 protein	-1.41	-0.87	-2.38	-2.38	1.78E-06	1.51E-05
Psed_4701	Cyclopropane-fatty-acyl-phospholipid synthase	0.01	-0.03	1.27	3.38	1.78E-06	1.52E-05
Psed_6301	dienelactone hydrolase	-2.00	-2.27	-1.23	-1.75	1.79E-06	1.52E-05
Psed_3863	hypothetical protein	-1.39	-2.23	-1.39	-0.95	1.80E-06	1.52E-05
Psed_5870	hypothetical protein	-0.97	-0.54	-1.75	-0.68	1.80E-06	1.52E-05
Psed_2906	hypothetical protein	-0.27	-1.20	0.38	-0.64	1.82E-06	1.54E-05
Psed_3784	NAD(+) synthase (glutamine-hydrolyzing)	-0.52	-0.42	-0.16	-1.18	1.86E-06	1.57E-05
Psed_5778	cell divisionFtsK/SpoIIIE	0.08	0.14	0.12	1.33	1.89E-06	1.59E-05
Psed_5736	Transglycosylase-like domain protein	1.41	2.98	0.94	2.30	1.90E-06	1.59E-05
Psed_2871	OsmC family protein	-0.32	-1.09	-0.81	-0.93	1.90E-06	1.60E-05
Psed_0388	Acetylcholinesterase	0.41	1.56	-0.45	-0.38	1.93E-06	1.61E-05
Psed_1198	DoxX family protein	-0.85	-0.95	-0.75	-2.38	1.93E-06	1.61E-05
Psed_0130	amidohydrolase 2	0.12	-0.31	0.83	2.17	1.97E-06	1.64E-05
Psed_1966	Formyl-CoA transferase	-0.71	-0.42	-1.11	-1.08	1.99E-06	1.66E-05
Psed_6572	UDP-N-acetylglucosamine 2-epimerase	0.11	0.36	1.07	0.15	2.04E-06	1.69E-05

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_4601	regulatory protein TetR	0.12	1.00	-0.00	0.15	2.08E-06	1.73E-05
Psed_5335	ATP-dependent DNA helicase PcrA	-0.63	-0.23	-0.36	-1.25	2.08E-06	1.73E-05
Psed_3341	putative F420-dependent oxidoreductase	-0.69	-0.75	-0.46	-1.20	2.09E-06	1.73E-05
Psed_3124	Methylmalonyl-CoA mutase	-0.57	-0.89	-1.46	-1.10	2.14E-06	1.77E-05
Psed_3414	hypothetical protein	-0.18	0.29	1.06	-0.61	2.14E-06	1.77E-05
Psed_5773	hypothetical protein	-0.19	0.87	1.21	0.79	2.15E-06	1.77E-05
Psed_2318	regulatory protein MerR	-2.00	-1.92	-1.83	-2.40	2.18E-06	1.79E-05
Psed_1001	ABC-type transporter, periplasmic subunit	-0.14	-0.32	0.22	-1.12	2.18E-06	1.79E-05
Psed_0106	putative Fe-S oxidoreductase	0.69	-1.07	-1.96	-0.37	2.24E-06	1.82E-05
Psed_1450	nitrile hydratase b-subunit	-0.21	-0.21	1.00	0.59	2.29E-06	1.85E-05
Psed_4702	protein of unknown function DUF1295	-0.00	-0.02	0.52	3.20	2.29E-06	1.85E-05
Psed_4321	hypothetical protein	-0.15	-0.44	-0.63	1.55	2.30E-06	1.86E-05
Psed_3670	Butyryl-CoA dehydrogenase	-3.27	-4.05	-5.33	-4.90	2.31E-06	1.86E-05
Psed_0139	nuclear export factor GLE1	-2.76	-3.16	-3.23	-1.61	2.32E-06	1.87E-05
Psed_4712	1-deoxy-D-xylulose 5-phosphate reductoisomerase	-0.77	-0.23	-0.70	-1.28	2.38E-06	1.91E-05
Psed_5765	hypothetical protein	0.14	0.39	0.21	1.01	2.38E-06	1.91E-05
Psed_6612	ABC-type transporter, periplasmic subunit family 3	-0.89	-0.82	-0.40	-1.66	2.40E-06	1.92E-05
Psed_1623	argininosuccinate lyase	-0.65	-0.35	-0.15	-1.07	2.45E-06	1.96E-05
Psed_4562	amino acid permease-associated region	-0.28	-0.57	-0.75	-1.05	2.45E-06	1.96E-05
Psed_0382	hypothetical protein	-1.39	0.44	0.84	1.16	2.46E-06	1.96E-05
Psed_3108	hypothetical protein	-0.55	2.47	0.88	-0.57	2.47E-06	1.96E-05
Psed_4753	nitrogen regulatory protein P-II	0.38	1.47	0.06	1.15	2.56E-06	2.03E-05
Psed_1182	acyl-CoA dehydrogenase domain-containing protein	0.57	0.86	2.29	0.36	2.56E-06	2.03E-05

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_6857	protein of unknown function DUF156	0.63	1.76	0.72	1.03	2.66E-06	2.09E-05
Psed_6095	Fe(3+)-transporting ATPase	-0.58	-3.26	-2.70	-0.90	2.67E-06	2.10E-05
Psed_5875	2-nitropropane dioxygenase NPD	-0.37	-0.85	0.05	-1.04	2.69E-06	2.11E-05
Psed_6996	hypothetical protein	0.69	0.02	1.03	0.88	2.70E-06	2.12E-05
Psed_0385	Radical SAM domain protein	-0.52	0.95	1.14	0.43	2.72E-06	2.13E-05
Psed_1689	UDP-N-acetylglucosamine 1-carboxyvinyl-transferase	-0.81	-0.48	-0.32	-1.46	2.75E-06	2.15E-05
Psed_2612	Uncharacterised conserved protein UCP007542	0.12	0.04	1.13	0.20	2.81E-06	2.19E-05
Psed_1982	hypothetical protein	-0.31	0.40	1.75	1.59	2.82E-06	2.19E-05
Psed_3698	Phthalate 4,5-dioxygenase	-0.82	-0.89	1.01	-0.47	2.84E-06	2.20E-05
Psed_5473	Linalool 8-monooxygenase	0.10	0.28	1.87	-0.31	2.84E-06	2.20E-05
Psed_5568	NAD(P)H-quinone oxidoreductase subunit K	-0.95	-0.01	-1.08	-1.53	2.91E-06	2.24E-05
Psed_3388	ABC transporter related	0.59	1.18	0.22	-0.55	2.92E-06	2.25E-05
Psed_1050	ABC-type transporter, integral membrane subunit	-1.58	-1.90	0.54	-0.57	2.93E-06	2.25E-05
Psed_5856	alkylhydroperoxidase like protein, AhpD family	-0.89	-1.12	-0.78	-1.02	2.93E-06	2.25E-05
Psed_3671	GntR domain protein	-0.57	-0.51	-0.26	-1.01	2.93E-06	2.25E-05
Psed_1686	ATP synthase epsilon chain	-1.22	-0.55	-0.06	-1.40	2.97E-06	2.28E-05
Psed_6500	Endoribonuclease L-PSP	-0.49	-0.02	-0.44	-1.34	2.98E-06	2.28E-05
Psed_2809	cell division protein FtsZ	-1.03	-2.25	-0.72	-1.35	2.99E-06	2.29E-05
Psed_6973	hypothetical protein	1.53	-0.56	0.02	0.92	3.02E-06	2.31E-05
Psed_4176	GntR domain protein	-1.61	-1.82	-1.88	-2.14	3.05E-06	2.32E-05
Psed_2845	Quinolinate synthase A	-0.70	-1.19	-2.37	-1.48	3.07E-06	2.33E-05
Psed_3963	polar amino acid ABC transporter, inner membrane subunit	-0.37	-0.94	0.18	-1.40	3.09E-06	2.34E-05
Psed_3875	Extracellular ligand-binding receptor	-0.18	-0.94	-0.12	-1.70	3.10E-06	2.35E-05

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_2080	GCN5-related N-acetyltransferase	-0.12	1.54	-0.14	-0.56	3.13E-06	2.37E-05
Psed_4662	processing peptidase	0.10	1.14	1.03	-0.35	3.17E-06	2.40E-05
Psed_0541	Ethyl tert-butyl ether degradation EthD	-0.80	-0.94	0.05	-1.01	3.19E-06	2.40E-05
Psed_6172	MaoC domain protein dehydratase	0.60	0.10	3.72	-0.47	3.20E-06	2.41E-05
Psed_0166	Prephenate dehydratase	-0.68	-0.63	-0.79	-1.12	3.31E-06	2.48E-05
Psed_6951	hypothetical protein	0.35	-0.71	-1.04	0.26	3.33E-06	2.49E-05
Psed_2371	ABC-type transporter, periplasmic subunit	1.05	1.47	-0.80	0.14	3.35E-06	2.50E-05
Psed_2030	Linalool 8-monooxygenase	1.09	1.29	1.78	0.03	3.36E-06	2.50E-05
Psed_6854	protein of unknown function DUF81	0.50	1.22	-0.16	0.39	3.43E-06	2.55E-05
Psed_3668	hypothetical protein	-1.20	-2.01	-0.44	-0.59	3.57E-06	2.64E-05
Psed_6807	hypothetical protein	0.64	0.06	-0.11	2.79	3.59E-06	2.65E-05
Psed_5805	hypothetical protein	0.19	1.35	1.62	2.72	3.61E-06	2.66E-05
Psed_5176	hypothetical protein	-0.39	-0.52	0.66	1.61	3.63E-06	2.67E-05
Psed_3918	FAD linked oxidase domain protein	1.37	1.55	5.31	2.54	3.68E-06	2.71E-05
Psed_2132	hypothetical protein	-1.01	-2.12	-0.28	-0.95	3.70E-06	2.71E-05
Psed_2799	Ribosomal RNA small subunit methyltransferase H	-0.62	-1.85	-0.43	-1.48	3.70E-06	2.71E-05
Psed_2846	L-aspartate oxidase	-0.22	-0.60	-1.45	-0.60	3.76E-06	2.75E-05
Psed_6114	FAD-binding 9 siderophore-interacting domain protein	-0.21	-1.57	-0.02	-0.13	3.80E-06	2.78E-05
Psed_2823	Abortive infection protein	0.29	1.03	0.41	0.28	3.80E-06	2.78E-05
Psed_5485	phenylacetic acid degradation-related protein	-0.83	-0.96	-1.14	-0.96	3.81E-06	2.78E-05
Psed_3678	squalene-associated FAD-dependent desaturase	-0.25	-1.37	-0.72	-1.13	3.85E-06	2.80E-05
Psed_2913	ABC-type transporter, integral membrane subunit	-0.13	-1.49	0.31	-0.12	3.89E-06	2.83E-05
Psed_6591	Heat shock protein 70	-0.20	0.30	1.99	0.26	3.93E-06	2.86E-05

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_5781	hypothetical protein	-1.17	0.14	0.81	1.29	3.97E-06	2.87E-05
Psed_2039	RNA polymerase sigma factor, sigma-70 family	0.09	0.66	1.46	1.65	3.98E-06	2.87E-05
Psed_3163	Glycine dehydrogenase [decarboxylating]	-0.34	-0.06	-1.44	-1.00	3.97E-06	2.87E-05
Psed_3044	hypothetical protein	0.64	-0.26	1.68	0.46	3.97E-06	2.87E-05
Psed_1791	fatty acid desaturase	-0.39	0.82	1.90	-0.17	3.98E-06	2.87E-05
Psed_6753	hypothetical protein	1.77	-0.06	-1.23	0.89	3.99E-06	2.88E-05
Psed_6441	beta-lactamase	-1.26	-2.51	0.01	0.20	4.12E-06	2.96E-05
Psed_6636	Nitric-oxide synthase	-0.72	0.48	1.30	-0.49	4.15E-06	2.97E-05
Psed_5925	cation diffusion facilitator family transporter	-0.67	-1.18	0.36	-0.28	4.16E-06	2.98E-05
Psed_6542	hypothetical protein	-0.91	-1.28	0.04	-0.84	4.25E-06	3.03E-05
Psed_2385	hypothetical protein	0.26	1.97	0.53	0.04	4.32E-06	3.07E-05
Psed_3699	Dimethylmenaquinone methyltransferase	-0.14	-0.18	1.14	0.08	4.32E-06	3.07E-05
Psed_3941	Glyoxalase/bleomycin resistance protein/dioxy-genase	-1.59	-2.61	-0.99	-0.63	4.35E-06	3.09E-05
Psed_2888	1-deoxy-D-xylulose-5-phosphate synthase	-1.04	-0.90	0.11	-1.46	4.39E-06	3.11E-05
Psed_4547	asparagine synthase (glutamine-hydrolyzing)	-0.28	0.02	-0.47	-1.13	4.40E-06	3.11E-05
Psed_3376	hypothetical protein	0.14	-0.09	3.00	0.63	4.49E-06	3.18E-05
Psed_1564	4-hydroxybenzoate 3-monooxygenase	0.60	0.43	3.90	-0.13	4.51E-06	3.19E-05
Psed_4110	MmgE/PrpD family protein	-1.29	-1.75	-1.83	-2.01	4.52E-06	3.19E-05
Psed_2329	Peroxidase	-0.67	-2.49	-0.98	-1.09	4.62E-06	3.25E-05
Psed_0958	Fumarate hydratase class II	-1.05	-1.19	-1.50	-1.07	4.66E-06	3.27E-05
Psed_3488	Sulfate transporter/antisigma-factor antagonist STAS	0.50	0.33	1.79	0.81	4.67E-06	3.27E-05
Psed_6202	pyrimidine utilization protein A	-0.19	-1.84	-0.69	0.21	4.69E-06	3.28E-05
Psed_3109	hypothetical protein	0.02	2.44	0.89	0.59	4.70E-06	3.28E-05
Psed_1115	hypothetical protein	-0.65	0.12	-0.32	-1.21	4.74E-06	3.31E-05

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_5760	hypothetical protein	-0.09	1.13	0.26	2.22	4.88E-06	3.40E-05
Psed_6169	Glyoxalase/bleomycin resistance protein/dioxy-genase	-0.44	-1.00	-1.04	-0.24	5.13E-06	3.55E-05
Psed_0140	hypothetical protein	-1.85	-1.87	-2.57	-1.44	5.17E-06	3.57E-05
Psed_3666	regulatory protein DeoR	-0.86	-1.66	-0.26	-0.93	5.17E-06	3.57E-05
Psed_5624	Long-chain-fatty-acid--CoA ligase	-0.87	-0.74	-0.29	-1.41	5.21E-06	3.58E-05
Psed_1184	transcriptional regulator PadR family protein	-0.55	0.28	1.73	0.15	5.28E-06	3.63E-05
Psed_1348	GntR domain protein	-0.95	-0.94	-1.02	-0.97	5.29E-06	3.63E-05
Psed_2847	nicotinate-nucleotide pyrophosphorylase	-0.03	-0.32	-0.24	-1.00	5.35E-06	3.66E-05
Psed_5186	NUDIX hydrolase	-1.57	0.71	-1.10	-0.61	5.40E-06	3.69E-05
Psed_2798	Protein mraZ	-0.29	-1.14	0.25	-0.55	5.41E-06	3.69E-05
Psed_2777	hypothetical protein	-0.27	0.60	1.82	-0.47	5.39E-06	3.69E-05
Psed_3679	Dimethylallyltranstransferase	-0.14	-2.00	-1.24	-1.90	5.56E-06	3.78E-05
Psed_4085	Glyoxalase/bleomycin resistance protein/dioxy-genase	-0.67	-1.08	-0.69	-0.86	5.57E-06	3.78E-05
Psed_5881	Heme oxygenase	-0.10	-1.43	0.19	-0.49	5.71E-06	3.85E-05
Psed_4421	alpha/beta hydrolase fold	-0.14	-0.13	1.06	-0.13	5.81E-06	3.91E-05
Psed_6170	regulatory protein MerR	0.43	-0.31	-1.04	-0.59	5.97E-06	3.99E-05
Psed_3652	hypothetical protein	0.15	-0.37	-0.25	1.52	6.00E-06	4.01E-05
Psed_1587	Long-chain-fatty-acid--CoA ligase	0.13	-0.15	1.05	-0.06	6.04E-06	4.02E-05
Psed_6425	hypothetical protein	-0.42	0.60	1.51	0.65	6.12E-06	4.07E-05
Psed_0537	short-chain dehydrogenase/reductase SDR	-0.79	-1.41	0.68	-0.86	6.20E-06	4.11E-05
Psed_4807	ATP-binding cassette protein, ChvD family	-0.63	0.01	0.10	-1.29	6.29E-06	4.17E-05
Psed_1024	protein of unknown function DUF1470	-1.08	-1.39	-1.30	-1.37	6.35E-06	4.20E-05
Psed_4703	alkyl hydroperoxide reductase/ Thiol specific antioxidant/ Mal allergen	0.19	0.02	1.13	2.96	6.40E-06	4.22E-05
Psed_1633	hypothetical protein	-1.26	-1.53	-0.19	-1.58	6.50E-06	4.27E-05

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_2721	hypothetical protein	-0.88	-0.51	-0.49	-1.34	6.50E-06	4.27E-05
Psed_3917	nitrile hydratase, alpha subunit	-0.72	-0.51	-0.43	-1.13	6.56E-06	4.30E-05
Psed_5759	hypothetical protein	0.10	1.08	0.45	2.39	6.76E-06	4.42E-05
Psed_0001	Chromosomal replication initiator protein dnaA	-0.07	0.55	1.08	0.11	6.78E-06	4.43E-05
Psed_6893	hypothetical protein	-0.63	-1.67	-1.04	-0.64	6.84E-06	4.46E-05
Psed_6616	hypothetical protein	-0.89	-0.58	0.35	-1.01	6.88E-06	4.48E-05
Psed_5570	geranylgeranyl reductase	-0.77	-0.26	-0.88	-1.47	6.99E-06	4.53E-05
Psed_1663	diaminopimelate decarboxylase	-0.26	0.39	0.09	-1.04	7.08E-06	4.57E-05
Psed_5111	o-succinylbenzoate--CoA ligase	-1.85	-1.21	0.32	-0.85	7.11E-06	4.59E-05
Psed_5697	Carbon-monoxide dehydrogenase (acceptor)	-0.40	-0.46	0.65	-1.05	7.19E-06	4.63E-05
Psed_4861	cell shape determining protein MreB/Mrl	-2.05	-0.35	-0.40	-1.73	7.23E-06	4.65E-05
Psed_1713	crotonyl-CoA reductase	-0.35	-1.62	-0.05	-2.07	7.32E-06	4.70E-05
Psed_6813	hypothetical protein	0.15	1.14	0.54	0.90	7.35E-06	4.71E-05
Psed_4949	Hydroxypyruvate isomerase	0.39	0.24	1.04	-0.05	7.45E-06	4.76E-05
Psed_1658	Trimethylamine-N-oxide reductase (cytochrome c)	2.43	-0.74	-0.45	0.21	7.47E-06	4.77E-05
Psed_1185	Peptide chain release factor 2	-0.59	0.13	0.33	-1.25	7.51E-06	4.79E-05
Psed_0129	protein of unknown function DUF59	0.10	-0.19	0.67	1.62	7.52E-06	4.79E-05
Psed_3937	hypothetical protein	-1.00	-1.61	-1.22	-1.01	7.55E-06	4.81E-05
Psed_4172	ABC-type sugar transport system periplasmic component-like	-0.93	-0.98	-1.10	-1.00	7.58E-06	4.82E-05
Psed_2434	GAF domain protein	-1.82	-0.29	-0.80	-1.40	8.02E-06	5.06E-05
Psed_1519	Ribonuclease PH	-0.56	-0.16	-1.07	-1.17	8.03E-06	5.06E-05
Psed_6606	hypothetical protein	-0.45	-0.34	-1.52	-0.37	8.04E-06	5.06E-05
Psed_6852	Protein of unknown function DUF2078, membrane	0.42	1.18	0.04	0.49	8.36E-06	5.22E-05

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_1149	ThiJ/PfpI domain-containing protein	0.62	0.62	1.55	0.92	8.39E-06	5.24E-05
Psed_5488	FAD-dependent pyridine nucleotide-disulphide oxidoreductase	-0.92	-1.63	-1.84	-0.77	8.49E-06	5.29E-05
Psed_5080	putative transcriptional regulator	-1.05	-1.26	-0.02	-0.80	8.70E-06	5.41E-05
Psed_1003	ABC-type transporter, integral membrane subunit	-0.43	-0.01	-0.51	-1.16	8.75E-06	5.44E-05
Psed_2295	Domain of unknown function DUF1918	-2.83	-0.29	-1.06	-1.33	8.91E-06	5.52E-05
Psed_0301	Alcohol dehydrogenase	-0.86	-0.66	-0.78	-1.11	9.08E-06	5.61E-05
Psed_1899	Aldehyde Dehydrogenase	-1.23	-1.00	-0.56	-0.77	9.20E-06	5.65E-05
Psed_6309	hypothetical protein	-0.17	-0.05	1.03	0.40	9.19E-06	5.65E-05
Psed_5608	ResB family protein	-0.91	-0.27	-1.03	-1.91	9.22E-06	5.65E-05
Psed_6803	hypothetical protein	0.07	-0.81	0.25	-1.95	9.22E-06	5.65E-05
Psed_6740	hypothetical protein	1.12	0.68	1.57	0.97	9.38E-06	5.74E-05
Psed_6929	oxidoreductase domain protein	-0.45	-1.31	-0.17	-0.71	9.38E-06	5.74E-05
Psed_1993	ATP-dependent DNA helicase, RecQ family	-0.95	-1.34	-1.10	-1.13	9.46E-06	5.78E-05
Psed_1494	LmbE family protein	-0.86	-0.65	-0.75	-1.72	9.48E-06	5.78E-05
Psed_2725	Cobyrinic acid A,C-diamide synthase	-1.08	-0.65	-0.61	-0.80	9.49E-06	5.78E-05
Psed_1076	short-chain dehydrogenase/reductase SDR	1.35	0.79	1.99	0.28	9.53E-06	5.80E-05
Psed_4698	amine oxidase	0.03	0.33	1.57	2.78	9.55E-06	5.80E-05
Psed_3996	Methionyl-tRNA formyltransferase	0.93	-1.88	0.80	-0.19	9.67E-06	5.86E-05
Psed_4709	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase	-1.26	-0.91	-0.46	-1.09	9.72E-06	5.88E-05
Psed_2289	hypothetical protein	-2.42	-0.48	-0.59	-1.45	9.75E-06	5.90E-05
Psed_6185	drug resistance transporter, Bcr/CflA subfamily	0.09	-0.34	-1.16	-0.42	9.83E-06	5.93E-05
Psed_0332	Amidase	-0.80	-0.02	-0.59	-1.03	9.83E-06	5.93E-05
Psed_5246	uncharacterized peroxidase-related enzyme	0.14	0.08	0.01	1.64	1.02E-05	6.12E-05

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_0185	UDP-galactopyranose mutase	-0.73	-0.10	-0.19	-1.35	1.03E-05	6.14E-05
Psed_1095	alpha/beta hydrolase fold	-0.82	-0.47	-0.24	-1.25	1.03E-05	6.14E-05
Psed_4937	hypothetical protein	0.15	0.18	-0.61	1.06	1.03E-05	6.15E-05
Psed_0233	putative transglycosylase associated protein	-0.84	-1.14	-0.11	-1.18	1.03E-05	6.15E-05
Psed_3375	NLP/P60 protein	-0.67	-1.72	-0.61	-0.76	1.05E-05	6.27E-05
Psed_6176	Acyl-CoA oxidase	1.16	0.63	3.80	-0.23	1.06E-05	6.32E-05
Psed_5899	hypothetical protein	-0.82	-0.32	-1.23	-1.12	1.07E-05	6.34E-05
Psed_3405	Protoheme IX farnesyltransferase	-0.44	0.11	-0.99	-1.22	1.07E-05	6.34E-05
Psed_1037	Asp/Glu/hydantoin racemase	0.46	0.19	-1.02	0.07	1.09E-05	6.44E-05
Psed_5187	Adenosylhomocysteinase	-1.63	0.27	-1.18	-0.25	1.10E-05	6.50E-05
Psed_6174	acetyl-CoA acetyltransferase	1.27	0.95	3.21	-0.15	1.12E-05	6.57E-05
Psed_4597	Protein recA	-0.80	-1.23	-0.81	-0.77	1.13E-05	6.60E-05
Psed_3602	small GTP-binding protein	-2.04	-1.39	0.32	-1.18	1.13E-05	6.62E-05
Psed_0284	pyridoxamine 5'-phosphate oxidase-related FMN-binding	-0.77	-0.92	-0.62	-1.19	1.13E-05	6.63E-05
Psed_3136	hydrolase	-0.61	-1.06	-0.36	-0.88	1.14E-05	6.67E-05
Psed_6554	Protein grpE	-0.03	1.02	-1.54	-0.81	1.16E-05	6.78E-05
Psed_5404	NAD(P)(+) transhydrogenase (AB-specific)	1.02	1.18	1.28	1.29	1.17E-05	6.79E-05
Psed_1559	response regulator receiver	0.22	0.48	2.97	1.31	1.17E-05	6.82E-05
Psed_5567	NAD(P)H-quinone oxidoreductase subunit J	-1.03	-0.08	-1.07	-1.77	1.19E-05	6.88E-05
Psed_3919	regulatory protein GntR HTH	-0.38	-0.52	1.18	-0.28	1.22E-05	7.03E-05
Psed_6261	ABC-type transporter, integral membrane subunit	1.31	-0.01	0.02	0.18	1.23E-05	7.10E-05
Psed_5757	hypothetical protein	-0.09	0.73	0.18	1.79	1.24E-05	7.14E-05
Psed_3832	Ornithine carbamoyltransferase	-0.58	-0.63	0.10	-1.82	1.25E-05	7.21E-05
Psed_1453	Amidase	-0.82	-0.65	1.19	-0.35	1.27E-05	7.27E-05

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_4514	hypothetical protein	-1.00	-0.94	0.33	-0.45	1.27E-05	7.27E-05
Psed_6031	response regulator receiver	0.26	0.84	2.41	0.84	1.27E-05	7.27E-05
Psed_2869	pyruvate kinase	0.76	1.69	1.23	-0.20	1.29E-05	7.34E-05
Psed_0580	highly repetitive protein	0.13	-1.54	-1.37	0.19	1.29E-05	7.34E-05
Psed_1913	hypothetical protein	-0.22	-0.04	1.09	0.14	1.32E-05	7.52E-05
Psed_1051	ABC-type transporter, integral membrane subunit	-1.70	-2.03	0.13	-0.10	1.32E-05	7.52E-05
Psed_1107	peptidase C60 sortase A and B	0.29	-0.08	-1.38	0.30	1.36E-05	7.72E-05
Psed_0950	hypothetical protein	-0.50	-1.84	-1.94	0.45	1.37E-05	7.73E-05
Psed_2902	hypothetical protein	-0.76	-1.26	-0.48	-0.94	1.37E-05	7.74E-05
Psed_1513	Glutamate racemase	-0.49	-0.09	0.09	-1.08	1.39E-05	7.82E-05
Psed_4014	exodeoxyribonuclease V, gamma subunit	-0.53	-1.00	-0.71	-1.05	1.41E-05	7.90E-05
Psed_6552	regulatory protein MerR	-0.07	1.58	-0.47	-0.09	1.41E-05	7.92E-05
Psed_3521	hypothetical protein	-0.34	-0.13	0.54	-1.18	1.45E-05	8.12E-05
Psed_2129	DNA binding domain protein, excisionase family	0.32	-0.52	-1.36	0.32	1.48E-05	8.21E-05
Psed_1077	hypothetical protein	0.89	0.22	2.88	0.02	1.50E-05	8.30E-05
Psed_6728	Amidase	1.37	0.07	0.34	0.75	1.51E-05	8.34E-05
Psed_1492	Alcohol dehydrogenase zinc-binding domain protein	-1.40	-1.10	0.71	-0.31	1.54E-05	8.49E-05
Psed_5652	Long-chain-fatty-acid--CoA ligase	-0.20	-0.18	1.00	0.13	1.55E-05	8.52E-05
Psed_3176	helix-turn-helix HxlR type	-0.70	-0.56	-0.27	-1.12	1.55E-05	8.52E-05
Psed_5541	helix-turn-helix domain-containing protein AraC type	-0.93	-1.04	-0.63	-0.54	1.56E-05	8.56E-05
Psed_5752	hypothetical protein	0.08	0.58	0.32	1.04	1.57E-05	8.62E-05
Psed_6565	protein of unknown function DUF224 cysteine-rich region domain protein	-1.39	-1.15	-0.32	-1.47	1.57E-05	8.62E-05

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_0567	hypothetical protein	-0.33	-0.31	1.10	0.75	1.59E-05	8.67E-05
Psed_6241	hypothetical protein	1.05	0.07	-0.14	-0.23	1.60E-05	8.75E-05
Psed_3564	Aromatic-amino-acid transaminase	1.55	0.32	0.47	1.66	1.68E-05	9.10E-05
Psed_3971	glutamate--cysteine ligase GCS2	0.42	-1.00	0.69	-0.12	1.68E-05	9.12E-05
Psed_5616	Porphobilinogen synthase	-1.05	-0.61	-0.75	-0.94	1.70E-05	9.19E-05
Psed_1721	hypothetical protein	-0.57	-1.17	-0.94	-0.50	1.72E-05	9.28E-05
Psed_0391	metallophosphoesterase	-0.54	0.08	-0.23	-1.70	1.74E-05	9.38E-05
Psed_6304	FAD-dependent pyridine nucleotide-disulphide oxidoreductase	-1.53	-0.61	0.44	-0.88	1.75E-05	9.41E-05
Psed_5135	malic protein NAD-binding	1.40	0.47	1.97	1.23	1.75E-05	9.41E-05
Psed_5245	regulatory protein TetR	-0.13	-0.10	-0.15	1.13	1.78E-05	9.53E-05
Psed_6286	hypothetical protein	-0.31	0.19	0.21	-1.33	1.80E-05	9.64E-05
Psed_2122	LexA repressor	-1.16	-2.20	-1.71	-1.05	1.82E-05	9.76E-05
Psed_3594	Tellurite resistance TerB	-2.11	-1.52	-2.67	-1.40	1.85E-05	9.85E-05
Psed_6262	ABC-type transporter, integral membrane sub-unit	1.20	-0.00	0.16	0.09	1.89E-05	1.00E-04
Psed_5868	hypothetical protein	-0.20	-0.05	-1.82	0.16	1.89E-05	1.00E-04
Psed_5763	hypothetical protein	0.05	1.19	0.35	1.35	1.91E-05	1.01E-04
Psed_6578	hypothetical protein	-0.64	-1.14	-0.57	-0.47	1.94E-05	1.02E-04
Psed_6732	MaoC domain protein dehydratase	1.19	0.00	-0.45	0.83	1.95E-05	1.03E-04
Psed_2321	ABC-type sugar transport system periplasmic component-like	0.81	1.10	-0.29	1.22	1.95E-05	1.03E-04
Psed_6601	CBS domain containing protein	-0.52	-0.40	1.08	0.21	1.97E-05	1.04E-04
Psed_4697	hypothetical protein	-0.32	0.80	2.31	2.52	1.97E-05	1.04E-04
Psed_6173	3-oxoacyl-[acyl-carrier-protein] reductase	0.89	0.33	3.49	-1.07	2.02E-05	1.06E-04
Psed_5914	Hydantoinase B/oxoprolinase	-0.03	-0.59	1.04	0.00	2.02E-05	1.06E-04

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_5113	putative F420-dependent oxidoreductase	-0.25	0.02	1.01	-0.47	2.07E-05	1.08E-04
Psed_6730	Luciferase-like, subgroup	1.63	-0.39	-0.26	0.93	2.09E-05	1.09E-04
Psed_0207	Teichoic-acid-transporting ATPase	-0.81	-0.07	0.16	-1.27	2.10E-05	1.09E-04
Psed_1451	nitrile hydratase, alpha subunit	-0.66	-0.57	1.27	-0.29	2.11E-05	1.09E-04
Psed_4873	Domain of unknown function DUF1876	-3.05	-0.01	-1.23	-1.65	2.12E-05	1.10E-04
Psed_1790	ferredoxin	-0.62	0.54	1.82	-0.71	2.15E-05	1.11E-04
Psed_2717	Butyrate--CoA ligase	-0.81	-1.29	-0.35	-0.73	2.17E-05	1.12E-04
Psed_3337	peptidase M48 Ste24p	-0.09	0.06	-0.63	-1.19	2.17E-05	1.12E-04
Psed_2941	regulatory protein MarR	-1.63	-0.92	-0.68	-1.37	2.20E-05	1.13E-04
Psed_0389	hypothetical protein	-1.41	1.08	-0.45	-1.52	2.20E-05	1.13E-04
Psed_4513	cobalamin (vitamin B12) biosynthesis CbiX protein	1.24	1.25	0.22	0.23	2.21E-05	1.13E-04
Psed_2372	ABC-type transporter, integral membrane subunit	0.84	1.66	-0.70	0.23	2.23E-05	1.14E-04
Psed_3114	hypothetical protein	0.14	0.39	1.02	0.84	2.29E-05	1.17E-04
Psed_3111	Hemerythrin HHE cation binding domain protein	0.06	0.55	1.45	1.67	2.31E-05	1.18E-04
Psed_1872	glycosyl transferase group 1	-0.70	-0.46	-0.55	-1.34	2.31E-05	1.18E-04
Psed_6641	ABC-type transporter, integral membrane subunit	-1.42	-1.07	-2.65	-1.56	2.36E-05	1.19E-04
Psed_1590	Formyl-CoA transferase	-0.25	-0.26	1.24	-0.39	2.36E-05	1.20E-04
Psed_2800	penicillin-binding protein transpeptidase	-0.71	-1.95	-0.47	-0.67	2.38E-05	1.20E-04
Psed_6610	pyruvate dehydrogenase (acetyl-transferring) E1 component, alpha subunit	-0.89	0.01	1.15	-1.04	2.38E-05	1.20E-04
Psed_3651	hypothetical protein	0.48	-0.47	-1.22	-1.03	2.38E-05	1.20E-04
Psed_0381	hypothetical protein	-0.61	0.42	1.18	-0.13	2.40E-05	1.21E-04
Psed_3840	tRNA/rRNA methyltransferase (SpoU)	0.77	1.50	1.06	-0.24	2.40E-05	1.21E-04

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_6693	hypothetical protein	-0.20	-1.16	-0.18	-0.68	2.40E-05	1.21E-04
Psed_5344	hypothetical protein	0.00	0.14	1.31	0.25	2.42E-05	1.21E-04
Psed_5369	Alanine racemase	-0.59	-0.38	-0.73	-1.01	2.44E-05	1.22E-04
Psed_5732	Transglycosylase-like domain protein	0.39	0.35	-1.16	-0.38	2.46E-05	1.23E-04
Psed_0982	fibronectin-attachment family protein	0.88	-1.25	-1.17	0.53	2.47E-05	1.23E-04
Psed_6203	Luciferase-like, subgroup	0.10	-1.02	-0.71	-0.50	2.55E-05	1.27E-04
Psed_2143	alkyl hydroperoxide reductase/ Thiol specific antioxidant/ Mal allergen	-1.14	-0.96	0.01	-1.92	2.56E-05	1.28E-04
Psed_1493	ATPase associated with various cellular activities AAA_5	-0.63	-0.24	1.16	0.25	2.60E-05	1.29E-04
Psed_1694	ectoine/hydroxyectoine ABC transporter, permease protein EhuC	0.05	-0.24	-1.46	-0.71	2.60E-05	1.29E-04
Psed_1813	glucose dehydrogenase	-0.28	-0.33	-0.14	-1.03	2.63E-05	1.30E-04
Psed_6721	regulatory protein ArsR	-0.06	-0.07	-1.17	-0.22	2.65E-05	1.31E-04
Psed_3653	hypothetical protein	1.51	-0.02	-0.40	0.23	2.67E-05	1.32E-04
Psed_5314	Bifunctional protein folD	-0.75	-0.53	-0.60	-1.32	2.68E-05	1.32E-04
Psed_2303	Glutamate dehydrogenase (NADP(+))	-0.89	-0.78	-1.30	-0.99	2.73E-05	1.34E-04
Psed_7018	putative partitioning protein ParA	-0.34	-1.48	-0.61	-0.66	2.77E-05	1.36E-04
Psed_2040	putative transmembrane anti-sigma factor	0.14	0.30	1.27	1.03	2.77E-05	1.36E-04
Psed_0081	ABC-type transporter, integral membrane subunit	-0.57	-1.06	-1.38	-0.20	2.81E-05	1.38E-04
Psed_2908	hypothetical protein	-0.82	-1.38	0.42	0.29	2.82E-05	1.38E-04
Psed_2373	ABC-type transporter, integral membrane subunit	0.56	1.16	-0.20	-0.10	2.85E-05	1.39E-04
Psed_2024	aminoglycoside phosphotransferase	-0.37	-0.50	-0.77	-1.13	2.95E-05	1.43E-04
Psed_4109	HpcH/HpaI aldolase	-0.90	-1.24	-1.58	-1.40	2.97E-05	1.44E-04
Psed_3278	LmbE family protein	-1.73	-1.88	-1.29	-1.72	2.99E-05	1.45E-04

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_4843	UspA domain-containing protein	-2.58	-0.04	-1.28	-1.79	3.01E-05	1.45E-04
Psed_4578	Stearoyl-CoA 9-desaturase	0.94	1.78	2.34	1.14	3.01E-05	1.45E-04
Psed_3544	hypothetical protein	-0.60	-0.63	-0.48	-1.72	3.05E-05	1.47E-04
Psed_3071	Dihydroxy-acid dehydratase	-0.95	-0.31	-0.86	-1.01	3.06E-05	1.47E-04
Psed_0375	regulatory protein TetR	-0.69	-1.27	-0.80	-0.97	3.11E-05	1.49E-04
Psed_2929	Protein-tyrosine phosphatase, low molecular weight	0.08	-0.00	-0.29	-1.19	3.23E-05	1.54E-04
Psed_4031	iron-sulfur cluster binding protein	0.98	1.39	0.25	0.58	3.24E-05	1.54E-04
Psed_4067	protein of unknown function DUF1470	-0.92	-1.07	-1.06	-1.21	3.26E-05	1.55E-04
Psed_2437	CBS domain containing protein	-2.83	0.47	-1.72	-2.01	3.29E-05	1.57E-04
Psed_1950	SNARE associated Golgi protein	0.28	0.04	1.17	0.25	3.32E-05	1.58E-04
Psed_2101	S-adenosylmethionine synthase	-0.44	-1.11	-0.24	-0.58	3.32E-05	1.58E-04
Psed_5565	NADH dehydrogenase (quinone)	-1.04	-0.17	-0.91	-1.40	3.34E-05	1.58E-04
Psed_0889	Tat-translocated protein	0.81	-0.42	1.97	0.76	3.35E-05	1.59E-04
Psed_6561	hypothetical protein	0.14	0.71	1.39	0.04	3.45E-05	1.63E-04
Psed_1869	polysaccharide biosynthesis protein CelD	-0.62	-0.27	-0.26	-1.01	3.46E-05	1.63E-04
Psed_5114	Phosphoglycerate mutase	0.20	0.17	1.05	0.00	3.56E-05	1.67E-04
Psed_0180	glycosyl transferase family 2	-0.41	-0.42	0.05	-1.06	3.58E-05	1.68E-04
Psed_4557	hypothetical protein	-0.75	-0.27	-0.34	-1.60	3.59E-05	1.68E-04
Psed_3545	secreted protein	-0.61	-0.70	-0.05	-1.43	3.65E-05	1.70E-04
Psed_4300	nitrate reductase, alpha subunit	-1.90	-0.14	-0.66	-1.21	3.69E-05	1.72E-04
Psed_4872	hypothetical protein	-1.50	0.37	-0.43	-0.31	3.73E-05	1.74E-04
Psed_6259	FMN-dependent oxidoreductase, nitrilotriacetate monooxygenase family	1.51	0.21	0.16	0.41	3.76E-05	1.74E-04
Psed_3101	Acetamidase/Formamidase	-0.74	-1.32	0.25	-0.96	3.76E-05	1.75E-04
Psed_2032	50S ribosomal protein L31 type B	0.31	-2.15	-0.07	0.03	3.77E-05	1.75E-04

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_5563	NADH-quinone oxidoreductase, chain G	-1.14	-0.14	-1.13	-1.49	3.78E-05	1.75E-04
Psed_1731	alpha-glucan phosphorylase	-0.74	-0.12	-0.11	-1.62	3.82E-05	1.76E-04
Psed_4329	hypothetical protein	0.02	-0.02	1.28	-0.20	3.90E-05	1.79E-04
Psed_0012	hypothetical protein	-0.02	-1.48	-0.59	-0.74	3.96E-05	1.81E-04
Psed_2119	GTP-binding proten HflX	-0.60	0.88	-0.11	-1.68	4.00E-05	1.83E-04
Psed_4401	DoxX family protein	-0.03	1.05	-0.21	0.11	4.08E-05	1.86E-04
Psed_4749	signal recognition particle protein	-0.67	0.16	-0.20	-1.20	4.09E-05	1.86E-04
Psed_4657	protein of unknown function DUF1006	-0.91	-1.54	-0.95	-0.77	4.17E-05	1.89E-04
Psed_1772	Electron transfer flavoprotein alpha subunit	-1.85	-1.69	-1.44	-1.51	4.20E-05	1.90E-04
Psed_5264	Thiosulfate sulfurtransferase	-1.20	-0.60	-0.39	-1.32	4.31E-05	1.94E-04
Psed_2862	anthranilate synthase component I	-0.59	0.11	-0.19	-1.00	4.39E-05	1.97E-04
Psed_4483	FAD dependent oxidoreductase	-0.55	-0.17	-0.39	-1.70	4.42E-05	1.98E-04
Psed_3578	RelA/SpoT family protein	-0.69	-0.05	0.27	-1.17	4.45E-05	1.99E-04
Psed_5217	threonine dehydratase	-0.75	0.01	-0.48	-1.12	4.58E-05	2.05E-04
Psed_5520	hypothetical protein	-0.84	1.26	-0.53	-0.57	4.64E-05	2.07E-04
Psed_5701	hypothetical protein	-0.43	-0.62	-1.04	-0.38	4.67E-05	2.08E-04
Psed_2700	phenylacetate-CoA oxygenase, PaaG subunit	-0.96	-1.36	-1.11	-0.55	4.73E-05	2.10E-04
Psed_6861	hypothetical protein	0.00	-0.07	1.22	0.38	4.73E-05	2.10E-04
Psed_4517	Alkanesulfonate monooxygenase	-0.37	-0.87	-1.00	-1.24	4.79E-05	2.12E-04
Psed_5116	hypothetical protein	-0.50	-0.34	0.14	-1.25	4.79E-05	2.12E-04
Psed_4627	YceI family protein	-0.08	0.02	-1.24	-0.06	4.81E-05	2.13E-04
Psed_4756	signal recognition particle-docking protein FtsY	-0.56	-0.00	-0.60	-1.11	4.81E-05	2.13E-04
Psed_1558	protein of unknown function DUF125 trans-membrane	0.15	0.10	1.48	0.52	4.87E-05	2.15E-04
Psed_5180	ribosomal subunit interface protein	-1.49	-0.68	0.28	0.02	4.87E-05	2.15E-04
Psed_3066	hypothetical protein	-0.78	-0.72	-0.46	-1.31	4.90E-05	2.16E-04

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_5621	beta-Ig-H3/fasciclin	-0.45	0.75	1.45	1.66	4.95E-05	2.18E-04
Psed_4083	hypothetical protein	-0.07	-0.26	0.80	1.23	4.95E-05	2.18E-04
Psed_2690	DSBA oxidoreductase	-1.10	-0.96	-0.68	-1.34	4.99E-05	2.19E-04
Psed_6357	hydrogenase accessory protein HypB	-0.14	-0.26	1.16	1.05	5.06E-05	2.21E-04
Psed_5465	Aspartate transaminase	-1.31	-0.57	-0.73	-1.62	5.07E-05	2.21E-04
Psed_1452	Nitrile hydratase	-0.81	-0.73	1.50	-0.27	5.09E-05	2.22E-04
Psed_5110	Xenobiotic-transporting ATPase., Peptide-transporting ATPase	-1.04	-1.03	-0.98	-1.19	5.11E-05	2.22E-04
Psed_6508	hypothetical protein	-1.05	-0.63	-0.56	-1.18	5.25E-05	2.28E-04
Psed_6615	uncharacterized peroxidase-related enzyme	-0.47	-0.07	0.87	-1.33	5.41E-05	2.34E-04
Psed_5185	Thymidylate kinase	-1.28	0.76	-0.10	-0.77	5.44E-05	2.35E-04
Psed_4115	Protein of unknown function DUF2236	-0.19	0.43	1.35	1.14	5.45E-05	2.35E-04
Psed_5375	phosphoglucosamine mutase	0.15	1.05	0.93	-0.56	5.46E-05	2.35E-04
Psed_3370	Enoyl-[acyl-carrier-protein] reductase (NADH)	-0.59	-0.57	-0.75	-1.14	5.53E-05	2.38E-04
Psed_4693	GCN5-related N-acetyltransferase	-0.39	-0.17	1.04	-0.53	5.56E-05	2.39E-04
Psed_0903	hypothetical protein	-0.09	1.73	-0.14	0.16	5.57E-05	2.39E-04
Psed_2907	hypothetical protein	-0.74	-1.16	0.65	-0.34	5.61E-05	2.40E-04
Psed_5223	putative sporulation-associated protein	-0.53	-1.04	-0.81	-0.71	5.61E-05	2.41E-04
Psed_1771	Electron transfer flavoprotein alpha/beta-subunit	-1.46	-1.47	-1.48	-1.92	5.68E-05	2.43E-04
Psed_1562	hypothetical protein	-1.65	-0.78	-0.91	-2.14	5.70E-05	2.44E-04
Psed_6168	Phosphomethylpyrimidine synthase	-0.30	-0.97	-2.17	-1.18	5.78E-05	2.47E-04
Psed_4505	Sulfite reductase (ferredoxin)	0.59	0.38	-0.97	-1.25	5.78E-05	2.47E-04
Psed_5825	diaminobutyrate/2-oxoglutarate aminotransferase	-1.71	-0.48	-1.87	-2.19	5.96E-05	2.54E-04
Psed_3286	Sec-independent protein translocase, TatC subunit	-0.53	-0.72	-0.42	-1.22	6.00E-05	2.55E-04

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_4449	DNA primase	-1.01	-0.49	-0.76	-1.37	6.03E-05	2.56E-04
Psed_5374	hypothetical protein	0.04	1.18	1.30	-0.16	6.05E-05	2.57E-04
Psed_3871	hypothetical protein	-1.51	-1.70	-1.30	-1.60	6.06E-05	2.57E-04
Psed_4848	hypothetical protein	-3.53	-0.64	-1.33	-1.52	6.15E-05	2.60E-04
Psed_2370	hypothetical protein	0.80	1.77	0.49	-0.18	6.17E-05	2.60E-04
Psed_4111	Formyl-CoA transferase	-0.58	-0.73	-1.34	-0.72	6.17E-05	2.61E-04
Psed_3817	hypothetical protein	-0.45	-1.57	-0.46	-0.92	6.22E-05	2.62E-04
Psed_3610	lipid A biosynthesis acyltransferase	-0.32	-0.01	-0.36	-1.05	6.47E-05	2.71E-04
Psed_6121	hypothetical protein	-0.59	-0.33	-0.33	-1.32	6.52E-05	2.73E-04
Psed_0891	iron permease FTR1	1.10	-0.23	2.17	0.56	6.55E-05	2.74E-04
Psed_3494	hypothetical protein	0.27	0.30	1.01	0.67	6.56E-05	2.74E-04
Psed_6563	hypothetical protein	-0.11	0.54	1.17	0.06	6.59E-05	2.74E-04
Psed_2661	drug resistance transporter, EmrB/QacA sub-family	-0.17	1.24	-0.53	-0.88	6.61E-05	2.75E-04
Psed_5327	Bifunctional purine biosynthesis protein purH	-0.79	-0.39	-0.60	-1.16	6.63E-05	2.76E-04
Psed_3808	segregation and condensation protein B	-0.07	0.36	0.16	-1.04	6.64E-05	2.76E-04
Psed_3831	Arginine repressor	-0.49	-0.39	-0.05	-1.34	6.64E-05	2.76E-04
Psed_6160	heavy metal translocating P-type ATPase	0.98	0.14	0.92	1.24	6.68E-05	2.77E-04
Psed_3821	hypothetical protein	-0.51	-0.16	-0.02	-1.17	6.74E-05	2.79E-04
Psed_3017	precorrin-6y C5,15-methyltransferase (decar-boxylating), CbiE subunit	0.02	0.13	-1.22	0.14	6.84E-05	2.82E-04
Psed_2191	FMN-dependent oxidoreductase, nitrilotriac-etate monooxygenase family	0.60	1.57	0.03	0.29	6.91E-05	2.85E-04
Psed_4212	tyrosyl-tRNA synthetase	-0.69	-0.23	0.03	-1.20	6.92E-05	2.85E-04
Psed_5710	hypothetical protein	-0.53	-1.54	-0.45	-0.73	6.96E-05	2.86E-04
Psed_6806	hypothetical protein	-0.02	0.13	-0.12	1.31	6.96E-05	2.86E-04

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_3677	squalene synthase HpnD	-0.44	-1.34	-1.08	-0.93	6.98E-05	2.87E-04
Psed_2436	nitroreductase	-2.64	-0.32	-1.34	-1.93	7.05E-05	2.89E-04
Psed_3162	regulatory protein MerR	-0.80	-1.15	0.76	-0.60	7.09E-05	2.90E-04
Psed_5198	mannose-6-phosphate isomerase, class I	-0.41	-0.12	0.02	-1.10	7.11E-05	2.91E-04
Psed_0886	hypothetical protein	-1.27	-0.67	-1.16	-1.83	7.18E-05	2.93E-04
Psed_4106	Catalase	0.26	1.54	0.01	0.42	7.35E-05	2.99E-04
Psed_1722	Thioredoxin domain-containing protein	-0.89	-0.28	-0.49	-1.74	7.42E-05	3.02E-04
Psed_4146	Potassium-transporting ATPase B chain	1.58	0.10	-0.07	0.38	7.43E-05	3.02E-04
Psed_4461	hypothetical protein	0.15	1.14	0.17	0.81	7.46E-05	3.03E-04
Psed_4577	Cold-shock protein DNA-binding	1.20	1.93	2.48	0.47	7.50E-05	3.05E-04
Psed_4147	Potassium-transporting ATPase A chain	1.27	0.10	-0.25	0.44	7.53E-05	3.05E-04
Psed_5756	hypothetical protein	-0.10	1.13	0.61	1.16	7.57E-05	3.07E-04
Psed_4012	hypothetical protein	-0.50	-0.68	-1.12	-0.37	7.66E-05	3.09E-04
Psed_2038	sporulation and cell division protein SsgA	-0.75	-1.47	-1.23	-0.41	7.71E-05	3.11E-04
Psed_5153	transcription factor WhiB	-0.41	-0.69	-0.59	-1.27	7.72E-05	3.11E-04
Psed_4819	integral membrane protein	0.56	0.41	-1.28	0.47	7.79E-05	3.14E-04
Psed_3829	Argininosuccinate lyase	-0.40	-0.17	0.08	-1.13	7.80E-05	3.14E-04
Psed_1585	hypothetical protein	0.28	0.17	-1.04	0.39	7.93E-05	3.18E-04
Psed_4552	regulatory protein TetR	0.10	0.51	1.03	-0.08	8.03E-05	3.22E-04
Psed_4736	Protein of unknown function DUF2469	-0.45	-0.27	0.13	-1.21	8.05E-05	3.22E-04
Psed_4806	hypothetical protein	-0.50	-0.14	0.04	-1.31	8.08E-05	3.23E-04
Psed_3442	protein of unknown function DUF939	0.54	0.34	0.74	1.05	8.14E-05	3.25E-04
Psed_6110	Antibiotic biosynthesis monooxygenase	-0.54	-0.46	-0.17	-1.45	8.22E-05	3.27E-04
Psed_3836	N-acetyl-gamma-glutamyl-phosphate reductase	-0.01	-0.52	0.62	-1.20	8.35E-05	3.32E-04
Psed_4535	response regulator receiver	-0.72	-0.95	-0.74	-1.08	8.37E-05	3.32E-04
Psed_2705	hypothetical protein	-0.58	-0.90	-0.63	-1.00	8.43E-05	3.34E-04
Psed_6322	Peptide methionine sulfoxide reductase msrA	-1.01	-0.37	-0.00	0.26	8.56E-05	3.38E-04

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_3512	hypothetical protein	0.15	0.26	1.69	0.69	8.59E-05	3.39E-04
Psed_6805	hypothetical protein	-1.06	-2.94	-2.23	-0.95	8.68E-05	3.42E-04
Psed_5147	Methyltransferase type 11	-0.53	-0.94	-1.06	-0.46	8.68E-05	3.42E-04
Psed_4330	hypothetical protein	-0.05	0.20	1.36	0.21	8.68E-05	3.42E-04
Psed_1819	hypothetical protein	-0.51	-0.16	-0.39	-1.51	9.73E-05	3.77E-04
Psed_1632	regulatory protein MarR	-0.79	-1.11	0.27	-1.02	9.74E-05	3.78E-04
Psed_5524	hydrolase	1.08	0.35	0.79	0.84	9.78E-05	3.79E-04
Psed_0551	Glutaryl-CoA dehydrogenase	-0.11	0.87	1.63	1.33	9.80E-05	3.79E-04
Psed_6068	beta-ketoadipate pathway transcriptional regulators, PcaR/PcaU/PobR family	-0.90	-0.36	-0.40	-1.16	9.85E-05	3.81E-04
Psed_0397	Pyrophosphate-energized proton pump	-0.36	-0.29	0.19	-1.86	9.89E-05	3.82E-04
Psed_4149	hypothetical protein	1.14	0.36	-0.27	0.58	1.01E-04	3.88E-04
Psed_5395	tRNA pseudouridine synthase A	0.27	1.07	0.59	0.03	1.01E-04	3.90E-04
Psed_1682	ATP synthase subunit delta	-0.99	-0.40	-0.85	-1.82	1.03E-04	3.95E-04
Psed_5019	protein of unknown function DUF1707	-1.15	-0.75	-0.13	-1.19	1.03E-04	3.95E-04
Psed_3857	hypothetical protein	-0.59	-0.56	-0.07	-1.33	1.03E-04	3.96E-04
Psed_4487	PhoH family protein	-0.84	0.45	-0.22	-1.11	1.05E-04	4.03E-04
Psed_4397	Polyketide cyclase/dehydrase	-0.59	-1.13	-0.03	-0.52	1.07E-04	4.07E-04
Psed_7016	hypothetical protein	-1.01	-1.34	-0.87	-1.00	1.08E-04	4.12E-04
Psed_4628	cell divisionFtsK/SpoIIIE	-0.82	-0.87	-0.02	-1.31	1.08E-04	4.12E-04
Psed_4303	respiratory nitrate reductase, gamma subunit	-2.07	-0.84	-0.01	-0.93	1.11E-04	4.20E-04
Psed_6650	hypothetical protein	0.07	0.91	1.09	1.48	1.11E-04	4.23E-04
Psed_4271	hypothetical protein	0.53	0.30	1.07	0.66	1.12E-04	4.23E-04
Psed_0554	hypothetical protein	0.91	2.93	0.59	2.06	1.13E-04	4.28E-04
Psed_0450	hypothetical protein	-0.62	-0.73	-1.12	-0.68	1.15E-04	4.32E-04

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_4510	aliphatic sulfonates family ABC transporter, periplsmic ligand-binding protein	0.81	0.75	-0.20	-1.08	1.16E-04	4.36E-04
Psed_4021	Bifunctional protein folD	-1.43	-1.15	-1.15	-1.87	1.16E-04	4.37E-04
Psed_2021	DoxX family protein	-0.89	-0.32	-1.27	-1.04	1.17E-04	4.40E-04
Psed_4699	protein of unknown function DUF1365	0.01	0.15	0.83	1.62	1.23E-04	4.60E-04
Psed_0953	PhoH family protein	-1.33	-1.12	-0.15	-0.73	1.25E-04	4.65E-04
Psed_6005	nitrile hydratase, alpha subunit	0.03	0.49	1.16	0.54	1.25E-04	4.67E-04
Psed_4512	ABC-type transporter, integral membrane subunit	1.25	1.47	-0.24	-0.17	1.26E-04	4.68E-04
Psed_1046	Citryl-CoA lyase	-0.01	-0.82	-0.92	-1.38	1.29E-04	4.79E-04
Psed_3865	Protein of unknown function DUF2029	-0.34	0.21	-0.26	-1.12	1.30E-04	4.80E-04
Psed_0432	phosphoribosylformylglycinamidine synthase, purS	-0.37	0.02	-0.50	-1.05	1.30E-04	4.80E-04
Psed_2292	response regulator receiver	-2.21	-0.38	0.16	-0.51	1.31E-04	4.83E-04
Psed_5938	glycoside hydrolase family 13 domain-containing protein	1.21	0.54	0.75	0.39	1.32E-04	4.87E-04
Psed_4844	glyceraldehyde-3-phosphate dehydrogenase, type I	-3.05	-1.08	-2.66	-2.84	1.32E-04	4.87E-04
Psed_0986	phospho-2-dehydro-3-deoxyheptonate aldolase	0.52	0.72	1.13	0.73	1.34E-04	4.93E-04
Psed_5749	hypothetical protein	0.02	1.06	0.70	1.31	1.35E-04	4.95E-04
Psed_3161	protein of unknown function DUF151	-1.04	-0.86	0.36	-0.72	1.37E-04	5.03E-04
Psed_0428	adenylosuccinate lyase	-0.47	-0.18	-0.26	-1.29	1.38E-04	5.06E-04
Psed_4236	hypothetical protein	0.07	0.21	-0.01	1.03	1.41E-04	5.14E-04
Psed_6522	hypothetical protein	-0.79	-0.33	-0.33	-1.16	1.42E-04	5.17E-04
Psed_0160	3-oxoacyl-[acyl-carrier-protein] reductase	-0.58	-0.38	-0.64	-1.23	1.44E-04	5.22E-04
Psed_4874	Glyoxalase/bleomycin resistance protein/dioxygenase	-2.08	0.07	-1.54	-1.76	1.44E-04	5.23E-04

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_1934	protein of unknown function DUF214	0.10	0.35	-1.13	-0.32	1.45E-04	5.25E-04
Psed_6249	Ribulose bisphosphate carboxylase large chain	0.17	0.14	-0.20	1.15	1.45E-04	5.25E-04
Psed_1691	methylmalonyl-CoA mutase, large subunit	-0.34	-1.50	-0.23	-1.43	1.47E-04	5.31E-04
Psed_5368	alpha/beta hydrolase fold	-0.84	-0.39	-0.53	-1.38	1.57E-04	5.64E-04
Psed_0560	hypothetical protein	-1.34	-0.17	0.38	-1.03	1.63E-04	5.84E-04
Psed_5014	esterase/lipase	-0.85	-0.83	-0.23	-1.31	1.64E-04	5.86E-04
Psed_4301	nitrate reductase, beta subunit	-2.62	-0.60	0.00	-1.37	1.67E-04	5.95E-04
Psed_1021	DNA-3-methyladenine glycosylase I	-0.50	-0.49	-1.25	-0.51	1.68E-04	5.99E-04
Psed_3121	ABC transporter, CydDC cysteine exporter (CydDC-E) family, permease/ATP-binding protein CydD	-1.62	-0.85	-1.30	-2.85	1.69E-04	6.01E-04
Psed_2778	phospho-2-dehydro-3-deoxyheptonate aldolase	-0.86	-0.44	-1.03	-1.21	1.70E-04	6.04E-04
Psed_5265	Fe-S metabolism associated SufE	-0.86	-0.42	-0.31	-1.40	1.73E-04	6.11E-04
Psed_1785	Serine--pyruvate transaminase	-0.67	-0.72	-1.03	-1.24	1.74E-04	6.15E-04
Psed_2910	protein of unknown function DUF322	-0.56	-1.16	-0.07	0.47	1.79E-04	6.32E-04
Psed_0165	hypothetical protein	-0.75	-0.43	-0.78	-1.03	1.82E-04	6.39E-04
Psed_5091	Undecaprenyl-diphosphatase	-0.11	-0.04	0.06	-1.09	1.84E-04	6.47E-04
Psed_5659	(R)-benzylsuccinyl-CoA dehydrogenase	-0.72	-0.50	-0.29	-1.42	1.85E-04	6.48E-04
Psed_4722	hypothetical protein	-0.79	-0.48	-0.56	-1.06	1.86E-04	6.53E-04
Psed_5562	NADH dehydrogenase (quinone)	-1.27	-0.27	-1.30	-1.48	1.87E-04	6.54E-04
Psed_3761	Glutamate synthase (NADPH)	-0.40	-0.48	0.31	-1.07	1.87E-04	6.54E-04
Psed_4570	putative F420-dependent oxidoreductase	-1.07	-0.93	-0.93	-0.45	1.94E-04	6.75E-04
Psed_3123	cytochrome bd ubiquinol oxidase subunit I	-1.42	-0.95	-2.11	-2.50	1.95E-04	6.77E-04
Psed_6879	S-adenosylmethionine synthase	-0.03	0.39	-0.23	1.82	1.98E-04	6.86E-04
Psed_2033	ABC-type transporter, periplasmic subunit	-0.33	-1.97	0.09	-0.82	2.01E-04	6.93E-04
Psed_6509	endonuclease III	-1.07	-0.48	-0.65	-1.80	2.01E-04	6.96E-04

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_4268	membrane protein of uknown function UCP014873	0.25	0.49	0.12	2.06	2.03E-04	7.00E-04
Psed_7019	hypothetical protein	-0.19	-1.36	-0.07	-0.35	2.05E-04	7.07E-04
Psed_6302	hypothetical protein	-0.88	-1.14	-0.87	-1.11	2.09E-04	7.18E-04
Psed_3039	ABC-type transporter, integral membrane sub-unit	-0.62	-0.89	-1.05	-0.11	2.14E-04	7.33E-04
Psed_4841	UspA domain-containing protein	-2.86	-0.54	-1.82	-2.13	2.15E-04	7.35E-04
Psed_3536	Shikimate kinase	-0.21	0.58	-0.09	-1.21	2.17E-04	7.42E-04
Psed_1189	amidohydrolase 2	-0.54	0.19	-0.31	-1.12	2.17E-04	7.43E-04
Psed_5149	ErfK/YbiS/YcfS/YnhG family protein	-0.31	-0.87	-1.10	-0.50	2.18E-04	7.45E-04
Psed_4623	Ribosomal protein S12 methylthiotransferase rimO	-0.60	-0.12	-0.11	-1.10	2.19E-04	7.45E-04
Psed_1676	hypothetical protein	-0.53	-0.36	-0.37	-1.34	2.21E-04	7.52E-04
Psed_5479	beta-lactamase	-0.90	-0.92	-0.49	-1.21	2.22E-04	7.55E-04
Psed_0459	transcriptional regulator domain-containing protein	-1.24	-1.02	-1.18	-0.81	2.23E-04	7.56E-04
Psed_3049	Formamidase	-1.83	-0.15	-2.02	0.86	2.24E-04	7.58E-04
Psed_4148	K+-transporting ATPase, F subunit	1.81	0.35	-0.52	0.24	2.24E-04	7.60E-04
Psed_2351	helix-turn-helix domain protein	-1.08	-0.62	-0.50	-0.50	2.29E-04	7.72E-04
Psed_4805	NAD-glutamate dehydrogenase	-0.78	-0.53	-0.43	-1.28	2.34E-04	7.86E-04
Psed_3323	ATP phosphoribosyltransferase	-0.50	-0.07	-0.07	-1.12	2.35E-04	7.87E-04
Psed_5194	hypothetical protein	-1.08	-0.62	-0.64	-1.29	2.39E-04	7.97E-04
Psed_3198	hypothetical protein	-0.85	0.35	-1.13	-1.12	2.42E-04	8.06E-04
Psed_1567	GtrA family protein	-0.34	-0.65	2.03	-0.56	2.44E-04	8.09E-04
Psed_5302	tryptophanyl-tRNA synthetase	-0.43	-0.17	0.08	-1.07	2.48E-04	8.21E-04
Psed_2791	hypothetical protein	-0.57	-0.85	-1.01	0.00	2.48E-04	8.23E-04

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_5456	hypothetical protein	0.31	0.30	1.19	0.05	2.49E-04	8.23E-04
Psed_3383	glutamate/cysteine ligase family protein	-0.55	-0.75	-0.44	-1.12	2.54E-04	8.39E-04
Psed_4486	metalloprotease ybeY	-0.86	0.15	-0.45	-1.22	2.57E-04	8.47E-04
Psed_2433	DoxX family protein	-4.16	-1.20	-2.12	-2.39	2.57E-04	8.48E-04
Psed_5764	hypothetical protein	0.01	0.61	0.22	1.31	2.58E-04	8.49E-04
Psed_6224	hypothetical protein	0.01	0.65	1.46	0.49	2.70E-04	8.82E-04
Psed_3615	hypothetical protein	-1.16	-0.39	0.45	-0.65	2.71E-04	8.86E-04
Psed_4840	CBS domain containing protein	-2.63	-0.50	-2.60	-1.35	2.71E-04	8.86E-04
Psed_7014	hypothetical protein	-0.60	-1.34	-0.81	-0.40	2.73E-04	8.91E-04
Psed_2502	hypothetical protein	-1.15	-0.38	-0.39	-0.89	2.75E-04	8.97E-04
Psed_2933	protein of unknown function DUF164	-0.50	-0.33	0.19	-1.05	2.77E-04	9.00E-04
Psed_3613	threonyl-tRNA synthetase	-0.47	0.09	0.12	-1.22	2.78E-04	9.04E-04
Psed_1662	Arginyl-tRNA synthetase	-0.59	-0.07	-0.21	-1.41	2.79E-04	9.07E-04
Psed_3349	acyl-CoA dehydrogenase domain-containing protein	-0.49	-0.46	-0.55	-1.08	2.80E-04	9.08E-04
Psed_4299	major facilitator superfamily MFS_1	-1.51	0.37	-0.99	-1.28	2.81E-04	9.10E-04
Psed_3854	Antibiotic biosynthesis monooxygenase	-0.46	-0.81	0.15	-1.20	2.84E-04	9.19E-04
Psed_2985	regulatory protein ArsR	-0.98	-0.40	-0.22	-1.09	2.86E-04	9.24E-04
Psed_1942	Lipoprotein signal peptidase	-0.69	-0.52	-0.39	-1.12	2.92E-04	9.40E-04
Psed_1186	cell division ATP-binding protein FtsE	-0.61	-0.63	0.16	-1.24	2.93E-04	9.41E-04
Psed_1612	alpha/beta hydrolase fold	-0.62	-0.17	-0.37	-1.32	2.97E-04	9.53E-04
Psed_5604	DNA binding domain protein, excisionase family	0.35	0.57	1.07	0.19	2.97E-04	9.53E-04
Psed_3355	band 7 protein	-0.64	-0.86	-0.68	-1.01	2.99E-04	9.57E-04
Psed_1818	Dihydroxy-acid dehydratase	-0.57	-0.26	0.03	-1.56	3.06E-04	9.75E-04
Psed_2875	Malate dehydrogenase	-1.20	-0.80	-0.52	-1.04	3.07E-04	9.79E-04

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_2388	Gas vesicle synthesis GvpLGvpF	0.45	1.24	0.31	-0.19	3.08E-04	9.81E-04
Psed_1259	hypothetical protein	-0.43	-1.16	-0.03	0.03	3.09E-04	9.81E-04
Psed_2290	hypothetical protein	-2.20	-0.25	-1.65	-1.98	3.10E-04	9.86E-04
Psed_3576	beta-lactamase domain protein	-0.29	-0.01	-0.02	-1.01	3.19E-04	1.01E-03
Psed_6526	SNARE associated Golgi protein	-0.67	-0.01	-0.02	-1.17	3.25E-04	1.03E-03
Psed_1761	S-adenosylhomocysteine deaminase	-1.23	-1.02	-0.74	-1.12	3.27E-04	1.03E-03
Psed_6994	Cobyrinic acid ac-diamide synthase	0.04	-1.13	-0.55	-0.73	3.34E-04	1.05E-03
Psed_5770	hypothetical protein	0.10	0.19	0.29	1.29	3.38E-04	1.06E-03
Psed_2141	2-oxo-acid dehydrogenase E1 subunit, homodimeric type	-0.90	-0.39	-0.49	-1.35	3.39E-04	1.06E-03
Psed_0300	Pyridoxine 4-dehydrogenase	-0.62	-0.77	-0.83	-1.13	3.41E-04	1.07E-03
Psed_0390	hypothetical protein	-1.04	0.02	-1.24	-0.98	3.44E-04	1.07E-03
Psed_3410	OpcA protein	-0.63	-0.82	-0.42	-1.60	3.54E-04	1.10E-03
Psed_0016	Integrase catalytic region	0.96	0.49	-1.07	0.53	3.54E-04	1.10E-03
Psed_6523	fructose-bisphosphate aldolase, class II	-1.08	-0.64	-0.08	-1.37	3.60E-04	1.12E-03
Psed_2527	catechol 2,3 dioxygenase	-0.42	-0.41	1.06	-0.32	3.62E-04	1.12E-03
Psed_4842	UspA domain-containing protein	-1.97	0.25	-1.29	-1.32	3.64E-04	1.13E-03
Psed_3015	hypothetical protein	0.38	1.07	0.43	-0.02	3.70E-04	1.14E-03
Psed_1122	UPF0182 protein	-0.89	-0.41	-0.46	-1.05	3.70E-04	1.14E-03
Psed_2298	UspA domain-containing protein	-2.12	-0.25	-1.14	-2.35	3.72E-04	1.15E-03
Psed_3356	protein of unknown function DUF107	-0.74	-1.19	-0.78	-0.83	3.78E-04	1.17E-03
Psed_5312	isocitrate dehydrogenase, NADP-dependent	-1.07	-0.86	-0.36	-1.19	3.85E-04	1.18E-03
Psed_3751	hypothetical protein	-0.29	-0.63	1.65	-0.08	3.86E-04	1.19E-03
Psed_6628	hypothetical protein	-1.68	-0.65	-0.29	-1.08	3.95E-04	1.21E-03
Psed_6269	hypothetical protein	-0.21	-0.31	-0.89	-1.24	4.00E-04	1.22E-03

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_6811	type IV secretory pathway VirD4 protein-like protein	0.16	1.31	1.03	0.90	4.24E-04	1.28E-03
Psed_6847	hypothetical protein	0.29	0.33	0.08	1.11	4.28E-04	1.29E-03
Psed_2505	RNA polymerase sigma-70 factor, sigma-B/F/G subfamily	0.09	0.39	1.04	0.62	4.29E-04	1.29E-03
Psed_1752	Antibiotic biosynthesis monooxygenase	-0.24	-0.15	-0.61	-1.30	4.31E-04	1.30E-03
Psed_0890	Peptidase M75, Imelysin	0.89	-0.26	1.43	0.39	4.34E-04	1.31E-03
Psed_5208	LPPG:FO 2-phospho-L-lactate transferase	-0.76	-0.72	-0.74	-1.21	4.37E-04	1.31E-03
Psed_2866	Tryptophan synthase alpha chain	-0.61	-0.21	-0.37	-1.04	4.39E-04	1.32E-03
Psed_1888	oxidoreductase domain protein	-0.78	-0.35	-0.37	-1.03	4.40E-04	1.32E-03
Psed_2649	hypothetical protein	-1.35	-1.04	-0.83	-0.40	4.44E-04	1.33E-03
Psed_1908	hypothetical protein	-1.13	-1.40	-0.44	-1.65	4.49E-04	1.34E-03
Psed_4409	methylmalonyl-CoA mutase, large subunit	-1.06	-0.23	-0.52	-1.02	4.54E-04	1.35E-03
Psed_3377	aconitate hydratase 1	-1.35	-0.88	-0.63	-1.65	4.56E-04	1.36E-03
Psed_1830	Heat shock protein 70	-0.86	-0.26	-0.41	-1.42	4.59E-04	1.36E-03
Psed_2297	hypothetical protein	-2.12	-0.13	-1.72	-2.11	4.60E-04	1.37E-03
Psed_5044	isochorismatase hydrolase	0.74	1.23	-0.39	0.19	4.73E-04	1.40E-03
Psed_3120	ABC transporter, CydDC cysteine exporter (CydDC-E) family, permease/ATP-binding protein CydC	-1.98	-1.07	-1.23	-2.60	4.81E-04	1.42E-03
Psed_6926	ABC-type transporter, integral membrane subunit	-0.27	-0.95	1.03	-0.54	4.81E-04	1.42E-03
Psed_2826	pseudouridine synthase, RluA family	-0.53	-0.10	-0.51	-1.05	4.87E-04	1.43E-03
Psed_1078	alpha/beta hydrolase fold	0.38	0.14	2.11	-0.03	4.91E-04	1.45E-03
Psed_4008	hypothetical protein	-1.36	-0.43	-0.36	-0.70	5.03E-04	1.48E-03
Psed_2112	Cupin 2 conserved barrel domain protein	-0.94	-0.77	-1.50	-1.20	5.25E-04	1.54E-03
Psed_3408	glucose-6-phosphate isomerase	-0.45	-0.43	0.03	-1.16	5.32E-04	1.55E-03

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_5555	Trans-hexaprenyltranstransferase	-0.71	-0.18	-0.36	-1.50	5.33E-04	1.56E-03
Psed_3577	peptidyl-prolyl cis-trans isomerase cyclophilin type	-0.86	-0.54	-0.19	-1.31	5.37E-04	1.57E-03
Psed_4631	LexA DNA-binding domain protein	-0.91	0.04	-0.27	-1.78	5.43E-04	1.58E-03
Psed_4632	hypothetical protein	-0.86	0.04	-0.42	-1.59	5.50E-04	1.60E-03
Psed_5742	Thiazole synthase	-0.61	0.02	-0.37	-1.29	5.54E-04	1.61E-03
Psed_2125	ribonucleoside-diphosphate reductase, adenosylcobalamin-dependent	-0.63	-1.16	-1.21	-0.55	5.69E-04	1.65E-03
Psed_3350	Succinate-semialdehyde dehydrogenase	-0.63	-0.75	-1.10	-0.35	5.74E-04	1.66E-03
Psed_3635	GTP cyclohydrolase 1	-0.80	-1.17	-0.16	-0.14	5.93E-04	1.71E-03
Psed_5557	proton-translocating NADH-quinone oxidoreductase, chain M	-1.36	-0.56	-0.84	-1.86	6.06E-04	1.74E-03
Psed_3951	hypothetical protein	-0.91	-1.04	-0.74	-1.08	6.18E-04	1.77E-03
Psed_1769	xanthine permease	-1.20	-1.08	-1.20	-1.14	6.38E-04	1.81E-03
Psed_0205	GtrA family protein	-0.78	-0.02	-0.87	-1.02	6.48E-04	1.84E-03
Psed_1054	1-aminocyclopropane-1-carboxylate deaminase	-1.56	-1.25	-1.30	-0.82	6.50E-04	1.84E-03
Psed_4847	CBS domain containing protein	-1.97	-0.27	-1.31	-1.38	6.54E-04	1.85E-03
Psed_3754	[2Fe-2S]-binding domain-containing protein	0.11	-0.05	2.30	-0.08	6.63E-04	1.87E-03
Psed_1887	DegT/DnrJ/EryC1/StrS aminotransferase	-0.93	-0.33	-0.92	-1.24	6.78E-04	1.90E-03
Psed_5024	Mandelate racemase/muconate lactonizing protein	0.64	0.28	1.90	0.08	6.91E-04	1.93E-03
Psed_2265	hypothetical protein	0.54	0.31	-1.30	0.38	6.99E-04	1.95E-03
Psed_2190	ABC-type transporter, periplasmic subunit	0.73	1.08	0.12	0.42	7.16E-04	2.00E-03
Psed_5224	hypothetical protein	0.84	0.48	-0.55	1.19	7.19E-04	2.00E-03
Psed_1648	hypothetical protein	-0.68	-0.73	-1.14	-0.94	7.25E-04	2.02E-03
Psed_3833	Acetylornithine/succinyldiaminopimelate aminotransferase	-0.59	-0.62	-0.22	-1.52	7.25E-04	2.02E-03

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_0187	galactofuranosyl transferase	-0.90	-0.43	-0.25	-1.16	7.25E-04	2.02E-03
Psed_3530	major facilitator superfamily MFS_1	-0.05	-0.16	-1.14	-0.27	7.26E-04	2.02E-03
Psed_2283	UspA domain-containing protein	-1.81	-0.02	-1.30	-1.37	7.27E-04	2.02E-03
Psed_2631	DNA topoisomerase IB	0.24	1.40	0.00	0.59	7.28E-04	2.02E-03
Psed_6149	Enoyl-CoA hydratase	-0.97	-0.49	-0.17	-1.06	7.31E-04	2.03E-03
Psed_1644	6-phosphofructokinase	0.45	1.13	0.41	0.06	7.45E-04	2.06E-03
Psed_3282	peptidase M24	-0.88	-0.62	-0.37	-1.10	7.56E-04	2.09E-03
Psed_5076	Glycerol kinase	-1.04	-0.33	0.97	-0.04	7.59E-04	2.09E-03
Psed_5566	NAD(P)H-quinone oxidoreductase subunit H	-0.85	0.01	-0.94	-1.00	7.67E-04	2.11E-03
Psed_3116	YbhB YbcL family protein	-0.66	-1.06	0.21	-0.88	8.04E-04	2.20E-03
Psed_4468	glycyl-tRNA synthetase	-0.50	-0.20	-0.10	-1.18	8.08E-04	2.21E-03
Psed_0438	Phosphoribosylformylglycinamidine synthase 2	-0.44	-0.01	-0.63	-1.01	8.21E-04	2.24E-03
Psed_5373	hypothetical protein	0.29	0.92	1.03	0.67	8.33E-04	2.27E-03
Psed_1762	Carbon-monoxide dehydrogenase (acceptor)	-1.20	-1.11	-0.45	-1.05	8.43E-04	2.29E-03
Psed_7013	hypothetical protein	-0.58	-1.22	-0.18	-0.63	9.02E-04	2.43E-03
Psed_0897	Septum formation initiator	-0.61	-0.77	-0.83	-1.41	9.06E-04	2.44E-03
Psed_3122	cytochrome d ubiquinol oxidase, subunit II	-1.37	-0.83	-1.31	-2.58	9.59E-04	2.57E-03
Psed_4220	Luciferase-like, subgroup	0.08	0.00	1.62	0.12	9.63E-04	2.58E-03
Psed_1825	D-3-phosphoglycerate dehydrogenase	-1.10	-0.63	-1.70	-1.39	9.79E-04	2.61E-03
Psed_0325	Glyoxalase/bleomycin resistance protein/dioxy-genase	-1.11	-1.12	-0.21	-1.07	9.81E-04	2.62E-03
Psed_5405	nicotinamide nucleotide transhydrogenase alpha subunit 2 PntAB	1.04	1.10	1.22	1.37	9.85E-04	2.62E-03
Psed_5556	NAD(P)H-quinone oxidoreductase subunit 2	-1.13	-0.48	-0.69	-1.71	9.91E-04	2.63E-03
Psed_2991	type III restriction protein res subunit	-0.83	-0.46	-0.08	-1.21	1.06E-03	2.78E-03

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_4302	nitrate reductase molybdenum cofactor assembly chaperone	-1.42	-0.56	-0.38	-0.99	1.07E-03	2.82E-03
Psed_2374	Luciferase-like, subgroup	0.46	1.10	-0.47	0.42	1.09E-03	2.85E-03
Psed_6004	Nitrile hydratase	-0.27	0.47	1.21	0.78	1.09E-03	2.86E-03
Psed_5175	(S)-2-hydroxy-acid oxidase	-0.52	-0.32	0.16	-1.20	1.11E-03	2.90E-03
Psed_2615	regulatory protein TetR	0.77	0.93	1.11	0.29	1.14E-03	2.96E-03
Psed_3753	alpha/beta hydrolase fold	0.18	0.05	2.62	0.12	1.15E-03	2.99E-03
Psed_1685	ATP synthase subunit beta	-1.12	-0.48	-0.32	-1.37	1.16E-03	3.02E-03
Psed_3986	Peptidase M23	-0.12	0.26	0.54	1.05	1.16E-03	3.02E-03
Psed_4654	Thymidylate synthase thyX	-0.90	-0.51	-0.46	-1.19	1.20E-03	3.10E-03
Psed_2352	methionine aminopeptidase, type I	-1.12	-1.50	-1.27	-0.73	1.23E-03	3.16E-03
Psed_1880	glycosyl transferase family 2	-1.51	-1.06	-1.21	-1.47	1.24E-03	3.19E-03
Psed_4682	Prolyl-tRNA synthetase	-0.62	0.03	-0.37	-1.62	1.25E-03	3.21E-03
Psed_6817	hypothetical protein	-0.87	-1.23	-1.10	-0.68	1.28E-03	3.26E-03
Psed_0876	Bifunctional protein glmU	-0.57	-0.14	-0.51	-1.09	1.35E-03	3.42E-03
Psed_5709	hypothetical protein	-0.45	-1.30	-0.15	-0.25	1.36E-03	3.43E-03
Psed_4845	gluconate kinase	-1.10	-0.38	-1.03	-1.02	1.37E-03	3.45E-03
Psed_6656	Deoxyribonuclease IV (phage-T(4)-induced)	-0.92	-0.37	-0.06	-1.03	1.37E-03	3.45E-03
Psed_4802	alpha amylase catalytic region	-0.70	-0.92	-0.93	-1.01	1.40E-03	3.51E-03
Psed_4566	2-oxoglutarate dehydrogenase, E2 component, dihydrolipoamide succinyltransferase	-0.65	-0.46	-0.67	-1.33	1.41E-03	3.54E-03
Psed_1878	glycosyl transferase group 1	-0.66	-0.67	0.18	-1.13	1.43E-03	3.58E-03
Psed_3926	protein of unknown function DUF1707	-1.19	-1.25	-1.34	-0.82	1.44E-03	3.59E-03
Psed_4801	globin	-1.17	-0.31	-0.41	-0.50	1.47E-03	3.66E-03
Psed_3755	Xanthine dehydrogenase	0.10	0.05	3.32	0.27	1.51E-03	3.74E-03
Psed_2752	hypothetical protein	1.12	0.77	1.29	0.65	1.54E-03	3.80E-03

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_3979	hydro-lyase, Fe-S type, tartrate/fumarate sub-family, beta subunit	-0.71	-0.07	-1.00	-0.76	1.58E-03	3.88E-03
Psed_1674	Glycine hydroxymethyltransferase	-1.33	-0.67	-0.91	-1.76	1.58E-03	3.88E-03
Psed_0875	ribose-phosphate pyrophosphokinase	-0.52	-0.06	-0.25	-1.11	1.58E-03	3.88E-03
Psed_1871	polysaccharide deacetylase	-0.89	-0.29	-0.59	-1.25	1.64E-03	4.00E-03
Psed_5920	oxidoreductase molybdopterin binding	-1.07	-0.73	-0.02	-0.85	1.65E-03	4.02E-03
Psed_5027	FAD-binding 9 siderophore-interacting domain protein	0.44	0.60	1.09	-0.08	1.69E-03	4.10E-03
Psed_3524	carbamoyl-phosphate synthase, large subunit	-0.40	-0.71	-1.18	-0.30	1.69E-03	4.11E-03
Psed_5348	death-on-curing family protein	0.81	0.82	1.01	0.28	1.70E-03	4.13E-03
Psed_5460	Alpha-methylacyl-CoA racemase	-0.68	-1.15	-0.95	-0.96	1.74E-03	4.22E-03
Psed_5330	antigen 34 kDa family protein	-0.87	-0.58	-1.02	-1.07	1.79E-03	4.30E-03
Psed_5618	Porphobilinogen deaminase	-0.48	0.16	0.11	-1.26	1.81E-03	4.35E-03
Psed_0109	Peptidylprolyl isomerase	-0.48	0.05	0.05	-1.04	1.84E-03	4.40E-03
Psed_6602	VanW family protein	-0.62	-0.04	0.15	-1.06	1.95E-03	4.65E-03
Psed_3756	molybdopterin dehydrogenase FAD-binding	0.05	0.02	3.26	0.07	1.97E-03	4.69E-03
Psed_5419	ribosomal protein L14	0.12	0.51	0.12	-1.02	2.03E-03	4.80E-03
Psed_3048	AmiS/UreI transporter	-1.44	-0.39	-2.12	-0.07	2.04E-03	4.81E-03
Psed_7000	hypothetical protein	-0.34	-1.25	-0.76	-0.95	2.09E-03	4.92E-03
Psed_4765	protein of unknown function DUF177	-0.72	-0.06	-0.27	-1.05	2.11E-03	4.96E-03
Psed_2286	hypothetical protein	-2.44	-1.23	-1.53	-1.92	2.13E-03	4.99E-03
Psed_3047	hypothetical protein	-2.20	-0.70	-2.12	-0.73	2.17E-03	5.08E-03
Psed_4685	Homoserine O-acetyltransferase	-0.27	-0.28	0.41	-1.00	2.27E-03	5.26E-03
Psed_6792	hypothetical protein	-0.61	-0.73	-1.04	-0.23	2.28E-03	5.28E-03
Psed_4563	cytosol aminopeptidase	-0.55	-0.36	-0.67	-1.08	2.29E-03	5.31E-03
Psed_1829	glutamyl-tRNA synthetase	-0.53	-0.17	-0.07	-1.08	2.31E-03	5.35E-03

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_0157	Rhodanese-like protein	-0.91	-0.31	-0.21	-1.18	2.32E-03	5.36E-03
Psed_0281	hypothetical protein	0.53	-0.10	-1.12	0.00	2.33E-03	5.39E-03
Psed_2294	hypothetical protein	-1.06	-0.15	-0.82	-0.84	2.36E-03	5.45E-03
Psed_5640	2,3-bisphosphoglycerate-dependent phospho-glycerate mutase	-1.08	-0.88	-0.78	-0.55	2.37E-03	5.46E-03
Psed_2282	protein of unknown function UPF0089	-1.13	-0.45	-0.67	-1.04	2.46E-03	5.64E-03
Psed_1886	DegT/DnrJ/EryC1/StrS aminotransferase	-0.75	-0.38	-0.41	-1.25	2.49E-03	5.70E-03
Psed_3757	carbon monoxide dehydrogenase subunit G	0.06	0.05	2.97	0.16	2.52E-03	5.74E-03
Psed_3543	alanyl-tRNA synthetase	-0.28	-0.22	0.17	-1.03	2.56E-03	5.81E-03
Psed_3308	dihydrolipoamide dehydrogenase	-1.16	-1.05	-0.97	-0.51	2.68E-03	6.04E-03
Psed_3371	3-oxoacyl-(acyl-carrier-protein) reductase	-0.54	-0.47	-0.34	-1.06	2.69E-03	6.06E-03
Psed_2296	hypothetical protein	-2.71	-0.70	-2.60	-2.21	2.74E-03	6.14E-03
Psed_5564	NADH-quinone oxidoreductase, F subunit	-1.03	-0.09	-0.68	-1.30	2.79E-03	6.24E-03
Psed_5115	2-oxoglutarate dehydrogenase, E1 subunit	-0.96	-0.72	-0.54	-1.57	2.88E-03	6.40E-03
Psed_1794	Aspartyl/glutamyl-tRNA(Asn/Gln) amidotrans-ferase subunit C	-0.42	0.11	0.11	-1.24	2.97E-03	6.57E-03
Psed_2284	CBS domain containing protein	-1.25	-0.26	-1.89	-0.94	3.01E-03	6.66E-03
Psed_5824	L-ectoine synthase	-1.44	-0.37	-1.13	-1.83	3.03E-03	6.67E-03
Psed_1977	Nucleoside diphosphate kinase	-0.55	0.07	0.24	-1.09	3.10E-03	6.83E-03
Psed_3102	3-oxoacyl-[acyl-carrier-protein] reductase	-0.55	-1.12	0.09	-0.19	3.10E-03	6.83E-03
Psed_5569	NAD(P)H-quinone oxidoreductase subunit 3	-1.06	-0.08	-1.10	-0.81	3.16E-03	6.94E-03
Psed_4594	succinate dehydrogenase (or fumarate reduc-tase) cytochrome b subunit, b558 family	-0.32	-1.03	-0.69	-0.58	3.17E-03	6.95E-03
Psed_3759	major facilitator superfamily MFS_1	0.09	0.00	2.38	-0.02	3.21E-03	7.02E-03
Psed_5871	hypothetical protein	-0.64	-0.15	-1.16	-0.68	3.39E-03	7.36E-03
Psed_1495	cytochrome c oxidase, subunit I	-0.55	-0.21	-0.61	-1.10	3.44E-03	7.45E-03

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Psed_5464	preprotein translocase, SecE subunit	-0.29	0.11	-0.32	-1.04	3.49E-03	7.53E-03
Psed_4331	protein of unknown function DUF955	-0.15	0.32	1.11	0.00	3.50E-03	7.55E-03
Psed_1877	lipopolysaccharide biosynthesis protein	-0.76	-0.34	-0.24	-1.09	3.53E-03	7.61E-03
Psed_3977	Ureidoglycolate lyase	-1.16	-1.10	-1.41	-0.43	3.66E-03	7.83E-03
Psed_2130	transglutaminase domain-containing protein	-0.69	-0.66	-0.10	-1.07	3.70E-03	7.90E-03
Psed_1710	3-hydroxyacyl-CoA dehydrogenase., 3-hydroxybutyryl-CoA dehydrogenase	-0.81	-1.16	-1.07	-1.11	3.74E-03	7.97E-03
Psed_3752	UbiD family decarboxylase	0.24	0.16	2.88	0.32	3.77E-03	8.03E-03
Psed_5823	ectoine hydroxylase	-1.59	-0.64	-0.69	-1.79	3.91E-03	8.31E-03
Psed_6882	filamentation induced by cAMP protein Fic	0.44	0.17	1.16	-0.06	4.07E-03	8.61E-03
Psed_1879	exopolysaccharide biosynthesis polyprenyl glycosylphosphotransferase	-0.99	-0.90	-0.28	-1.62	4.14E-03	8.73E-03
Psed_1509	Mov34/MPN/PAD-1 family protein	-0.21	-0.15	-0.33	-1.18	4.21E-03	8.85E-03
Psed_4839	phosphoketolase	-2.70	-1.07	-1.82	-1.73	4.34E-03	9.08E-03
Psed_4849	heavy metal translocating P-type ATPase	-1.93	-0.36	-1.08	-1.31	4.38E-03	9.15E-03
Psed_4860	hypothetical protein	-1.57	0.04	-0.74	-0.61	4.51E-03	9.36E-03
Psed_0174	phosphoesterase PA-phosphatase related	-0.79	-0.23	-0.43	-1.16	4.52E-03	9.38E-03
Psed_5406	NAD(P)(+) transhydrogenase (AB-specific)	1.06	1.09	1.15	0.67	4.66E-03	9.62E-03
Psed_4711	peptidase M50	-0.43	-0.15	-0.14	-1.06	4.75E-03	9.76E-03
Psed_5202	hypothetical protein	-0.73	-1.20	-0.14	-0.61	4.81E-03	9.87E-03

Appendix 11. Log_2FCs of differentially expressed genes from all growth conditions relative to pyruvate.

Appendix 12

Direct comparison versus a comparison relative to pyruvate- of 1,4-dioxane- and glycolate-grown *P. dioxanivorans* CB1190.

Appendix 12. Direct comparison versus a comparison relative to pyruvate- of 1,4-dioxane- and glycolate-grown *P. dioxanivorans*. The list is based upon genes differentially expressed and up-regulated genes ($log_2FC \geq 1$, FDR < 0.01) on 1,4-dioxane versus glycolate. Y = Yes, N = No.

Gene	Protein	log2FC Dioxane vs. Glycolate	Up-regulated on Dioxane vs. Pyruvate and not Glycolate vs. Pyruvate	log_2FC Dioxane vs. Pyruvate	log_2FC Glycolate vs. Pyruvate
Psed_0038	regulatory protein ArsR	2.53	Y	1.2	-1.3
Psed_1658	Trimethylamine-N-oxide reductase (cytochrome c)	3.17	Y	2.4	-0.7
Psed_3564	Aromatic-amino-acid transaminase	1.22	Y	1.5	0.3
Psed_3653	hypothetical protein	1.53	Y	1.5	-0.0
Psed_3935	GntR domain protein	1.24	Y	1.5	0.2
Psed_4146	Potassium-transporting ATPase B chain	1.48	Y	1.6	0.1
Psed_4147	Potassium-transporting ATPase A chain	1.17	Y	1.3	0.1
Psed_6259	FMN-dependent oxidoreductase, nitrilotriacetate monooxygenase family	1.29	Y	1.5	0.2
Psed_6261	ABC-type transporter, integral membrane subunit	1.32	Y	1.3	-0.0
Psed_6262	ABC-type transporter, integral membrane subunit	1.21	Y	1.2	-0.0
Psed_6263	Extracellular ligand-binding receptor	1.38	Y	1.2	-0.2
Psed_6728	Amidase	1.30	Y	1.4	0.1
Psed_6729	hypothetical protein	1.88	Y	1.9	0.0
Psed_6730	Luciferase-like, subgroup	2.02	Y	1.6	-0.4
Psed_6732	MaoC domain protein dehydratase	1.19	Y	1.2	0.0
Psed_6742	NLP/P60 protein	1.42	Y	1.5	0.1
Psed_6743	hypothetical protein	3.19	Y	2.5	-0.7
Psed_6744	hypothetical protein	2.22	Y	2.0	-0.2

Psed_6745	ATP-binding protein	2.06	Y	1.9	-0.2
Psed_6746	hypothetical protein	3.38	Y	2.6	-0.8
Psed_6747	hypothetical protein	2.46	Y	1.9	-0.5
Psed_6748	hypothetical protein	2.99	Y	2.3	-0.7
Psed_6749	hypothetical protein	3.78	Y	3.0	-0.7
Psed_6750	hypothetical protein	2.34	Y	2.1	-0.2
Psed_6751	Transglycosylase-like domain protein	2.99	Y	2.3	-0.7
Psed_6752	hypothetical protein	2.44	Y	2.2	-0.3
Psed_6753	hypothetical protein	1.82	Y	1.8	-0.1
Psed_6754	hypothetical protein	2.23	Y	1.9	-0.4
Psed_6755	hypothetical protein	1.96	Y	1.8	-0.1
Psed_6757	hypothetical protein	1.67	Y	1.0	-0.6
Psed_6758	hypothetical protein	1.17	Y	1.2	-0.0
Psed_6888	C-5 cytosine-specific DNA methylase	1.38	Y	1.2	-0.2
Psed_6913	hypothetical protein	1.28	Y	1.3	-0.0
Psed_6970	D-lactate dehydrogenase (cytochrome)	3.39	Y	2.8	-0.6
Psed_6971	Hydroxyacid-oxoacid transhydrogenase	3.02	Y	2.5	-0.6
Psed_6972	GntR domain protein	2.64	Y	1.9	-0.7
Psed_6973	hypothetical protein	2.09	Y	1.5	-0.6
Psed_6974	Ethyl tert-butyl ether degradation EthD	2.32	Y	1.8	-0.5
Psed_6975	Betaine-aldehyde dehydrogenase	1.88	Y	1.5	-0.4
Psed_6977	Ferredoxin--NAD(+) reductase	2.70	Y	2.3	-0.4
Psed_6978	methane/phenol/toluene hydroxylase	1.89	Y	1.6	-0.3
Psed_6979	monooxygenase component MmoB/DmpM	2.36	Y	2.0	-0.4
Psed_6980	hypothetical protein	3.22	Y	2.6	-0.6
Psed_6981	Aldehyde Dehydrogenase	3.70	Y	3.4	-0.3

Appendix 12. Direct comparison versus a comparison relative to pyruvate- of 1,4-dioxane- and glycolate-grown *P. dioxanivorans* CB1190

Psed_6982	Mn2+/Fe2+ transporter, NRAMP family	3.95	Y	3.9	-0.1
Psed_7002	transcription factor WhiB	2.08	Y	1.5	-0.6
Psed_7003	hypothetical protein	1.57	Y	1.5	-0.1
Psed_7007	hypothetical protein	1.22	Y	1.4	0.2
Psed_7008	hypothetical protein	1.29	Y	1.2	-0.1
Psed_2204	50S ribosomal protein L33	5.34	N	-0.1	-5.4
Psed_0249	L-lysine 6-monooxygenase (NADPH)	3.48	N	0.6	-2.8
Psed_3903	hypothetical protein	3.32	N	0.1	-3.2
Psed_3904	CO dehydrogenase maturation factor-like protein	3.31	N	0.1	-3.2
Psed_0164	hypothetical protein	3.22	N	0.6	-2.6
Psed_0011	hypothetical protein	3.19	N	0.0	-3.1
Psed_6094	ABC-type metal ion transporter, periplasmic subunit	3.10	N	-0.6	-3.7
Psed_0250	Xenobiotic-transporting ATPase	3.00	N	0.4	-2.6
Psed_6161	Siderophore-interacting protein	2.84	N	0.3	-2.5
Psed_3996	Methionyl-tRNA formyltransferase	2.82	N	0.9	-1.9
Psed_0251	Xenobiotic-transporting ATPase	2.78	N	0.3	-2.5
Psed_5844	hypothetical protein	2.76	N	-1.1	-3.8
Psed_6095	Fe(3+)-transporting ATPase	2.68	N	-0.6	-3.3
Psed_0247	ABC-type transporter, integral membrane subunit	2.57	N	0.8	-1.8
Psed_0252	amino acid adenylation domain protein	2.54	N	0.4	-2.1
Psed_2032	50S ribosomal protein L31 type B	2.47	N	0.3	-2.2
Psed_3667	SSS sodium solute transporter superfamily	2.46	N	-1.1	-3.5
Psed_0248	ABC-type transporter, periplasmic subunit	2.43	N	0.4	-2.1
Psed_6162	MbtH domain protein	2.37	N	0.1	-2.3
Psed_5471	hypothetical protein	2.29	N	-0.9	-3.2
Psed_5843	hypothetical protein	2.22	N	-1.0	-3.3

Appendix 12. Direct comparison versus a comparison relative to pyruvate- of 1,4-dioxane- and glycolate-grown *P. dioxanivorans* CB1190

Psed_0982	fibronectin-attachment family protein	2.13	N	0.9	-1.3
Psed_2203	cobalamin synthesis CobW domain protein	2.04	N	-0.2	-2.2
Psed_0013	hypothetical protein	1.97	N	-0.1	-2.1
Psed_6096	ABC-type transporter, integral membrane subunit	1.95	N	-0.2	-2.2
Psed_6968	transposase mutator type	1.94	N	-0.0	-1.9
Psed_0021	isochorismatase hydrolase	1.91	N	0.2	-1.7
Psed_1656	hypothetical protein	1.90	N	-1.3	-3.2
Psed_6805	hypothetical protein	1.88	N	-1.1	-2.9
Psed_3679	Dimethylallyltranstransferase	1.86	N	-0.1	-2.0
Psed_1657	Cobalt transporter subunit CbtB putative	1.82	N	-1.3	-3.1
Psed_2329	Peroxidase	1.82	N	-0.7	-2.5
Psed_0246	ABC-type transporter, integral membrane subunit	1.82	N	0.7	-1.2
Psed_6756	hypothetical protein	1.80	N	0.9	-0.9
Psed_3440	SH3 type 3 domain protein	1.80	N	-0.3	-2.1
Psed_3174	hypothetical protein	1.79	N	-0.9	-2.7
Psed_0106	putative Fe-S oxidoreductase	1.76	N	0.7	-1.1
Psed_6599	N-acetylmuramoyl-L-alanine amidase family 2	1.69	N	-1.2	-2.9
Psed_3682	hopanoid biosynthesis associated radical SAM protein HpnH	1.68	N	-0.2	-1.9
Psed_0580	highly repetitive protein	1.67	N	0.1	-1.5
Psed_6889	hypothetical protein	1.67	N	0.0	-1.7
Psed_6202	pyrimidine utilization protein A	1.65	N	-0.2	-1.8
Psed_2033	ABC-type transporter, periplasmic subunit	1.64	N	-0.3	-2.0
Psed_6914	amidohydrolase	1.64	N	-0.6	-2.2
Psed_6890	hypothetical protein	1.64	N	0.1	-1.6
Psed_0998	hypothetical protein	1.60	N	0.8	-0.8

Appendix 12. Direct comparison versus a comparison relative to pyruvate- of 1,4-dioxane- and glycolate-grown *P. dioxanivorans* CB1190

Psed_3726	Xylose isomerase domain-containing protein TIM barrel	1.56	N	-2.2	-3.7
Psed_3681	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	1.55	N	-0.0	-1.6
Psed_2812	Cell division protein sepF	1.53	N	-1.4	-2.9
Psed_0010	hypothetical protein	1.53	N	1.0	-0.6
Psed_3680	squalene-hopene cyclase	1.52	N	0.1	-1.4
Psed_0282	esterase	1.52	N	0.0	-1.5
Psed_6948	transposase mutator type	1.51	N	-0.6	-2.1
Psed_3894	ErfK/YbiS/YcfS/YnhG family protein	1.47	N	-0.3	-1.8
Psed_0012	hypothetical protein	1.46	N	-0.0	-1.5
Psed_3725	Extracellular ligand-binding receptor	1.44	N	-1.9	-3.4
Psed_3971	glutamate--cysteine ligase GCS2	1.42	N	0.4	-1.0
Psed_3676	squalene synthase HpnC	1.41	N	-0.8	-2.2
Psed_2914	Iron-chelate-transporting ATPase	1.37	N	0.1	-1.3
Psed_1654	hypothetical protein	1.37	N	-1.2	-2.6
Psed_5355	regulatory protein LuxR	1.37	N	-0.3	-1.7
Psed_2913	ABC-type transporter, integral membrane subunit	1.36	N	-0.1	-1.5
Psed_6114	FAD-binding 9 siderophore-interacting domain protein	1.36	N	-0.2	-1.6
Psed_0951	hypothetical protein	1.35	N	-0.4	-1.7
Psed_2328	high-affinity nickel-transporter	1.35	N	0.6	-0.8
Psed_0950	hypothetical protein	1.34	N	-0.5	-1.8
Psed_3972	nitroreductase	1.34	N	0.3	-1.0
Psed_5881	Heme oxygenase	1.33	N	-0.1	-1.4
Psed_2995	monosaccharide-transporting ATPase	1.27	N	-1.2	-2.5

Appendix 12. Direct comparison versus a comparison relative to pyruvate- of 1,4-dioxane- and glycolate-grown *P. dioxanivorans* CB1190

Psed_1713	crotonyl-CoA reductase	1.27	N	-0.3	-1.6
Psed_2800	penicillin-binding protein transpeptidase	1.24	N	-0.7	-2.0
Psed_2799	Ribosomal RNA small subunit methyltransferase H	1.24	N	-0.6	-1.9
Psed_1655	hypothetical protein	1.23	N	-0.7	-1.9
Psed_5496	regulatory protein MerR	1.23	N	0.3	-1.0
Psed_0889	Tat-translocated protein	1.23	N	0.8	-0.4
Psed_2809	cell division protein FtsZ	1.22	N	-1.0	-2.2
Psed_6737	alpha/beta hydrolase fold	1.22	N	0.8	-0.4
Psed_6892	hypothetical protein	1.21	N	-0.5	-1.7
Psed_3729	3-oxoacyl-[acyl-carrier-protein] reductase	1.21	N	-2.0	-3.2
Psed_3995	ABC-type transporter, periplasmic subunit	1.20	N	-1.4	-2.6
Psed_5293	hypothetical protein	1.19	N	0.4	-0.8
Psed_2715	sporulation and cell division protein SsgA	1.19	N	0.4	-0.8
Psed_3043	formate dehydrogenase, alpha subunit	1.17	N	0.4	-0.8
Psed_7019	hypothetical protein	1.17	N	-0.2	-1.4
Psed_6994	Cobyrinic acid ac-diamide synthase	1.17	N	0.0	-1.1
Psed_2887	diguanylate cyclase	1.17	N	-0.7	-1.8
Psed_3271	BFD domain protein [2Fe-2S]-binding domain protein	1.17	N	0.8	-0.4
Psed_1691	methylmalonyl-CoA mutase, large subunit	1.16	N	-0.3	-1.5
Psed_7018	putative partitioning protein ParA	1.14	N	-0.3	-1.5
Psed_6958	hypothetical protein	1.13	N	1.0	-0.2
Psed_6987	Integrase catalytic region	1.13	N	0.7	-0.5
Psed_0079	ABC-type transporter, periplasmic subunit	1.13	N	-2.7	-3.9
Psed_6203	Luciferase-like, subgroup	1.13	N	0.1	-1.0
Psed_0023	regulatory protein GntR HTH	1.13	N	-0.3	-1.5

Appendix 12. Direct comparison versus a comparison relative to pyruvate- of 1,4-dioxane- and glycolate-grown *P. dioxanivorans* CB1190

Psed_3817	hypothetical protein	1.12	N	-0.4	-1.6
Psed_3722	hypothetical protein	1.12	N	-2.3	-3.4
Psed_3678	squalene-associated FAD-dependent desaturase	1.12	N	-0.3	-1.4
Psed_3042	NADH dehydrogenase (quinone)	1.11	N	0.9	-0.2
Psed_2132	hypothetical protein	1.11	N	-1.0	-2.1
Psed_4688	polysaccharide deacetylase	1.10	N	-1.9	-3.0
Psed_2893	hypothetical protein	1.09	N	-0.1	-1.2
Psed_0022	hypothetical protein	1.09	N	-0.0	-1.1
Psed_6891	Relaxase/mobilization nuclease family protein	1.09	N	0.2	-0.9
Psed_6961	hypothetical protein	1.09	N	-0.4	-1.5
Psed_3728	Pyruvate dehydrogenase (acetyl-transferring)	1.08	N	-2.1	-3.2
Psed_6988	IstB domain protein ATP-binding protein	1.06	N	0.6	-0.5
Psed_5215	cell envelope-related function transcriptional attenuator, LytR/CpsA family	1.06	N	-1.1	-2.1
Psed_6951	hypothetical protein	1.05	N	0.3	-0.7
Psed_2491	hypothetical protein	1.05	N	0.5	-0.6
Psed_2763	hypothetical protein	1.05	N	-1.2	-2.2
Psed_3375	NLP/P60 protein	1.05	N	-0.7	-1.7
Psed_6893	hypothetical protein	1.03	N	-0.6	-1.7
Psed_0078	Methyltransferase type 12	1.03	N	-2.6	-3.7
Psed_3175	hypothetical protein	1.02	N	0.0	-1.0
Psed_5710	hypothetical protein	1.01	N	-0.5	-1.5
Psed_3723	oxidoreductase domain protein	1.01	N	-2.0	-3.0

Appendix 12. Direct comparison versus a comparison relative to pyruvate- of 1,4-dioxane- and glycolate-grown *P. dioxanivorans* CB1190

Appendix 13

Direct comparison versus a comparison relative to pyruvate- of THF- and succinate-grown *P. dioxanivorans* CB1190.

Appendix 13. Direct comparison versus a comparison relative to pyruvate- of THF- and succinate-grown *P. dioxanivorans*. The list is based upon genes differentially expressed and up-regulated genes ($log_2FC \geq 1$, FDR < 0.01) on THF versus succinate Y = yes, N = No.

Gene	Protein	log_2FC THF vs. Succinate	Up-regulated on THF vs. Pyruvate and not Succinate vs. Pyruvate	log_2FC THF vs Pyruvate	log_2FC Succinate vs. Pyruvate
Psed_0094	YCII-related	1.69	Y	2.46	0.77
Psed_0095	RNA polymerase sigma factor, sigma-70 family	1.32	Y	1.52	0.21
Psed_0130	amidohydrolase 2	1.34	Y	2.17	0.83
Psed_0131	Alcohol dehydrogenase GroES domain protein	1.39	Y	1.99	0.60
Psed_0288	S-(hydroxymethyl)glutathione dehydrogenase	1.58	Y	1.76	0.18
Psed_0355	hypothetical protein	1.67	Y	1.26	-0.41
Psed_0357	helix-turn-helix domain protein	1.02	Y	1.08	0.06
Psed_0934	YCII-related	2.74	Y	2.58	-0.16
Psed_1301	Redoxin domain protein	1.08	Y	1.03	-0.05
Psed_1303	Heavy metal transport/detoxification protein	1.77	Y	1.05	-0.72
Psed_1584	6-phosphofructokinase	1.54	Y	1.05	-0.49
Psed_1967	major facilitator superfamily MFS_1	1.76	Y	1.85	0.09
Psed_1968	hypothetical protein	1.84	Y	2.11	0.27
Psed_2321	ABC-type sugar transport system periplasmic component-like	1.51	Y	1.22	-0.29
Psed_3256	hypothetical protein	2.35	Y	3.34	0.99
Psed_3564	Aromatic-amino-acid transaminase	1.19	Y	1.66	0.47
Psed_3652	hypothetical protein	1.77	Y	1.52	-0.25
Psed_3669	hypothetical protein	1.63	Y	2.28	0.66
Psed_4236	hypothetical protein	1.04	Y	1.03	-0.01

Appendix 13. Direct comparison versus a comparison relative to pyruvate- of THF- and succinate-grown *P. dioxanivorans*. CB1190

Psed_4268	membrane protein of uknown function UCP014873	1.94	Y	2.06	0.12
Psed_4321	hypothetical protein	2.18	Y	1.55	-0.63
Psed_4322	Dihydrolipoyl dehydrogenase	2.63	Y	2.21	-0.42
Psed_4702	protein of unknown function DUF1295	2.69	Y	3.20	0.52
Psed_4753	nitrogen regulatory protein P-II	1.08	Y	1.15	0.06
Psed_4937	hypothetical protein	1.67	Y	1.06	-0.61
Psed_4938	regulatory protein MarR	2.99	Y	2.83	-0.16
Psed_4939	lipase	6.88	Y	6.70	-0.18
Psed_4940	fumarylacetoacetate (FAA) hydrolase	5.23	Y	5.21	-0.01
Psed_4941	Formyl-CoA transferase	2.76	Y	2.78	0.02
Psed_5224	hypothetical protein	1.74	Y	1.19	-0.55
Psed_5245	regulatory protein TetR	1.28	Y	1.13	-0.15
Psed_5246	uncharacterized peroxidase-related enzyme	1.63	Y	1.64	0.01
Psed_5247	hypothetical protein	2.23	Y	2.10	-0.13
Psed_5248	nitroreductase	4.41	Y	4.37	-0.04
Psed_5249	NADPH:quinone reductase	3.70	Y	3.45	-0.25
Psed_5736	Transglycosylase-like domain protein	1.36	Y	2.30	0.94
Psed_5747	hypothetical protein	1.30	Y	1.83	0.53
Psed_5753	hypothetical protein	1.45	Y	1.73	0.29
Psed_5757	hypothetical protein	1.61	Y	1.79	0.18
Psed_5758	hypothetical protein	1.68	Y	2.27	0.60
Psed_5759	hypothetical protein	1.94	Y	2.39	0.45
Psed_5760	hypothetical protein	1.96	Y	2.22	0.26
Psed_5761	hypothetical protein	1.41	Y	2.02	0.61
Psed_5762	hypothetical protein	1.30	Y	1.63	0.33
Psed_5763	hypothetical protein	1.00	Y	1.35	0.35

Appendix 13. Direct comparison versus a comparison relative to pyruvate- of THF- and succinate-grown *P. dioxanivorans.* CB1190

Psed_5764	hypothetical protein	1.09	Y	1.31	0.22
Psed_5767	hypothetical protein	1.36	Y	1.50	0.14
Psed_5769	hypothetical protein	1.37	Y	1.55	0.19
Psed_5778	cell divisionFtsK/SpoIIIE	1.22	Y	1.33	0.12
Psed_5779	hypothetical protein	1.93	Y	2.01	0.08
Psed_6249	Ribulose bisphosphate carboxylase large chain	1.34	Y	1.15	-0.20
Psed_6665	NLP/P60 protein	1.92	Y	2.19	0.27
Psed_6780	hypothetical protein	1.66	Y	1.57	-0.10
Psed_6806	hypothetical protein	1.43	Y	1.31	-0.12
Psed_6807	hypothetical protein	2.90	Y	2.79	-0.11
Psed_6847	hypothetical protein	1.02	Y	1.11	0.08
Psed_6879	S-adenosylmethionine synthase	2.04	Y	1.82	-0.23
Psed_6970	D-lactate dehydrogenase (cytochrome)	2.91	Y	2.71	-0.20
Psed_6971	Hydroxyacid-oxoacid transhydrogenase	1.73	Y	2.17	0.44
Psed_6972	GntR domain protein	1.35	Y	1.66	0.31
Psed_6977	Ferredoxin--NAD(+) reductase	1.78	Y	2.48	0.70
Psed_6978	methane/phenol/toluene hydroxylase	1.05	Y	1.59	0.54
Psed_6979	monooxygenase component MmoB/DmpM	1.55	Y	2.23	0.68
Psed_6981	Aldehyde Dehydrogenase	2.48	Y	3.35	0.88
Psed_6982	Mn2+/Fe2+ transporter, NRAMP family	3.21	Y	3.46	0.25
Psed_4700	Cyclopropane-fatty-acyl-phospholipid synthase	3.85	N	4.86	1.02
Psed_1422	Ferritin Dps family protein	3.54	N	-0.69	-4.23
Psed_0079	ABC-type transporter, periplasmic subunit	3.41	N	-0.39	-3.80
Psed_2204	50S ribosomal protein L33	3.02	N	-0.53	-3.54
Psed_3049	Formamidase	2.87	N	0.86	-2.02

Appendix 13. Direct comparison versus a comparison relative to pyruvate- of THF- and succinate-grown *P. dioxanivorans.* CB1190

Psed_6094	ABC-type metal ion transporter, periplasmic sub-unit	2.79	N	-0.52	-3.31
Psed_5471	hypothetical protein	2.59	N	-1.41	-4.00
Psed_2789	hypothetical protein	2.51	N	0.29	-2.22
Psed_5783	hypothetical protein	2.47	N	4.12	1.65
Psed_2203	cobalamin synthesis CobW domain protein	2.46	N	-0.43	-2.89
Psed_0078	Methyltransferase type 12	2.44	N	-1.77	-4.21
Psed_0950	hypothetical protein	2.39	N	0.45	-1.94
Psed_3571	Radical SAM domain protein	2.37	N	-0.06	-2.43
Psed_6640	extracellular solute-binding protein family 1	2.27	N	-1.54	-3.81
Psed_1655	hypothetical protein	2.24	N	-0.26	-2.50
Psed_6753	hypothetical protein	2.12	N	0.89	-1.23
Psed_1657	Cobalt transporter subunit CbtB putative	2.12	N	-0.22	-2.34
Psed_4701	Cyclopropane-fatty-acyl-phospholipid synthase	2.11	N	3.38	1.27
Psed_3048	AmiS/UreI transporter	2.05	N	-0.07	-2.12
Psed_5868	hypothetical protein	1.98	N	0.16	-1.82
Psed_3037	ABC-type metal ion transporter, periplasmic sub-unit	1.97	N	-0.03	-1.99
Psed_3440	SH3 type 3 domain protein	1.90	N	-0.76	-2.66
Psed_3038	Sulfate-transporting ATPase	1.86	N	0.70	-1.16
Psed_5317	Error-prone DNA polymerase	1.85	N	0.46	-1.39
Psed_6096	ABC-type transporter, integral membrane subunit	1.82	N	-0.07	-1.89
Psed_4703	alkyl hydroperoxide reductase/ Thiol specific anti-oxidant/ Mal allergen	1.82	N	2.96	1.13
Psed_6095	Fe(3+)-transporting ATPase	1.80	N	-0.90	-2.70
Psed_6751	Transglycosylase-like domain protein	1.78	N	0.97	-0.81

Appendix 13. Direct comparison versus a comparison relative to pyruvate- of THF- and succinate-grown *P. dioxanivorans.* CB1190

Psed_6980	hypothetical protein	1.76	N	2.93	1.18
Psed_4819	integral membrane protein	1.75	N	0.47	-1.28
Psed_3904	CO dehydrogenase maturation factor-like protein	1.73	N	-1.02	-2.75
Psed_1302	protein of unknown function DUF156	1.71	N	1.20	-0.51
Psed_0982	fibronectin-attachment family protein	1.70	N	0.53	-1.17
Psed_1107	peptidase C60 sortase A and B	1.68	N	0.30	-1.38
Psed_2265	hypothetical protein	1.68	N	0.38	-1.30
Psed_2129	DNA binding domain protein, excisionase family	1.67	N	0.32	-1.36
Psed_3985	hypothetical protein	1.65	N	0.89	-0.76
Psed_1656	hypothetical protein	1.64	N	-0.55	-2.19
Psed_0139	nuclear export factor GLE1	1.62	N	-1.61	-3.23
Psed_5784	hypothetical protein	1.60	N	3.79	2.19
Psed_0016	Integrase catalytic region	1.60	N	0.53	-1.07
Psed_0106	putative Fe-S oxidoreductase	1.59	N	-0.37	-1.96
Psed_0137	copper resistance D domain protein	1.59	N	-0.60	-2.18
Psed_2397	putative acyl-CoA dehydrogenase-related protein	1.57	N	0.87	-0.71
Psed_3023	cobaltochelatase, CobN subunit	1.56	N	-0.14	-1.70
Psed_0580	highly repetitive protein	1.56	N	0.19	-1.37
Psed_1421	ferric-uptake regulator	1.56	N	-2.00	-3.56
Psed_2652	carbon-monoxide dehydrogenase, large subunit	1.55	N	0.57	-0.98
Psed_0082	Monosaccharide-transporting ATPase	1.53	N	0.22	-1.31
Psed_5320	thioredoxin	1.51	N	0.69	-0.81
Psed_6544	hypothetical protein	1.51	N	0.66	-0.85
Psed_5844	hypothetical protein	1.50	N	-1.19	-2.68
Psed_6799	transposase IS4 family protein	1.49	N	0.91	-0.58
Psed_1653	hypothetical protein	1.48	N	-0.36	-1.84

Appendix 13. Direct comparison versus a comparison relative to pyruvate- of THF- and succinate-grown *P. dioxanivorans.* CB1190

Psed_0138	copper resistance protein CopC	1.47	N	-1.36	-2.83
Psed_6409	Endonuclease/exonuclease/phosphatase	1.46	N	0.76	-0.69
Psed_5324	hypothetical protein	1.46	N	-0.06	-1.52
Psed_5814	hypothetical protein	1.45	N	3.71	2.26
Psed_1585	hypothetical protein	1.43	N	0.39	-1.04
Psed_4359	hypothetical protein	1.39	N	0.61	-0.77
Psed_2491	hypothetical protein	1.37	N	0.65	-0.73
Psed_2123	Peptidoglycan-binding lysin domain	1.37	N	-0.66	-2.03
Psed_3017	precorrin-6y C5,15-methyltransferase (decarboxyl-ating), CbiE subunit	1.36	N	0.14	-1.22
Psed_2650	hypothetical protein	1.36	N	0.16	-1.20
Psed_0951	hypothetical protein	1.36	N	-0.30	-1.65
Psed_6737	alpha/beta hydrolase fold	1.36	N	0.85	-0.51
Psed_1268	Radical SAM domain protein	1.35	N	0.68	-0.67
Psed_2688	major facilitator superfamily MFS_1	1.34	N	0.72	-0.62
Psed_2788	hypothetical protein	1.32	N	0.73	-0.59
Psed_2939	putative acyltransferase	1.31	N	0.42	-0.90
Psed_0369	GCN5-related N-acetyltransferase	1.31	N	0.61	-0.70
Psed_1362	hypothetical protein	1.31	N	1.14	-0.17
Psed_5323	hypothetical protein	1.30	N	0.35	-0.95
Psed_6951	hypothetical protein	1.30	N	0.26	-1.04
Psed_3069	ErfK/YbiS/YcfS/YnhG family protein	1.29	N	0.70	-0.59
Psed_6732	MaoC domain protein dehydratase	1.28	N	0.83	-0.45
Psed_3594	Tellurite resistance TerB	1.26	N	-1.40	-2.67
Psed_0055	non-ribosomal peptide synthetase domain protein	1.26	N	0.30	-0.96
Psed_6856	Rhodanese-like protein	1.26	N	0.53	-0.73

Appendix 13. Direct comparison versus a comparison relative to pyruvate- of THF- and succinate-grown *P. dioxanivorans.* CB1190

Psed_5967	4Fe-4S ferredoxin iron-sulfur binding domain-containing protein	1.26	N	0.83	-0.43
Psed_6855	beta-lactamase domain protein	1.26	N	0.79	-0.46
Psed_4276	hypothetical protein	1.25	N	0.93	-0.32
Psed_4360	heavy metal translocating P-type ATPase	1.23	N	0.39	-0.84
Psed_4583	hypothetical protein	1.22	N	0.96	-0.26
Psed_6193	Xenobiotic-transporting ATPase	1.22	N	0.31	-0.91
Psed_6754	hypothetical protein	1.22	N	0.42	-0.80
Psed_3059	hypothetical protein	1.20	N	0.56	-0.64
Psed_2647	hypothetical protein	1.20	N	0.45	-0.75
Psed_6730	Luciferase-like, subgroup	1.20	N	0.93	-0.26
Psed_0081	ABC-type transporter, integral membrane subunit	1.19	N	-0.20	-1.38
Psed_2937	HNH endonuclease	1.18	N	0.72	-0.46
Psed_2411	FAD-dependent pyridine nucleotide-disulphide oxidoreductase	1.18	N	0.54	-0.64
Psed_0080	ABC-type transporter, integral membrane subunit	1.18	N	-0.31	-1.49
Psed_4627	YceI family protein	1.18	N	-0.06	-1.24
Psed_5811	hypothetical protein	1.17	N	3.75	2.58
Psed_5166	hypothetical protein	1.15	N	0.75	-0.40
Psed_2444	PDZ/DHR/GLGF domain protein	1.15	N	0.74	-0.42
Psed_6755	hypothetical protein	1.15	N	0.44	-0.71
Psed_6606	hypothetical protein	1.15	N	-0.37	-1.52
Psed_5786	phage terminase	1.15	N	3.43	2.28
Psed_6401	heavy metal translocating P-type ATPase	1.14	N	0.32	-0.82
Psed_6645	hypothetical protein	1.14	N	-0.85	-1.99
Psed_5800	hypothetical protein	1.13	N	3.80	2.67

Appendix 13. Direct comparison versus a comparison relative to pyruvate- of THF- and succinate-grown *P. dioxanivorans.* CB1190

Psed_4339	hypothetical protein	1.13	N	0.17	-0.96
Psed_3317	hypothetical protein	1.13	N	0.26	-0.87
Psed_0140	hypothetical protein	1.12	N	-1.44	-2.57
Psed_4881	hypothetical protein	1.12	N	0.40	-0.71
Psed_0037	hypothetical protein	1.11	N	0.70	-0.42
Psed_5688	hypothetical protein	1.11	N	0.60	-0.51
Psed_3105	membrane protein AbrB duplication	1.10	N	0.16	-0.94
Psed_1292	alkyl hydroperoxide reductase/ Thiol specific anti-oxidant/ Mal allergen	1.09	N	0.36	-0.73
Psed_6944	Xylose isomerase domain-containing protein TIM barrel	1.09	N	0.55	-0.53
Psed_1037	Asp/Glu/hydantoin racemase	1.08	N	0.07	-1.02
Psed_3903	hypothetical protein	1.08	N	-1.21	-2.29
Psed_6735	flavoprotein WrbA	1.08	N	0.89	-0.19
Psed_1571	Glutathione transferase	1.08	N	0.55	-0.54
Psed_4445	regulatory protein TetR	1.07	N	0.60	-0.47
Psed_5516	ferric-uptake regulator	1.07	N	0.27	-0.81
Psed_5809	hypothetical protein	1.07	N	3.02	1.95
Psed_4390	hypothetical protein	1.07	N	0.38	-0.69
Psed_6008	hypothetical protein	1.06	N	0.77	-0.29
Psed_5870	hypothetical protein	1.06	N	-0.68	-1.75
Psed_5488	FAD-dependent pyridine nucleotide-disulphide oxidoreductase	1.06	N	-0.77	-1.84
Psed_4443	hypothetical protein	1.06	N	0.18	-0.89
Psed_6197	major facilitator superfamily MFS_1	1.06	N	0.28	-0.78
Psed_5592	hypothetical protein	1.06	N	0.50	-0.56

Appendix 13. Direct comparison versus a comparison relative to pyruvate- of THF- and succinate-grown *P. dioxanivorans.* CB1190

Psed_5775	hypothetical protein	1.05	N	0.96	-0.10
Psed_5741	hypothetical protein	1.05	N	0.71	-0.34
Psed_6403	Heavy metal transport/detoxification protein	1.05	N	0.60	-0.45
Psed_0096	Uncharacterised protein family UPF0126	1.05	N	0.74	-0.30
Psed_2202	class II aldolase/adducin family protein	1.05	N	0.63	-0.41
Psed_6963	replicative DNA helicase	1.05	N	0.46	-0.58
Psed_5802	hypothetical protein	1.04	N	2.79	1.74
Psed_1898	CBS domain containing protein	1.04	N	0.89	-0.15
Psed_5480	small multidrug resistance protein	1.04	N	0.24	-0.80
Psed_0289	hypothetical protein	1.04	N	0.65	-0.39
Psed_6213	2'-5' RNA ligase	1.03	N	0.39	-0.65
Psed_4382	hypothetical protein	1.03	N	0.66	-0.38
Psed_6746	hypothetical protein	1.03	N	0.20	-0.84
Psed_5788	peptidase S14 ClpP	1.03	N	3.39	2.36
Psed_6736	Amidase	1.03	N	0.83	-0.20
Psed_1129	hypothetical protein	1.02	N	0.54	-0.49
Psed_1300	cytochrome c biogenesis protein transmembrane region	1.02	N	0.08	-0.94
Psed_6897	hypothetical protein	1.02	N	0.89	-0.13
Psed_2206	hypothetical protein	1.02	N	0.51	-0.51
Psed_1297	haloacid dehalogenase, type II	1.01	N	0.57	-0.44
Psed_1284	hypothetical protein	1.01	N	0.47	-0.54
Psed_1654	hypothetical protein	1.01	N	-0.92	-1.93
Psed_6818	hypothetical protein	1.01	N	0.15	-0.86
Psed_2791	hypothetical protein	1.01	N	0.00	-1.01
Psed_1102	ROK family protein	1.01	N	0.98	-0.03

Appendix 13. Direct comparison versus a comparison relative to pyruvate- of THF- and succinate-grown *P. dioxanivorans.* CB1190

Psed_5527	hypothetical protein	1.01	N	0.42	-0.59
Psed_4899	hypothetical protein	1.01	N	0.47	-0.54
Psed_2549	L-serine dehydratase 1	1.00	N	0.27	-0.74
Psed_3589	stress protein	1.00	N	-1.78	-2.78

Appendix 13. Direct comparison versus a comparison relative to pyruvate- of THF- and succinate-grown *P. dioxanivorans.* CB1190