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Publication Date
2018-04-01

Peer reviewed

Metabolic Engineering xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Metabolic Engineering

journal homepage: www.elsevier.com/locate/meteng

Escherichia coli as a host for metabolic engineering

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A B S T R A C T

Over the past century, *Escherichia coli* has become one of the best studied organisms on earth. Features such as genetic tractability, favorable growth conditions, well characterized biochemistry and physiology, and availability of versatile genetic manipulation tools make *E. coli* an ideal platform host for development of industrially viable productions. In this review, we discuss the physiological attributes of *E. coli* that are most relevant for metabolic engineering, as well as emerging techniques that enable efficient phenotype construction. Further, we summarize the large number of native and non-native products that have been synthesized by *E. coli*, and address some of the future challenges in broadening substrate range and fighting phage infection.

1. Introduction

Escherichia coli is a Gram-negative, facultative anaerobic bacterium originally discovered in the human colon in 1885 by German bacteriologist Theodor Escherich (Feng et al., 2002). Owing to extensive investigation and development, *E. coli* has become the best characterized organism on earth, the workhorse in molecular biology laboratories, and one of the most important organisms used in industry. Because of its rapid growth, easy culture conditions, metabolic plasticity, wealth of biochemical and physiological knowledge, and the plethora of tools for genetic and genomic engineering, *E. coli* has also become one of the best host organisms for metabolic engineering and synthetic biology. Commonly used *E. coli* strains are generally considered harmless. Laboratory strains, such as K-12, are classified as Risk Group 1 for lack of *O*-antigens, virulence factors, colonization factors, and association with disease in healthy human adults (NIH, 2016). These non-pathogenic strains have been widely used for productions of pharmaceuticals, food, chemicals, and fuels.

E. coli by no means is the most versatile organisms for industrial purposes. However, the large body of knowledge in *E. coli* biochemistry, physiology, and genetics has enabled rapid progress in *E. coli* metabolic engineering and synthetic biology. As such, many previously perceived limitations have been overcome, and new phenotypes have been engineered in *E. coli* that surpass the traditional native producers. For example, *E. coli* has been engineered for industrial production of amino acids which has traditionally been produced from the natural producer *Corynebacterium glutamicum* (Gusyatiner et al., 2017). *E. coli* production of n-butanol has also been demonstrated to the level similar to that produced in *Clostridia* (Shen et al., 2011; Ohtake et al., 2017). In cases where no natural producers exist, *E. coli* has been among the top choices as the host for metabolic engineering. In addition to serving as a proof-of-concept model organism, *E. coli* has been used extensively as an industrial producer. Lysine (Kojima et al., 2000), 1,3-propanediol (PDO) (Sabra et al., 2016) and 1,4 butandiol (Burgard et al., 2016; Sanford et al., 2016) productions are prominent and successful examples. As such, *E. coli* is clearly the most preferred prokaryote for both laboratory and industrial applications. Here, we review the recent progress in physiology, genomics, and other aspects relevant to *E. coli* metabolic engineering, particularly for industrial applications.

This review is divided into four main sections. We first start by discussing aspects of cell physiology that have proven to be most relevant to metabolic engineering with focus on relatively recent progress. Next, we discuss modern tools used for strain engineering techniques with an emphasis on CRISPR related technologies and directed cell evolution. Following this, we provide an overview of chemicals that have been produced in *E. coli*, with the aim of conveying the versatility of metabolism for exploitation beyond common chemical products such as alcohols and amino acids. Lastly, we describe the most common compounds targeted as alternative carbon sources that can be further used for biochemical production, as well as efforts to engineer *E. coli* to

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https://doi.org/10.1016/j.ymben.2018.04.008
Received 17 February 2018; Received in revised form 11 April 2018; Accepted 12 April 2018

utilize them.

2. Why *E. coli*?

E. coli strains are easily cultured, have a short doubling time, and can thrive under a variety of growth conditions. Most importantly, *E. coli* can be easily manipulated genetically, enhancing our ability both to study its physiology and engineer new phenotypes. A large number of molecular cloning techniques and genetic tools have been developed using *E. coli*. Rapid strain development allowed by these techniques can significantly reduce costs for industrial development (Meyer and Schmidhalter, 2012).

E. coli has been used as the host for industrial production of various chemicals, including trypotophan, phenylalanine, threonine, lysine, nucleotides, succinate, itaconic acid, polyhydroxybutyate, and 1,3-PDO, as discussed below. In terms of biopharmaceutical applications, it was reported that of more than 100 recombinant protein products in the US and European markets, 34% of these are expressed in *E. coli* (Meyer and Schmidhalter, 2012). In 1998, *E. coli* K strain MG-1655 was sequenced (Blattner et al., 1997). To date, there are 484 strains of *E. coli* completely sequenced (NCBI, 2018). In 2006, an in-frame, single-gene deletion mutant collection, the Keio collection, was created (Baba et al., 2006) using *E. coli* K-12 BW25113. This collection has greatly facilitated *E. coli* physiological studies and metabolic engineering. Most general applications of *E. coli* are carried out using K strains. However, BL21 (B strain) and its derivative BL21(DE3), containing λDE3 lysogen harboring the gene for T7 RNA polymerase, are popular for recombinant protein production (Studier and Moffatt, 1986). Applications that involve high protein expression, such as production of protein biopharmaceuticals, most frequently use BL21 as a host because of its lack of *lon* and *ompT* proteases, as well as its insensitivity to high glucose concentrations (Meyer and Schmidhalter, 2012). The insensitivity to glucose concentration stems from high activity of the glyoxylate shunt, gluconeogenesis, anaplerotic pathways, and the TCA cycle, leading to reduced acetate production and efficient glucose utilization (Phue et al., 2008). Origami strains, K-12 derivatives, with *trxB* and *gor* mutations, have lower reducing power in the cytoplasm (Novagen) to allow better formation of disulfide bonds. DH5α is a frequently chosen host for production of plasmid DNA due to lack of endonuclease 1 (endA) and recombinase (recA) (Phue et al., 2008). W strains exhibit several valuable traits such as extensive substrate range, less acetate production, and higher product tolerances (Park et al., 2011; Prieto et al., 1996). The best known ethanol-producing strain KO11 (ATCC 55124) is a W strain (ATCC 9637) engineered with *Zymomonas mobilis pdc* and *adhB* genes (Ohta et al., 1991; Jarboe et al., 2007). The strain contains extensive chromosomal rearrangements, and multiple tandem copies of the *Z. mobilis pdc* and *adhB* genes (Turner et al., 2012).

However, there are some disadvantages for the use of *E. coli* as a host. *E. coli* is incapable of producing glycosylated biopharmaceutical products, proteins that require complex assembly, or proteins with high numbers of disulfide bonds (Meyer and Schmidhalter, 2012). In addition, *E. coli* is not suitable for culturing conditions at high (Tao et al., 2005) and low pH (Wernick et al., 2016), and high temperatures (Hasunuma and Kondo, 2012; Bhalla et al., 2013). These conditions present advantages for contamination resistance (Tao et al., 2005; Wernick et al., 2016), reduced titration requirements, and consolidated bioprocesses (Hasunuma and Kondo, 2012; Bhalla et al., 2013; Olson et al., 2012) in which certain substrates can be broken down and consumed simultaneously. Phage attack may also present a major threat to industrial production using bacteria (de Melo et al. (2018); Samson and Moineau, 2013) in non-sterile conditions.

3. *E. coli* physiology

To adapt to changing environments, living organisms have evolved extensive regulatory mechanisms to control metabolic pathways. In addition to the metabolic network itself, the regulatory mechanisms often play a critical role in metabolic engineering. In contrast to metabolic networks that can be designed rationally, it remains a challenge to predict *a priori* the exact alternations that need to be made in regulatory networks to achieve a desired phenotype or production goal. As a reminder of this significance, we will briefly discuss regulations that are most relevant to metabolic engineering and newly identified features in *E. coli* physiology.

3.1. Phophotransferase system

E. coli, as well as some facultative anaerobic microorganisms, use the phosphoenolpyruvate (PEP)-carbohydrate phosphotransferase system (PTS) to import and phosphorylate various sugars using PEP as the phosphoryl group donor. This system also irreversibly converts PEP to pyruvate and provides a driving force for sugar uptake. PTS is comprised of four phosphotransferases: Enzyme I (EI); Histidine Protein (HPr, Heat-stable Protein), carbohydrate-specific Enzyme IIA (EIIA), and carbohydrate-specific Enzyme II BC (EIIBC, which transfer the phosphoryl group on PEP to the importing sugar) (Pflüger-Grau and Görke, 2010). Steps from EI to EIIA are presumed to be in micro-equilibrium, which leads to the hypothesis that the PEP/pyruvate ratio serves as a flux sensor (Liao et al., 1996). In addition, PEP also allosterically inhibits phosphofructokinase (encoded by *pfkA* and *pfkF*) activity (Blangy et al., 1968). Most importantly, PTS is also closely involved in global carbon regulation (Deutscher et al., 2006). The phosphorylated form of glucose-specific EIIA has been reported to activate adenylate cyclase (CyaA), which synthesizes cAMP from ATP. In the absence of glucose or glucose-specific EIIBC, CyaA is activated (Crasnier-Mednansky et al., 1997; Notley-mcrobb et al., 2006; Reddy and Kamireddi, 1998). Although the detailed mechanism remains unclear, deletion of the gene (*ptsG*) coding for glucose-specific EIIBC has been shown to increase the cAMP level (Steinsiek and Bettenbrock, 2012), which activates the cAMP-CRP regulon. Through such mechanisms, PTS indirectly regulates the expression of a large number of genes involved in carbon metabolism.

Regulation of *ptsG* post-transcriptionally is carried out by the “sugar transport related” system, comprised of genes *sgrR, sgrS* and *sgrT* (Gabor et al., 2011). The small RNA SgrS depletes the abundance of mRNA of *ptsG* and the secondary glucose transporter *manXYZ*. SgrS transcription itself is positively regulated by glucose or glucose analogue, α-methyl glucoside (α-MG). However, the physiological effect was demonstrated in *E. coli* B, but not in K-12, during growth on high levels of glucose (Negrete et al., 2010). As expected, overexpression of SgrS in a K-12 strain was demonstrated to reduce the growth rate and acetate secretion during growth on glucose (Negrete et al., 2013; Ross et al., 2014). Extensive studies have been conducted to characterize SgrS and its role in regulating “glucose-phosphate stress response” (Vanderpool and Gottesman, 2007). Interestingly, SgrS could also encode a 43-amino-acid protein SgrT which interacts and blocks ptsG to allow non-preferred carbon source transport and utilization under glucose phosphate stress (Chelsea and Lloyd, 2017).

3.2. Glucose utilization

Glucose affects *E. coli* physiology at many levels. Glucose enters the cell through PTS, and as a result of using PEP as the phosphoryl group donor, PTS simultaneously splits the resources into two pools. The first pool is defined by PEP and all metabolites derived from PEP, including those of the TCA cycle, upper glycolysis, and the pentose phosphate pathway. The other pool is defined by pyruvate and all metabolites derived from pyruvate, including acetyl-CoA derived compounds. These are listed in Table 1. All other metabolites and building blocks required for growth are formed from these pools. Interestingly, the demands of the PEP and pyruvate pools for biosynthesis are roughly the same (Table 1), and the two pools are separated by a thermodynamic barrier.

Table 1
Building blocks required for growth.

Precursor metabolite	Amount required (μmol/g cells)[a]
PEP derived compounds	
Glucose 6-phosphate	205
Fructose 6-phosphate	70.9
Ribose 6-phosphate	897.7
Erythrose 4-phosphate	361
Triose phosphate	129
3-phosphoglycerate	1496
Phosphoenol pyruvate	519.1
α-ketoglutarate	1078.9
Oxaloacetate	1786.7
Total	**6539.6**
Pyruvate derived compounds	
Pyruvate	2832.8
Acetyl-CoA	3747.8
Total	**6580.6**

Data obtained from Neidhardt et al. (1990).

[a] These values are calculated from multiplication of the amount of each compound by the precursor metabolite molar requirement or other components required to produce them.

The pyruvate pool is not readily converted back to the PEP pool unless energy expenditure is involved, for example, by use of the gluconeogenesis enzyme PEP synthetase (Pps). It appears that PTS allocates the carbon source properly to ensure that the demand for biosynthesis is met. However, if one wishes to overproduce a metabolite that is derived from the PEP pool, such as aromatics or succinate, the maximum yield will be limited by the availability of PEP. This limitation can be removed by artificially inducing Pps (Patnaik and Liao, 1994a), or by use of a non-PTS glucose transport system, such as galactose permease (Hernández-Montalvo et al., 2003). Pyruvate also provides the two-carbon building block acetyl-CoA through either pyruvate decarboxylation complex or pyruvate-formate lyase. These two enzymes decarboxylate pyruvate to produce either CO_2 or formate, resulting in a loss of 1/3 of carbon. Thus, production of compounds derived from acetyl-CoA have a maximum theoretical carbon yield no greater than 66.7%. This is not a problem unique to *E. coli*, but to all organisms that derive acetyl-CoA from pyruvate. Recently, a synthetic non-oxidative glycolysis (NOG) pathway has been developed to avoid such a carbon loss (Bogorad et al., 2013). This pathway utilizes phosphoketolase, the non-oxidative part of pentose phosphate pathway, and gluconeogenic enzymes for carbon rearrangement. The net result is the production of three acetyl-CoA from one glucose. Initial proof of concept has been performed in *E. coli*, and further development of NOG-dependent *E. coli* strains is currently underway (Lin et al., 2018).

3.3. Respiration and fermentation

E. coli can grow and metabolize various substrates with or without oxygen. For industrial production, many traits are essential in strain development including rapid growth, product and substrate tolerance and high titers, yields, and productivities. Oxygen allows efficient ATP production in using reducing equivalents for oxidative phosphorylation. While this is favorable for growth, these reducing equivalents may otherwise be needed for production of reduced compounds. Thus, the facultative anaerobic feature of *E. coli* is beneficial, and regulations of aerobic and anaerobic respiration are important.

Other than oxygen, *E. coli* is capable of using several terminal electron acceptors for anaerobic respiration: nitrate, dimethyl sulfoxide (DMSO), trimethylamine-N-oxide (TMAO), and fumarate (Gunsalus, 1992). Although these terminal electron acceptors may not be practical for industrial use, they serve as useful tools for metabolic studies. In *E. coli*, anaerobic respiration connects a dehydrogenase and a terminal reductase or oxidase by a quinone pool (Unden and Bongaerts, 1997a). *E. coli* contains a large variety of each of these components. Therefore, the exact composition of a functional respiratory chain can be variable and depends on specific environmental conditions. Proteins involved in the respiratory chain have been discussed previously (Unden and Bongaerts, 1997a). Some examples of dehydrogenases that can contribute to anaerobic respiration include formate dehydrogenases N and O, hydrogenases 1 and 2, glycerol-3-phosphate dehydrogenase, and pyruvate oxidase. Some examples of terminal reductases include fumarate reductase, nitrite reductases and quinol oxidases (Unden and Bongaerts, 1997b).

Several global regulators induce systematic physiological changes in response to changing environmental oxygen conditions. The two major regulators involved in respiration are the ArcAB (anoxic redox control) two-component system and DNA-binding transcriptional regulator FNR. The ArcAB two component system directly senses oxygen limitations to invoke a system wide response (Iuchi and Lin, 1995; Salmon et al., 2005). ArcB is a membrane bound sensory histidine kinase that is constitutively expressed, and phosphorylates DNA-binding transcriptional regulator ArcA during anaerobic or microaerobic conditions (Iuchi and Weiner, 1996). ArcAB has been shown to regulate the expression of a wide range of genes and metabolic processes. This includes the TCA cycle, glyoxylate shunt, and fatty acid degradation (Alexeeva et al., 2003). Responses generated by the ArcAB system are initiated solely by the ArcB sensor protein (Iuchi and Lin, 1995). FNR contains an oxygen sensitive iron sulfur cluster that can directly sense oxygen concentrations to alter its regulatory capabilities (Kiley and Beinert, 2003). FNR activates dehydrogenases involved in anaerobic respiration, as well as terminal oxidases and reductases that utilize alternative terminal electron acceptors (Kang et al., 2005). FNR also represses genes used for aerobic respiration.

In the absence of oxygen and active respiratory pathways, ATP production is severely limited. Anaerobic growth is characterized by limited energy, leading to an increase in glycolytic flux (Koebmann et al., 2002). The TCA cycle becomes downregulated, leading to the incomplete oxidation of a consumed carbon source. Under these conditions, fermentation byproducts are excreted. These include succinate, formate, acetate, lactate and ethanol. It has been shown that the ratios in which fermentation byproducts are produced provides the cell with the ability to regulate redox balance and ATP formation. Ethanol is produced from acetyl-CoA and consumes two NADH. Lactate is produced from pyruvate and consumes one NADH. Acetate is produced from acetyl-CoA with an ATP generation. Succinate is formed from PEP, which ultimately consumes one NADH and an electron from an anaerobic respiratory chain, but prevents the formation of ATP by preventing PEP from completing glycolysis.

The conversion of pyruvate into acetyl-CoA is catalyzed primarily by two separate reactions depending on the presence of external electron acceptors. In aerobic conditions, pyruvate dehydrogenase converts pyruvate into acetyl-CoA while producing one molecule of NADH (Fig. 1a). This NADH can ultimately be used in the respiratory chain. However, excess NADH production under anaerobic conditions is unfavorable as it will require additional carbon expenditure to provide an electron sink. Under anaerobic conditions, PDH is downregulated and further, inherently higher NADH/NAD+ ratios inhibit its activity (Mark and Amsterdam, 1999; Kim et al., 2008). Therefore, pyruvate formate-lyase (*pflB*) is primarily used in anaerobic conditions, which produces formate in lieu of CO_2 and NADH (Fig. 1b). As a result, formate is also produced as a major fermentation byproduct. These fermentation pathways are main targets for metabolic engineering, and can be replaced by desired pathways to achieve high yield of product formation. Examples include the production of ethanol, n-butanol (Shen et al., 2011; Ohtake et al., 2017; Nitta et al., 2017), lactate (Kim et al., 2013, butyrate (Baek et al., 2013a; Lim et al., 2013) and succinate (Sánchez et al., 2005; Zhu et al., 2014).

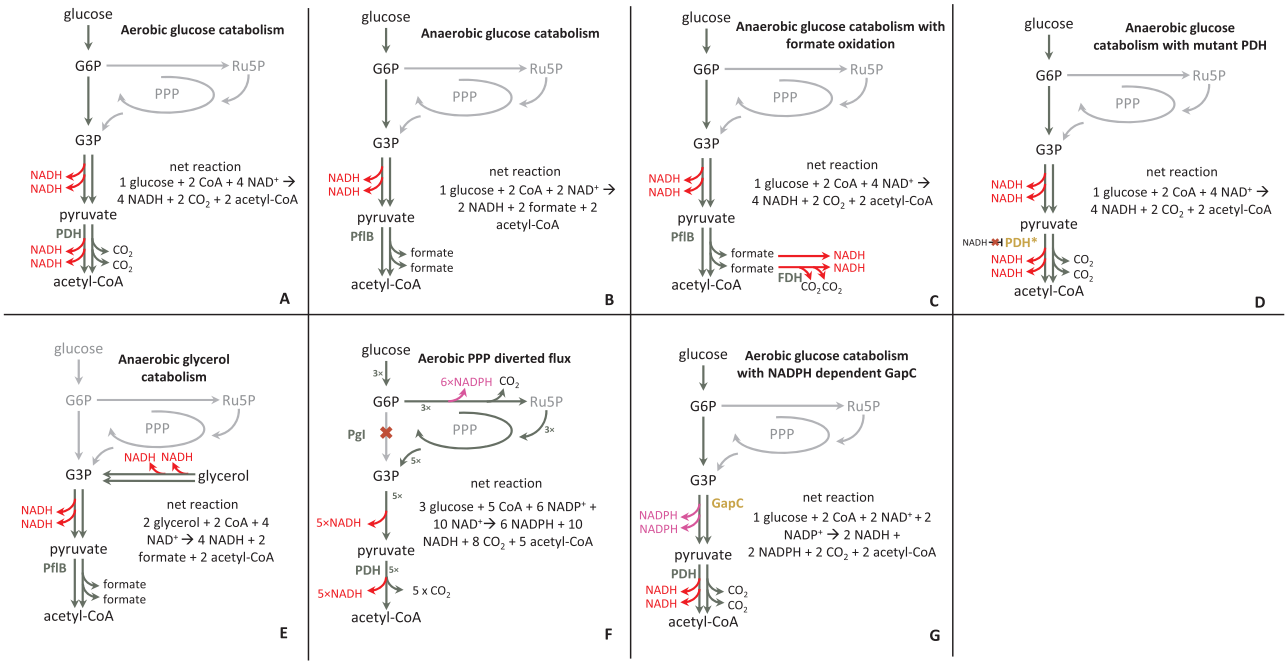

Fig. 1. Altering cofactor supply. Altering cofactor supply from glucose catabolism to acetyl-CoA. a) Aerobic glucose catabolism using glycolysis and PDH, pyruvate dehydrogenase. b) In anaerobic conditions, PDH is NADH sensitive and pyruvate formate-lyase (PflB) is used instead, producing formate instead of NADH. c) Overexpression of formate dehydrogenase, FDH, can oxidize formate to recover NADH (Shen et al., 2011; Ohtake et al., 2017). d) A mutant of PDH (LPD E353K) decreases sensitivity to NADH and can increase NADH production anaerobically (Kim et al., 2008). e) Catabolism of glycerol creates an additional NADH molecule per acetyl-CoA produced compared to glucose catabolism through glycolysis (Dharmadi et al., 2005). f) Knocking out *pgi*, glucose-6-phosphate isomerase, forces flux through the pentose phosphate pathway (PPP) to increase NADPH yield (Siedler et al., 2011). g) Replacement of native NAD dependent glyceraldehyde-3-phosphate dehydrogenase (GapA), with NADP dependent GapC from *Clostridium acetobutylicum* increases NADPH supply (Martínez et al., 2008). Other abbreviations: G6P, glucose-6-phosphate; G3P, glyceraldehyde-3-phosphate; Ru5P, ribulose-5-phosphate.

3.4. Adapting cofactor supply

In engineering microbes for production of certain chemicals, especially under anaerobic conditions, a critical consideration is to ensure sufficient cofactor supply to drive product formation. For instance, a modified *Clostridial* CoA-dependent butanol pathway was implemented that requires four NADH for the conversion of two acetyl-CoA into one butanol (Shen et al., 2011). However, after glycolysis and decarboxylation of pyruvate using PflB, only two NADH are produced with two formate. Therefore, formate must be oxidized into CO_2 in order to retrieve these extra electrons. *E. coli* contains several native formate dehydrogenases, however, none of these enzymes utilize NAD + as an electron acceptor. To solve this problem, NAD + dependent Fdh from *Candida boidinii* was adopted for its ability to oxidize formate while increasing supply of NADH (Berríos-Rivera et al., 2002) (Fig. 1c).

Several other approaches have been demonstrated to recover additional NADH in anaerobic conditions. A mutant of pyruvate dehydrogenase complex, which is normally sensitive to high NADH/NAD + ratios, was isolated that has decreased sensitivity to NADH (Kim et al., 2008, 2007). A mutation within the *lpd* gene, encoding for the PDH subunit dihydrolipoamide dehydrogenase LPD E353K, was the cause of this decreased sensitivity (Fig. 1d). Contrasting to the wild-type strain, the presence of this mutation allowed production of homoethanol and anaerobic growth in strains deficient of *ldhA* (lactate dehydrogenase) and *pflB* (pyruvate formate-lyase). In another approach, glycerol has been demonstrated as an alternative carbon source for production of highly reduced products (Dharmadi et al., 2005; Shams Yazdani and Gonzalez, 2008) (Fig. 1e). In the catabolism of glycerol into PEP, twice as many reducing equivalents are generated compared to glucose catabolism within glycolysis. Therefore, even without dominant pyruvate dehydrogenase activity under anaerobic conditions, production of ethanol or succinate from glycerol are redox-balanced processes.

Ensuring that sufficient cofactors are supplied is a crucial consideration for pathway design. Many reduced compounds are formed from pathways that specifically require NADH or NADPH. While some enzymes utilize either as a cofactor equally well, most enzymes strongly prefer one over the other. For example, the ketol-acid reductoisomerase in the isobutanol pathway (Atsumi et al., 2008a) prefers NAPDH over NADH (Brinkmann-Chen et al., 2013). Furthermore, the *Clostridial* CoA-dependent butanol production pathway relies on formation of NADH (Ohtake et al., 2017), while compounds derived from the mevalonate (MVA) pathway require NADPH inputs (Wada et al., 2017). NADH is easily supplied, as it is produced during catabolism of sugars through glycolysis, or through the TCA cycle during aerobic growth conditions. However, NADPH is primarily formed through the oxidative branch of the pentose phosphate pathway and isocitrate dehydrogenase in the TCA cycle under aerobic conditions. NADPH can also be formed from transhydrogenases, which can interconvert between NADH and NADPH. These methods of producing NADPH have been exploited in microbial hosts. In order to divert flux through the pentose phosphate pathway from glycolysis, deletion of phosphoglucose isomerase (*pgi*) has proven successful (Siedler et al., 2011; Lee et al., 2010) (Fig. 1f). Transhydrogenase PntAB was used to improve NADPH supply for production of 3-hydroxypropionic acid (Rathnasingh et al., 2012). Several other strategies have been employed. One strategy to increase NADPH entails replacing native *E. coli* NAD-dependent glyceraldehyde-3-phosphate dehydrogenase, with an NADP-dependent version from *Clostridium acetobutylicum* (Martínez et al., 2008)(Fig. 1g). This was previously employed to enhance production of lycopene and ε-caprolactone. In another study, changing NADPH dependency to NADH dependency through directed protein evolution has also proven useful (Brinkmann-Chen et al., 2013). Further, concentrations of NADPH were improved by using constrained-based modeling that identified three knockouts to yield a *ΔpgiΔpplΔppc* strain of *E. coli*. As a result, NADPH concentrations were 1.5 fold higher than a sole *pgi* deletion strain. This strain was further used to improve

production of flavan-3-ols, which selectively require NADPH for production (Chemler et al., 2010).

3.5. Sigma factors

E. coli uses various sigma factors to recruit RNA polymerase (RNAP) to initiate transcription and regulate a specific set of genes/operons (Feklístov et al., 2014). These include the house keeping sigma factor σ70 (RpoD), heat shock sigma factor σ32 (RpoH), extreme heat stress sigma factor σ24 (RpoE), stationary phase sigma factor σ38 (RpoS), nitrogen regulation sigma factor σ54 (RpoN), and the flagellar sigma factor σ28 (RpoF). Among them, the house-keeping and stationary phase sigma factors, RpoD and RpoS, control the largest number of genes relevant to metabolic engineering. The total number of genes controlled by each sigma factor is a function of environmental conditions and growth phase (Wade and Struhl, 2004; Jishage et al., 1996). Within *E. coli* strain MC4100 during exponential phase, RpoD accounts for roughly 93% of the total pool of sigma factors, while RpoS was undetectable (Jishage et al., 1996). However, during stationary phase, RpoD accounts for roughly 70%, while RpoS accounts for 26% of the total pool. Deleting *rpoS* has been demonstrated to increase expression of the TCA cycle and glyoxylate shunt during exponential growth (Rahman et al., 2009). Activating *rpoS* has been shown to improve acid tolerance and provide protection against carboxylic acid and oxidative stresses (Gaida et al., 2013). RpoH and RpoE are particularly important for protein production. When both carbon and nitrogen are limited, σ24 (RpoE) is the major one coordinating the complex regulatory networks (Löffler et al., 2017).

Sigma factors can simultaneously control the expression of many genes, which makes them appealing as targets for engineering microbial phenotypes. This is an approach known as global transcription machinery engineering (gTME) (Alper et al., 2006a). Initial work on gTME in *E. coli* involved creation of a random mutagenesis library of *rpoD* (Alper and Stephanopoulos, 2007). This was then transformed into various production strains to witness variation in phenotypes. This strategy was used to improve phenotypes such as ethanol tolerance (Alper and Stephanopoulos, 2007), lycopene production (Alper et al., 2005), solvent tolerance (Zhang et al., 2015), and production titers of L-tyrosine and hyaluronic acid (Goldfarb et al., 2015). Manipulating RpoS by deletion and random mutagenesis has been applied to improve 1-propanol,putrescine and isobutanol production (Bettenbrock et al., 2007; Qian et al., 2009; Smith and Liao, 2011). In another study, various attempts were made to increase production of L-tyrosine in *E. coli*, predominantly in engineering global transcription machinery (Santos et al., 2012). This was done by introducing a plasmid based mutagenesis library of RNA polymerase subunits *rpoA* and *rpoD*, with a novel selection strategy to test for enhanced tyrosine production. Three strains were isolated that exhibited a 91–113% improvement in L-tyrosine production. Transcriptome analysis within these strains revealed significant perturbations of a set of genes that are normally altered within stringent response; these are decrease in ribosomal proteins and RNA formation, fatty acid elongation, *de novo* nucleotide biosynthesis, and DNA replication. Because of the commonality of these perturbations within all strains with improved production, it was hypothesized that stringent response was a major contributor to this increased production phenotype.

3.6. Post translational modification

A growing amount of research is highlighting the abundance of post translational modifications (PTM) on proteins within *E. coli*, as well as their relation to cell physiology (Wang et al., 2010; Brown et al., 2017; Soares et al., 2013; Zhang et al., 2009; Schmidt et al., 2016; Hentchel and Escalante-Semerena, 2015). Lysine acetylation, which is a highly studied PTM, was shown to have major effects on enzymes in central carbon metabolism in *Salmonella enterica* (Wang et al., 2010). In response to growth on different carbon sources, acetylation of metabolic enzymes was coordinated with flux of carbon through glycolysis, gluconeogenesis, TCA cycle, and glyoxylate shunt. Acetylation, as well as other PTMs, have been linked to metabolic states that are altered by glucose and nutrient availability (Brown et al., 2017; Schmidt et al., 2016), growth phase (Soares et al., 2013; Schmidt et al., 2016; Weinert et al., 2013) and stress conditions (Schmidt et al., 2016).

In *E.* coli, lysine acetylation can proceed either enzymatically or non-enzymatically (Hentchel and Escalante-Semerena, 2015). To dynamically control acetylation, a pair of enzymes is capable of either attaching or removing an acetyl-group from the ε-amino moiety of a lysine side chain (Linda I et al., 2010; Thao and Escalante-Semerena, 2011). Using acetyl-CoA as a donor, the acetyl group is attached using lysine acetyltransferase, and removed using lysine deacetylase, encoded by *patZ* and *cobB*, respectively (Abouelfetouh et al., 2015; Castaño-Cerezo et al., 2011). Non-enzymatic acetylation proceeds spontaneously from the donation of an acetyl group from acetyl-phosphate (Weinert et al., 2013). It is believed that acetyl-phosphate acts as an intermediate between carbon metabolism and metabolic enzymes; excess acetyl-phosphate is produced from carbon overflow, as flux through central carbon metabolism exceeds the capacity of other metabolic pathways (Schilling et al., 2015). This in turn results in non-enzymatic acetylation, which regulates the flow of carbon through central metabolism.

Certain lysine residues that can be acetylated are dependent on acetylation from either enzymatic or non-enzymatic mechanisms (Weinert et al., 2013; Garwin et al., 1980). Interestingly, it was reported that although CobB is able to reverse acetyl-phosphate dependent acetylation, it is only capable of deacetylating a fraction of sites that are sensitive to acetyl-phosphate (Abouelfetouh et al., 2015). While the acetylation profile in *E. coli* increases with increased glucose, it was demonstrated that much of this acetylation is non-enzymatic (Schilling et al., 2015). Deletion of *pta* (encoding for phosphate acetyltransferase), which is responsible for conversion of acetyl-CoA into acetyl-phosphate, eliminated glucose induced acetylation during growth. When measuring acetylation during growth, the amount of acetylation progresses over time starting at exponential growth phase and increasing substantially with entry into stationary phase. As glucose was consumed, more proteins became acetylated. Because the majority of lysine acetylated sites varied with time and glucose concentrations, it was hypothesized that acetylation over time is a response to glucose consumption. This was confirmed, as a strain with deletion of *ptsG*, an essential component of the phosphotransferase system within *E. coli*, produced a similar acetylation profile to cells growing in rich media without glucose supplementation (Schilling et al., 2015). Further, it was demonstrated that over an extended period of glucose exposure, lysine residues within the same protein exhibited both a variable susceptibility to acetylation as well as different acetylation rates.

In addition to acetylation, there are many different types of PTM that have been identified including phosphorylation (Dworkin, 2015; Vincent et al., 1999), propionylation (Sun et al., 2016), succinylation (Colak et al., 2013) and formylation (Wilkins et al., 1999). Recently, Sun et al. identified lysine propionylation in *E. coli* (Sun et al., 2016). In contrast to succinylation and acetylation, the propionyl-modification is decreased under high glucose. Propionylation also plays a role in propionate metabolism and propionyl-CoA degradation. Both CobB and acetyltransferase PatZ seem to be involved in regulating propionylation.

A comprehensive analysis was performed to determine the differences in PTM across 22 experimental conditions (Schmidt et al., 2016). 11 different types of PTM were identified including novel methylations and terminal N^{α}-acetylations. It was determined that certain modifications can be enriched within specific protein classes and pathways. Some of these include lysine acetylation sites which were enriched in enzymes of glycolysis, gluconeogenesis, the TCA cycle, and pyruvate metabolism. ABC transporters were enriched for lysine methylation.

Nucleotide binding proteins were enriched for N^{α}-acetylation. Further, enrichment of certain modifications was observed as protein abundance changed. For example, lysine acetylation was observed at high protein abundance levels, while N^{α}-acetylation was observed at low protein abundance. It was also observed that different types of PTMs can be carried within the same residue.

3.7. Toxicity

Metabolic engineering modifies native pathways or to introduce non-native enzymes for production of desired compounds. These processes may involve accumulation of toxic intermediates, toxic product, or byproducts from unwanted side reactions that exhibit toxicity (Jarboe et al., 2011; Nicolaou et al., 2010). Metabolite toxicity is a complex phenomenon that may result from general stress response, inhibition or inactivation of essential enzymes, or damage of cell membranes. In most cases, the toxicity effect is non-specific and condition dependent. Toxicity can reduce either growth or product formation, or both. It is common that the cell can accumulate products to a titer exceeding the toxicity level that inhibits growth (Shen et al., 2011; Atsumi et al., 2008a; Atsumi et al., 2010) because the toxicity to growth and production are uncoupled.

Hydrophobic compounds exhibit toxicity as they can enter into the membrane, increase permeability and fluidity, decrease energy transduction, and alter membrane protein function (Dunlop, 2011). It was demonstrated that hexanoic and octanoic acids induce changes to membrane fluidity and integrity when exposed to *E. coli* at millimolar concentrations (Royce et al., 2013). Interestingly, a fatty acid producing strain exhibited no change in membrane fluidity, but there was an increase in membrane leakage alongside increasing product titers. These results demonstrate that the effects of a toxic product is difficult to pinpoint and dependent on the method of characterization. A similar case was observed for styrene (Lian et al., 2016).

There are several common strategies used to alleviate toxicity (Jarboe et al., 2011; Dunlop, 2011). Efflux pumps have been used (Dunlop et al., 2011; Takatsuka et al., 2010), which use the proton motive force to export toxic compounds, as well as heatshock proteins (Rutherford et al., 2010) that can aid in protein folding. Microbial evolution (discussed below) is also an established strategy for improving tolerance and has been demonstrated in many cases including isobutanol (Atsumi et al., 2010), serine (Mundhada et al., 2016, 2017), octanoic acid (Royce et al., 2015) and sugars produced by biomass pyrolysis (Jin et al., 2017). It was shown that improved tolerance to isobutanol also resulted in tolerance to *n*-butanol and 2-methyl-1-butanol (Atsumi et al., 2010). However, the same strain showed no improvement in ethanol tolerance and higher sensitivity to hexane and chloramphenicol. This demonstrates the existence of evolutionary tradeoffs that may come with acquisition of certain phenotypes, and in this case, it must be noted that tolerance mechanisms to the tested compounds rely on different reaction mechanisms. Another broadly-applicable strategy for improving tolerance lies in altering the membrane composition at the lipid level, or by adjusting the distribution of phospholipid heads (Tana et al., 2017; Tan et al., 2016).

3.8. Side reactions and metabolite damage

While many metabolic enzymes have evolved to catalyze only a specific chemical reaction, a significant number of enzymes are promiscuous: they contain the ability to catalyze the same mechanistic reaction on a different substrate. Many secondary activities have been discovered and published in public databases such as BRENDA and MetaCyc (Schomburg et al., 2002; Keseler et al., 2005). In *E. coli*, more than 260 secondary, or underground reactions have been discovered that are outside of known metabolic networks (Guzmán et al., 2014; Notebaart et al., 2018). Further study of these capabilities will not only provide deeper understanding into the physiology of microbes, but also provide us with new molecular tools with which we can design metabolic pathways.

On the other hand, many of these side reactions are increasingly being discovered that contribute to metabolite damage within the cell, which is a serious factor for metabolic engineering (Linster et al., 2013). Metabolite damage refers to side reactions to metabolites that can occur either enzymatically or non-enzymatically to produce wasteful or toxic products. To give an example, methylglyoxal is one of the major metabolites that causes metabolite damage (Richard, 1984). Methylglyoxal is formed from the spontaneous degradation of glyceraldehyde 3-phosphate and dihydroxyacetone phosphate. It is also formed as a side product from triose phosphate isomerase during glycolysis (Richard, 1993). Excess methylglyoxal can covalently link with lysine, arginine, and cysteine that is either free within the cytosol or bound within a protein (Richarme et al., 2015). Methylglyoxal metabolite toxicity therefore presents a major concern for maintenance of optimal metabolic function. Another prevalent example is a side reaction of carbon fixing RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase), which poorly discriminates oxygen and CO_2 (Erb and Zarzycki, 2016). A 2:5 ratio is present in the consumption of oxygen and CO_2, resulting in production of toxic side product glycolate-2-phospate rather than the desired 3-phosphoglycerate product (Walker et al., 2016). When engineering high flux metabolic pathways, metabolite damage becomes an increasingly relevant concern, as increasing flux and pool sizes of metabolites can exacerbate metabolite damage reactions (Sun et al., 2017). Repair enzymes are being discovered that can reverse metabolite damage. For example, to combat methylglyoxal metabolite damage in *E. coli*, YhbO and YajL can repair glycated proteins including glyceraldehyde-3-phoaphate dehydrogenase and fructose bisphosphate aldolase. Strains deficient in *yhbO* and *yajL* are sensitive to glucose containing media and methylglyoxal (Abdallah et al., 2016).

There are many other forms of metabolite damage (Sun et al., 2017). For instance, glyceraldehyde 3-phosphate dehydrogenase is known to produce hydrates of NADH and NADPH, which inhibit certain dehydrogenases (Oppenheimer and Kaplan, 1974). The reaction, which produces NADHX and NADPHX, can also occur simultaneously. However, many organisms contain ATP or ADP dependent NAD(P)H dehydrases and epimerases (Marbaix et al., 2011) which can reverse the metabolite damage. In *E. coli*, these reactions are catalyzed by ADP dependent YjeF (Marbaix et al., 2011). The promiscuous nature of many transaminases produces a deaminated glutathione molecule which adversely affects function of glutathione-dependent enzymes. A eukaryotic protein, Nit1, was recently identified that can hydrolyze deaminated glutathione into cysteinylglycine and α-ketoglutarate (Peracchi et al., 2017). In other cases, 5,10-Methenyltetrahydrofolate can form a 5-Formyltetrahydrofolate side product from serine hydroxymethyltransferase (Stover and Schirchs, 1990), and glutamine or glutamate can spontaneously cyclize to form 5-oxoproline (Park et al., 2001).

3.9. Omics tools used to explore physiology

System-wide analysis of a host can be performed using a variety of omics techniques that include genomics, transcriptomics, proteomics, metabolomics, and fluxomics. These techniques have been used in a variety of ways to aid in strain engineering. Recently, seven industrially important strains of *E. coli* (BL21, C, Crooks, DH5α, MG1655, W, W3100) were compared using multi-omic data (Monk et al., 2016). The overall dataset included genomic sequencing, transcriptomics and phenotypic measurements. Strain specific genome-scale models were constructed. Based on multiomic data, these models were used to characterize traits that differentiate each strain by metabolic fluxes, gene expression, and gene regulation. These strains showed variability in growth rates under anaerobic and aerobic conditions and organic acid secretion profiles. Comparing genome sequencing revealed that of 6626 unique protein-coding sequences, roughly only half were shared

between all seven strains. In constructing strain specific genome-scale models, genetic variance corresponded to 211 reactions that were variably present within different strains. Presence or absence of these reactions may enable, or limit, production of certain compounds. Transcriptomics was also used to demonstrate that these strains have unique flux and gene expression patterns. Overall, this multi-omic data served as the basis of a strategy to determine if certain strains may be better suited for specific engineering applications.

Omics strategies are now routinely being applied to study how and why transcriptional changes are affected under specific conditions, and how these changes can coordinate microbial metabolism (Chubukov et al., 2014; Nichols et al., 2011; Sévin et al., 2015; Fuhrer and Zamboni, 2015). With more advanced techniques, these coordinated responses are being understood with a higher resolution. One study used metabolomics, in combination with a library of fluorescent transcriptional reporters, to study how central metabolic promoters respond to different environmental conditions (Kochanowski et al., 2017). While it was deduced that 70% of the total variance in activity of the selected promoters can be explained by global transcriptional regulation, the remaining transcriptional regulation was correlated with metabolomic response. This led to the identification of cyclic AMP, fructose 1,6-bisphosphate, and fructose 1-phosphate as connected to much of this regulation, mediated by their interaction with the transcription factors Crp and Cra.

A variety of metabolomics strategies have been developed for identification of novel enzyme functions. Recently, a high-throughput strategy has been developed for identification of new enzyme function using non-targeted metabolomics with supplemented metabolome extract (Sévin et al., 2017). Purified protein, or cell lysate containing an overexpressed protein is incubated with a metabolite cocktail that consists of metabolite extracts from cultivated *E. coli* cells with the addition of a select amount of additional cofactors. Change in metabolite concentrations were determined after incubation. Through screening 1275 uncharacterized *E. coli* proteins, 241 potential novel enzymes were identified. Through elucidation of activity of uncharacterized enzymes, in addition to determining side activities of enzymes with known function, it is possible to piece together new metabolic pathways for applications in bioproduction (Notebaart et al., 2018). Alternatively, through identification of reactions which may cause undesirable flux away form a target compound, further genetic manipulation of a host can be done to enhance pathway performance.

Omics techniques have also been employed to identify targets for improvement of specific metabolic pathways (Ohtake et al., 2017; Nitta et al., 2017; George et al., 2014). Previously, a combination of proteomics and targeted metabolomics were used to enhance production of isopentanol (George et al., 2014). Strain variants that were expressing varying amounts of pathway proteins were assayed for concentrations of select metabolites and proteins. Through additional quantification of product formation, a correlation analysis was performed to identify relationships between protein levels and metabolites. Using this data, a pathway bottleneck was identified, which when addressed, improved production titers. Using metabolomic analysis, titers of 1-butanol were enhanced in *E. coli* using a modified *Clostridial* CoA dependent pathway (Ohtake et al., 2017). In doing so, a CoA imbalance was identified that was addressed by increasing the release of CoA from the pathway. An RBS library was used to optimize expression of the rate limiting enzyme, effectively enhancing the recycling of CoA, to enhance titers. While omics technology has proven useful in developing applications that can aid in further understanding and manipulation of *E. coli*, current methods of analysis have room for improvement as useful information may be difficult to extract from inherently large datasets (Haas et al., 2017).

4. Strain engineering techniques

Many genetic tools have been developed to modify the genome of *E. coli*, and have been discussed in detail elsewhere (Makrides, 1996; Tyo et al., 2007; Muyrers et al., 2001; Andrianantoandro et al., 2006). Below are techniques that have been widely adopted for their efficient usage and potential for development into further applications.

4.1. λ-Red recombinase

The λ-Red recombinase system is based on homologous recombination of linear fragments, and has proven to be an extremely efficient and versatile strategy for genomic modifications (Datsenko and Wanner, 2000). The λ-Red system is constructed based on the λ bacteriophage and consists of several genetic components: Exo, Beta, and Gam. Gam prevents degradation of small linear fragments of DNA that would otherwise be digested from native nucleases RecBCD or SbcCD. Exo is an exonuclease that degrades double stranded DNA in a 5' to 3' manner. Beta binds to the single stranded DNA, created by Exo, to promote annealing to a complementary genomic region. Genes can be knocked out with selection by simultaneously replacing a target gene with a kanamycin cassette that is flanked with short flippase recognition sites (FRT). This kanamycin cassette can be removed by flippase (FLP) recombinase. However, this leaves an FRT scar within the genome, which can be problematic for use in some applications. λ-Red recombinase has been broadly used in many metabolic engineering applications. The Keio Collection, which contains individual gene deletions of all non-essential genes within *E. coli* (Baba et al., 2006), was constructed using this system. The λ-Red recombinase system has been widely used in strain construction for gene deletions (Shen et al., 2011), introduction of point mutations (Heermann et al., 2008), and insertions (Kuhlman and Cox, 2010), and for applications such as promoter engineering (Alper et al., 2006b) and enhancing CRISPR efficiency (Jiang et al., 2015).

4.2. Multiplex automated genome engineering (MAGE)

Multiplex automated genome engineering (MAGE) is a strategy aimed at systematically imposing genetic mutations, often in parallel, to bypass other time-consuming engineering strategies (Wang et al., 2009). The overall strategy of MAGE entails electroporation of a host with short single stranded DNA (ssDNA) that has regions homologous to a specific genomic location. By over-expressing λ-Red ssDNA binding protein beta, the ssDNA is prevented from degradation once it has entered the cell, where it can undergo homologous recombination to result in the modified host genome. Designing the ssDNA to bind to the lagging strand of the replication fork results in efficiency 10–100 times higher than the leading strand (Ellis et al., 2001). An optimal size is between 70 and 90 nucleotides in length, but can be as short as 30 nucleotides (Erler et al., 2009). Deletion of *mutS* from the host genome is an important factor. MutS is a component of the methyl-directed MutHLS mismatch repair system (MMR) that functions to repair mismatches, insertions and deletions 1–6 nucleotides in length (Modrich, 1991). It has been demonstrated that the presence of endogenous MMR significantly reduces the efficiency of oligonucleotide mediated allelic replacement by repairing introduced mutations (Modrich, 1991). Therefore, with use of an MMR-deficient strain, mutations introduced through recombination of oligonucleotides have an improved bias towards the substitution mutation (Wang et al., 2011). On the other hand, lack of MMR renders the host more prone to accumulation of undesired mutations which may pose a challenge for certain applications.

The entire process is designed to be rapid, with a start to finish time within 2–2.5 h per cycle. The strains can subsequently undergo additional rounds of MAGE to introduce genomic modifications in combination or to generate strain variants with different combinations of modifications (Wang et al., 2009). Therefore, MAGE enables rapid directed evolution targeted to specific regions of the genome. One major tradeoff is that no selection is available for many genetic modifications. Therefore, individual colonies must be screened for the correct

sequence. A strategy called multiplex-allele specific colony PCR has been developed to aid in this process (Isaacs et al., 2011).

Another challenge of MAGE compared to λ-Red recombinase is that base pair insertions drastically reduce the efficiency. While λ-Red has been used to insert fragments in excess of 7 kb (Kuhlman and Cox, 2010), the efficiency of MAGE drops below 2% with inserts greater than 20 bp (Wang et al., 2009). It was later discovered that the insertion rate can be greatly improved using "coselection" MAGE (CoS-MAGE), as certain subpopulations appear to be more susceptible to the oligo-based allelic replacement strategy (Wang et al., 2012). CoS-MAGE was performed to insert T7 promoters into 12 genomic operons to enhance expression of genes used in aromatic amino acid biosynthesis. First, genes were inactivated within the base strains (such as *bla* or *cat*) that conferred antibiotic susceptibility. The CoS-MAGE protocol differentiated from the MAGE protocol mainly in that a small amount of an oligo (called CoS oligo) that restored antibiotic susceptibility was included within the electroporation in addition to oligos that target all 12 T7 insertions. Cells were then selected on antibiotic plates and tested for any T7 insertions. Remarkably, without coselection, only 10–25% contained a T7 promoter after four MAGE cycles. Contrasting to this, with coselection more than 40% of cells had at least one T7 promoter insertion and 5% has 3 insertions.

4.3. CRISPR

Clustered regularly interspaced short palindromic repeats (CRISPR), along with CRISPR-associated genes (Cas), form the basis of a bacterial immune system to create site-specific, double-stranded breaks (DSBs) on invading phage DNA (Choi and Lee, 2016). Bacteria that use CRISPR/Cas systems contain CRISPR arrays that are composed of CRISPR repeats and spacers that match foreign DNA from previous infections (Bolotin et al., 2005; Mojica et al., 2005). These arrays are transcribed as CRISPR RNAs (crRNAS), which form complexes with Cas proteins to recognize complementary sequences of foreign nucleotides (Brouns et al., 2006). Upon binding to the foreign nucleotide, called a protospacer, the complex induces a DSB (Jinek et al., 2012).

Over the past several years, extensive development of CRISPR/Cas systems has led to the emergence of efficient nucleotide manipulation strategies that function in eukaryotes and prokaryotes (Choi and Lee, 2016; Mougiakos et al., 2018; Donohoue et al., 2017) (Fig. 2). There are multiple types of CRISPR/Cas systems (Makarova et al., 2011). CRISPR/Cas for use in *E. coli* was first developed using a system adapted from *S. pyogenes* (Jiang et al., 2013). This is a type II CRISPR/Cas9 system, which requires a dual RNA complex that consists of a CRISPR RNA (crRNA) and a *trans*-activating crRNA (tracrRNA) (Jiang et al., 2013; Deltcheva et al., 2011) Crucial to this is an adjacent protospacer-adjacent motif (PAM) which is present at the 3' end of the protospacer (NGG in the case of *S. pyogenes* CRISPR/Cas system (Sternberg et al., 2014). A Cas9: dual RNA complex forms which binds to the PAM located adjacent to the target DNA sequence to introduce a DSB within the protospacer region (Jinek et al., 2012; Sternberg et al., 2014). To simplify this process, the tracrRNA and crRNA are hybridized into a single guide RNA (sgRNA) that is expressed as one unit. A region within the sgRNA containing a 20-bp complementary region and PAM sequence is modified to specify the location of the DSB (Jinek et al., 2012). These components can all be introduced by a plasmid (Jiang et al., 2015).

E. coli and many other bacteria lack non-homologous end joining (NHEJ) mechanisms to repair DSBs. Therefore, a chromosomal DSB will either induce cell death or dormancy (Cui and Bikard, 2016). *E. coli* can undergo homology-directed recombination (HDR) to repair this break (Jiang et al., 2013; Shuman and Glickman, 2007). This requires supply of a template DNA strand to act as a complementary strain for HDR. The template can be introduced as plasmid DNA (Jiang et al., 2015) or a linear (Pyne et al., 2015; Li et al., 2015) fragment, and can either be double stranded or single-stranded (Reisch and Prather, 2015). The template DNA contains the modification intended for introduction into the genome. Using CRISPR/Cas9 for only point mutations or small insertions does not include the introduction of a selection marker to isolate a successfully modified host. However, because induced DSBs are lethal to the host, this serves as a form of selection to enrich strains that successfully incorporated template DNA using HDR (Jiang et al., 2015). Furthermore, the rate at which HDR can occur can be enhanced with expression of other recombination machinery (Jiang et al., 2015, 2013). Commonly, the λ-Red recombinase system is used in conjunction with a CRISPR/Cas9 system in *E. coli* and other bacteria.

CRISPR/Cas9 has frequently been used for genomic modifications, including knockouts, point mutations, and integration of metabolic pathways into *E. coli* genomes (Dong et al., 2017; Wu et al., 2017). In one case, a CRISPR/Cas9 protocol was optimized for scarless integration of large DNA fragments; 2.4, 3.9, 5.4, and 7 kb fragments were inserted with respective efficiencies of 91%, 92%, 71% and 61% (Chung et al., 2017). CRISPR/Cas9 has also been employed for optimizing expression of chromosomally contained metabolic pathways (Alonso-Gutierrez et al., 2017; Zhu et al., 2017; Liang et al., 2017). For example, a CRISPR/Cas9-facilitated multiplex pathway optimization (CFPO) technique was developed to alter expression of chromosomally integrated genes (Zhu et al., 2017). Using CFPO, expression of multiple genes can be altered simultaneously. Here, a regulatory library was designed using the artificial promotor M1-93 with 6 random nucleotides within the RBS region. A plasmid was constructed (pRBSL-genes) that contains the pathways genes and upstream regions, each with the regulatory library within the upstream region. With co-transformation of pRBSL-genes and pRedCas9 (containing Cas9 and λ-Red recombinase), a combinatorial library of strains was generated to be further tested for desired properties. Similar strategies have been developed, taking advantage of the high efficiency offered by CRISPR/Cas9, to develop large numbers of strain variants for further testing (Liang et al., 2017).

Applications of CRISPR have also been developed to provide favorable characteristics to bacterial strains for industrial batch fermentations or other uses. One application involves elimination of engineered DNA at a specific time point within a largescale fermentation or in response to a change of environment. This could aid in preventing environmental contamination of engineered DNA, preventing of DNA contamination in a final product, allowing efficient biomass disposal, and protecting genetic trade secrets (Caliando and Voigt, 2015) (Fig. 2e). The spread of engineered DNA into the environment is likely not prevented by applications which induce cell death, as is evident by the prevalence of recombinant DNA within biomass treated with heat and chemicals (Andersen et al., 2001). Caliando et al. reported the development of a genetic device (DNAi) which induces CRISPR-mediated nucleotide degradation depending on the presence of a transcriptional signal (Caliando and Voigt, 2015). DNAi was shown to have a dynamic range with low basal activity, to efficiently knockout plasmids with a range of copy numbers, and to function for inducible cell killing. Authors employed the type I-E CRISPR-Cas system. Genes of the Cas system were integrated into the genome under control of a P_{BAD} promoter for arabinose inducible expression. A CRISPR array with 29-bp sequence repeats and 32-bp spacer sequences is carried on a pUC19 plasmid with a constitutively expressed P_{J23117} promoter. Two spacers within the array were used to target a Str^R gene within a target plasmid to observe a 2.1×10^{-9} fraction of cells carrying the plasmid. The system was further demonstrated to target genomic DNA to induce cell killing. By using a spacer that targets multiple regions on the genome, a viable cell ratio of 2.1×10^{-9} was achieved. This adds the additional benefit of being able to preferentially degrade genomic DNA associated with a highly engineered phenotype.

Another application involves specific selection of certain strains. Established methods of selection include use of antibiotics, specific growth conditions, antimicrobial peptides, auxotrophic markers, lytic bacteriophages, and use of specific toxins (Parisien et al., 2008).

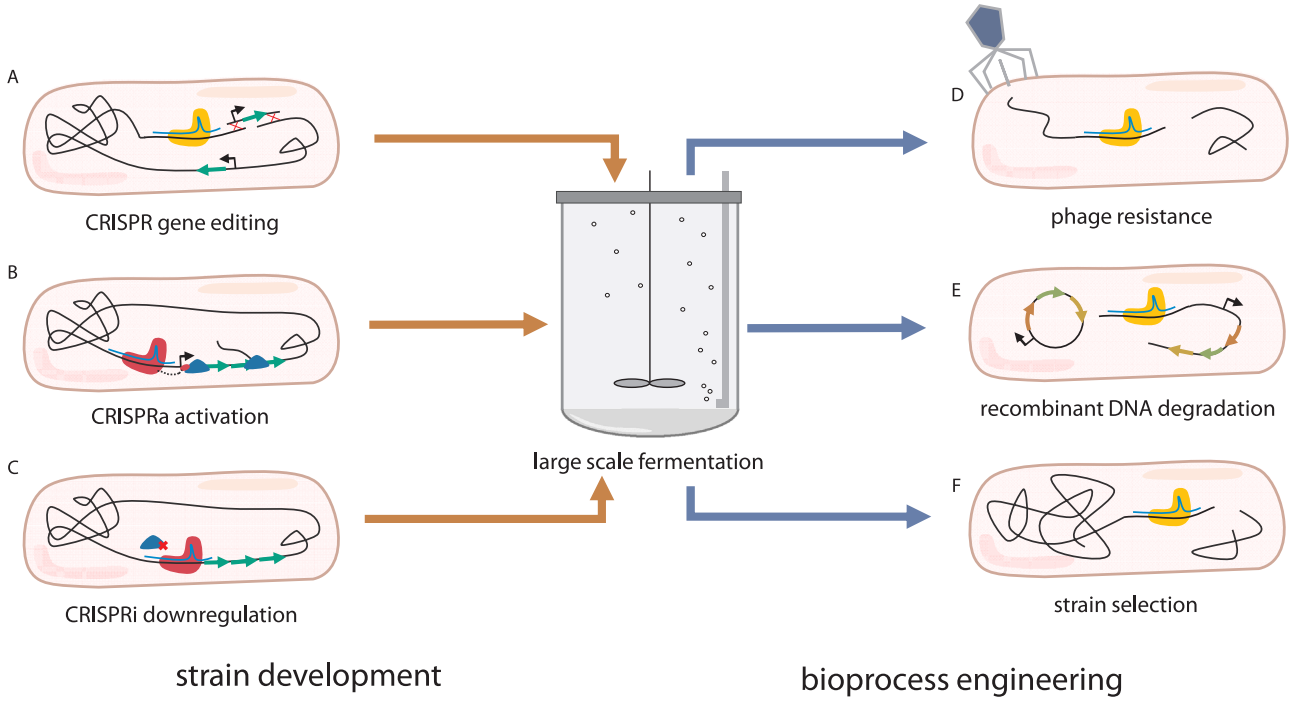

Fig. 2. CRISPR based applications. Applications of CRISPR for strain development and bioprocess engineering. a) CRISPR/Cas9 (yellow) used for genome modifications in strain construction and optimization. b) Activation of gene expression using CRISPRa (Bikard et al., 2013). dCas9 is fused to a transcriptional activator (pink) to stabilize binding of RNAP complex (blue). c) CRISPRi used for gene repression. dCas9 (pink) binds to promoter region, preventing RNAP from initiating transcription (Nielsen et al., 2014). d) CRISPR/Cas can be used for enhanced phage immunity to prevent failure during large scale fermentation (Barrangou et al., 2013). e) CRISPR/Cas systems can break down recombinant DNA to prevent environmental contamination of genes, to protect trade secrets, and to decrease DNA contamination within a fermentation product (Caliando and Voigt, 2015). f) CRISPR/Cas systems can be used to induce cell death (Gomaa et al., 2014). This can aid in altering the composition of cells within a co-culture, prevent environmental contamination of genetically modified strains, and inducing death following fermentation.

However, these methods may not be suitable for some applications, especially when targeting closely related strains. Gomaa et al. demonstrated an application of the type I-E CRISPR-Cas system to induce cell death of closely related *E. coli* strains separately in co-cultures (Gomaa et al., 2014) (Fig. 2e). Authors demonstrated that inducing DSBs can provoke strain selective killing regardless of genomic location or targeted gene. This can aid in higher control of population dynamics within fermentation co-cultures.

4.4. CRISPR controlled gene expression

The efficiency and specificity offered by the CRISPR/Cas system has allowed it to be repurposed for applications in gene regulation. One of the main deviations from the typical CRISPR/Cas9 applications is use of a variant of Cas9, called dCas9, that lacks endonuclease activity (Qi et al., 2013). The dCas mutant harbors two mutations within the nuclease domains RuvC1 and HNH (D10A and H841A) (Jinek et al., 2012; Qi et al., 2013). While it is unable to induce double stranded breaks, dCas9 is still able to bind to specific DNA sequences based on the sgRNA sequence. Therefore, instead of targeting specific nucleotides to be cut, dCas9 only binds to specific nucleotides and can be programmed to do so at locations that interact with gene transcription (Qi et al., 2013). To block transcription, dCas9 has been programmed to target either movement of an RNA polymerase (Qi et al., 2013; Bikard et al., 2012), or to prevent the binding of RNA polymerase to a promoter (Bikard et al., 2012; Rath et al., 2015). It has been demonstrated that binding of dCas9: sgRNA ribonucleoprotein complex (RNP) at regions near the RBS binding site is more effective at decreasing gene expression than binding further downstream (Qi et al., 2013; Bikard et al., 2012; Rath et al., 2015). This offers a means to variably alter the regulatory effects of dCas9: sgRNA RNPs.

CRISPRi presents a promising platform for development of orthogonal promoters (Fig. 2c); promoters that may be induced independently of one another without crosstalk. Orthogonal promoters are useful in development of complex genetic circuits (Nielsen et al., 2014). Circuits that use natural and synthetic transcription factors have been developed that exhibit orthogonality (Tamsir et al., 2011; Stanton et al., 2014; Moon et al., 2012). In addition, orthogonal transcriptional regulators have also been developed using zinc finger proteins (Beerli and Barbas, 2002; Miller et al., 2007) (ZFPs) and transcriptional activator-like effectors (Morbitzer et al., 2010) (TALEs). However, these systems often exhibit high levels of crosstalk, as there are unintended interactions of transcriptional regulators with non-cognate operator sites (Cress et al., 2016; Nielsen and Voigt, 2014). As a result, constructed genetic circuits may exhibit unpredictable behavior. The high specificity offered by dCas9 makes it an appealing choice for further development of orthogonal transcription regulators.

Several approaches have been taken to develop orthogonal promoters. In one case, variants of the BBa_J23101 σ70 promoter were designed in *E. coli* that are controlled by the presence of corresponding sgRNA (Nielsen and Voigt, 2014). A unique 13 bp sgRNA operator is placed in between the -35 and -10 σ_{70} binding sites directly adjacent to a PAM. A dCas9: sgRNA complex can then base pair with a cognate promoter to cause steric repression of transcription initiation. This and other reports utilize *de novo* design to obtain orthogonality with the intent of maximizing the number of mismatches between sgRNA and any non-cognate dCas9 binding sites (Didovyk et al., 2016; Farzadfard et al., 2013). However, it was also reported that only by varying a small number of mismatches directly adjacent to PAM is sufficient to allow promoter orthogonality (Cress et al., 2016). Only with 2 or 3 mismatches, crosstalk between different promoters can be eliminated. This region is named the PAM-proximal seed nucleation region (PPSNR).

Activation of target genes can be mediated by CRISPR activation (CRISPRa). This strategy is also based on dCas9 (Fig. 2a). However, instead of blocking transcription, dCas9 is fused to ω subunit of *E. coli* RNA polymerase, RpoZ (Bikard et al., 2013). RpoZ has been shown to aid in assembling an RNA polymerase as well as enhancing binding to the promoter (Mathew and Chatterji, 2006). CRISPRa exert a higher relative change in expression when enacting on weak promoters. CRISPRa improved production of a target protein by 2.5 fold (Bikard et al., 2013).

CRISPRi is often used as a tool to efficiently modulate expression of metabolic pathways (Kim et al., 2017; Cress et al., 2017). Strain optimization can also be performed concurrently with use of CRISPR for gene editing (Dong et al., 2017). In one study, CRISPRi was used to enhance butanol productivities and yields by downregulating pathways that compete for carbon usage and NADH (Kim et al., 2017). CRISPR and CRISPRi were used simultaneously to improve production of 1,4-butanediol (Wu et al., 2017). In this case, CRISPR was used for initial strain construction for point mutations, knockouts, and knockins of non-native pathway genes. CRISPRi was then used to suppress competing pathways that may divert flux from the biosynthesis pathway.

4.5. RNA based strategies to control gene expression

While gene deletions provide a reliable, stable genetic manipulation in organisms, they can only completely eliminate gene expression, but cannot modulate expression. Many RNA-based strategies have been developed to positively, negatively, or dynamically regulate gene expression. Several of these include riboregulators (Liu et al., 2012; Rodrigo et al., 2012; Mutalik et al., 2012; Lucks et al., 2011), Small Transcriptional Activating RNAs (STARs) (Chappell et al., 2017), toehold switches (Green et al., 2014), CRISPR interference (CRISPRi), and synthetic small regulatory RNAs (sRNAs) (Na et al., 2013). Riboregulators, STARs, and toehold switches function to regulate gene expression, primarily by binding upstream a target gene to affect translation or transcription. Each exhibit certain advantages or disadvantages for specific applications and have been reviewed elsewhere (Lee and Moon, 2018; Vigar and Wieden, 2017; MacDonald and Deans, 2016; Qi et al., 2015).

Synthetic small regulatory RNAs (sRNAs) can be used to knockdown expression of target genes in bacteria without the need for genetic modifications. The rational designed small regulatory RNA (sRNA) is composed of a target-binding sequence and a scaffold sequence which can be used to target and fine-tune expression of key enzymes in metabolic pathways. sRNA is designed to bind to the translation initiation region of target mRNA to repress expression of these genes (Na et al., 2013). sRNA can be used for development of high-throughput screening of gene knockdowns. Experiments can be designed to screen them individually, in combination with one another, or in combination with different modified hosts. Furthermore, sRNA also offers the advantage in that they can repress essential genes, those that cannot be deleted. In *E. coli* sRNA methods have been employed to enhance production of a variety of compounds including L-tyrosine (Na et al., 2013), cadaverine (Na et al., 2013), short-chain alkanes (Choi and Lee, 2013), 1,3-diaminopropane (Chae et al., 2015), and phenol (Kim et al., 2014).

4.6. Directed cell evolution

Directed cell evolution involves the continuous or repeated culturing of a strain with a selection pressure, allowing the accumulation of mutations that give rise to a desired phenotype. There are many factors that must be considered when designing a methodology for directed cell evolution. These include selection pressure, growth medium, timescale, mutagenesis rate, culture and propagation size, and maintenance of growth phases (LaCroix et al., 2015). Evolution can be accelerated by different strategies that enhance mutagenesis. These strategies include chemical mutagenesis (Lee et al., 1997), UV mutagenesis (Mundhada et al., 2016), compromising DNA repair mechanisms (Antonovsky et al., 2016), and synthetic variable mutagenesis systems (Chou and Keasling, 2013).

Directed cell evolution has previously been employed for generating a variety of industrially relevant phenotypes. *E. coli* and other microbes have been evolved to exhibit enhanced tolerances to conditions such as heat (Tenaillon et al., 2012), pH (Hughes et al., 2007), salt concentrations (Dhar et al., 2011), and substrate or product toxicity (Atsumi et al., 2010; Mundhada et al., 2016, 2017; Almario et al., 2013). Directed cell evolution has also been used to enhance growth rates (Sandberg et al., 2017), and to evolve *E. coli* to utilize alternating carbon sources, reducing lag caused diauxic growth phases (Sandberg et al., 2017).

Substrate consumption or product formation have also been enhanced using directed cell evolution (Mundhada et al., 2016; Mundhada et al., 2017; Sandberg et al., 2017; Argyros et al., 2011). Increasing product tolerance formed the basis in attempts to improve production of serine and isobutanol (Atsumi et al., 2010), as existing titers exceeded toxicity limits for these compounds. In the case of isobutanol, increased tolerance did not alter production titers because the product was produced mainly in the non-growing phase. However, enhancing tolerance of serine was quite successful in improving production titers and yields (Mundhada et al., 2016, 2017). Interestingly, two successive studies that both aimed at evolving higher production of serine achieved different results using different strategies for enhancing mutagenesis. In one example, UV mutagenesis was used to enhance tolerance to 25 g/l from 1.6 g/l (Mundhada et al., 2017). This improved production titers 20% to 11.3 g/l. A further study used adaptive laboratory evolution to enhance tolerance to 100 g/l, and further enhanced titers to 37 g/l (Mundhada et al., 2017). Directed cell evolution has also been used in combination with OptKnock to enhance product formation. OptKnock is an algorithm used to identify which genes should be deleted in order to optimize biomass formation and product secretion within a host strain (Burgard et al., 2003). In one example, strains that were constructed based on predictions made by OptKnock were further subjected to directed cell evolution to simultaneously increase growth rates and product formation (Fong et al., 2005).

Many directed cell evolution applications for metabolic engineering require the direct coupling of fitness and production. Apart from those mentioned above, one innovative strategy to enhance production of carotenoids in *Saccharomyces cerevisiae* exploits the antioxidant properties of carotenoids (Reyes et al., 2014) which protect against periodic hydrogen peroxide shocking. However, reliance on fitness coupled production greatly limits applications of evolution to production of only a few compounds. Several synthetic biology based strategies have been employed to decouple growth and production that result in improved titers of a target compound. One strategy, termed feedback-regulated evolution of phenotype (FREP), was developed to employ a molecular sensor to gauge the concentration of a target metabolite that in turn alters mutation rates (Chou and Keasling, 2013). The assembly of synthetic transcription factors that serve as actuators of operons that govern a selection mechanism allow the ability to evolve certain traits that normally have no natural selection mechanism. Unfortunately, this strategy is susceptible to spontaneous mutations that can alter the efficacy of synthetic constructs. “Escapees” are cells that recover normal growth by overcoming induced stress conditions. While strategies have been developed to prevent the formation of escapees (Liu et al., 2017), one recent example employs a synthetic circuit that controls the expression of a maltose-utilizing enzyme using a biosensor of a target metabolite. Tight coupling of growth and production is yielded in a parent strain that is deficient of enzymes required for maltose utilization.

Besides acquiring a specific phenotype, directed cell evolution also offers insight into cell physiology that can aid in further engineering efforts. In one example, *E. coli* was evolved for enhanced succinate fermentation (Zhang et al., 2009). *E. coli*’s native succinate

Table 2
Production of biochemicals by *E. coli*[a].

Chemical	Uses	Base *E. coli* strain used	titer	Approach and notes	Reference
dihomo-methionine	nutraceutical	BL21(DE3)	57 mg/L with methionine feeding	Expression of plant methionine elongation cycle. Dihomo-methionine is a precursor to glucoraphanin, which is associated with health promoting properties of broccoli	(Mirza et al., 2016).
2-pyrrolidone	solvents	BL21 Star (DE3)	1.1 g/L produced from 7.7 g/L glutamate	Novel 2-pyrrolidone synthase ORF27 was identified and improved for solubility in E.coli by MBP fusion	(Zhang et al., 2016).
peonidin 3-O-glucoside	Antioxidant, colorants for food	BL21 Star (DE3)	56 mg/L from (+)-catechin feeding	First to achieve methylated anthocyanin. CRISPRi is used for deregulation of SAM biosynthesis to improve yield	(Cress et al., 2017).
callistephin	Antioxidant, colorants for food	Co-culture of 4 strains: BL21star(DE3), BL21star(DE3) derivative, rpoA14(DE3) (K12 derivative), and ATCC 31884[b] derivative	10 mg/L	Separated an extensive pathway of 15 enzymes and transcriptional factors into four individual strains for functional production from sugar	(Jones et al., 2017).
Benzyl alcohol	solvents	ATCC 31884[b]	114 mg/L	Development of a novel pathway via conventional exogenous gene overexpression and competing pathway knockouts	(Pugh et al., 2015).
valerenadiene	Pharmaceutical component	BL21(DE3)	62 mg/L	Strong expression of valerenadiene synthase gene with media optimization	(Nybo et al., 2017).
Vanillin	Flavor and fragrance	MG1655(DE3)	119 mg/L	Removal of native aldehyde reductases and expression of necessary enzymes for converting 3-dehydroshikimate to vanillin	(Kunjapur et al., 2014).
Cinnamaldehyde	Flavor and fragrance	W3110	75 mg/L	Expression of genes for increased flux to phenylalanine. Three exogenous genes were used for converting phenylalanine to cinnamaldehyde	(Bang et al., 2016).
Isobutyl acetate	Flavor and fragrance	BW25113 derivative	36 g/L	Screened several alcohol transferases. Subsequently optimized production via bioreactor	(Tai et al., 2015).
		BW25113 derivative	19.7 g/L	Expression of acetate assimilation genes and external addition of acetate increases yield	(Tashiro et al., 2015).
Acetate, propionate, and butyrate esters	Flavor and fragrance	BW25113 derivative	Varying titer depending on ester	Demonstrated the ability of using *S. cerevisiae* ATF1 in *E. coli* for synthesis of various short chain esters with different length acyl-CoA and alcohols	(Rodriguez et al., 2014).
2-Phenylethylacetate	Flavor and fragrance	MG1655	268 mg/L from phenylalanine feeding	Combined the synthesis pathway of 2-phenylethanol from phenylalanine and ester formation using alcohol transferase ATF1	(Guo et al., 2017).
2-phenylethanol	Flavor and fragrance	BW25113	9.14 g/L from phenylalanine	Conversion of phenylalanine to 2-phenylethanol generates glutamate from α-ketoglutarate. A glutamate recycling pathway was integrated and ammonia secreted was removed by zeolite	(Wang et al., 2017).
		DH5α derivative	285 mg/L	Overexpression of genes leading to synthesis of phenylpyruvate, followed by genes for keto acid decarboxylase and alcohol dehydrogenase	(Kang et al., 2014).
		BW25113(DE3) derivative	940 mg/L	Expression of feedback resistant AroF and PheA allowed flux to enter phenylpyruvate. Phenylpyruvate is subsequently decarboxylated and reduced	(Koma et al., 2012).
limonene	Flavor and fragrance; biofuel	DH1	435 mg/L	Expression of mevalonate pathway with geranyl diphosphate (GPP) synthase and limonene synthase from *Abies grandis* (grand fir)	(Alonso-Gutierrez et al., 2013).
perillyl alcohol	Flavor and fragrance	DH1	100 mg/L	Expression of mevalonate pathway with geranyl diphosphate (GPP) synthase, limonene synthase from Abies grandis (grand fir), and a cytochrome P450 from *Mycobacterium*	(Alonso-Gutierrez et al., 2013).
myrcene	Flavor and fragrance; biofuel	DH1	58 mg/L	Expression of mevalonate pathway. with geranyl diphosphate (GPP) synthase expression was optimized. Myrcene synthase from *Quercus ilex* (oak) was expressed	(Kim et al., 2015).
caryophyllene	Flavor and fragrance; biofuel	DH1	100 mg/L	Expressed endophytic caryophyllene synthase together with mevalonate pathway enzymes and geranyl diphosphate (GPP) synthase	Wu et al.,
1,8-cineole	Flavor and fragrance; biofuel	DH1 derivative	653 mg/L	Expression of genes necessary for GPP biosynthesis. Reduced GPP consumption through mutations in *IspA*. Cineole synthase was overexpressed	(Mendez-Perez et al., 2017).
linalool	Flavor and fragrance; biofuel	DH1 derivative	505 mg/L	Expression of truncated 3R-linalool synthase with the same engineering as in the production of 1,8-cineole	(Mendez-Perez et al., 2017).

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Table 2 (*continued*)

Chemical	Uses	Base *E. coli* strain used	titer	Approach and notes	Reference
geraniol	Flavor and fragrance; biofuel	BL21 (DE3)	2 g/L	This work observed conversion of geraniol to geraniol acetate. Expression of an acetylesterase together with a two phase production allows reconversion of geraniol acetate back to geraniol through glucose starvation induced acetate utilization	(Liu et al., 2016).
α-Pinene	Flavor and fragrance; biofuel	BL21 (DE3)	970 mg/L	Expression of mevalonate pathway with geranyl diphosphate (GPP) synthase and limonene synthase from *Pinus taeda* (loblolly pine)	(Yang et al., 2013)
Tyrosol	Pharmaceutical precursor	BW25113(DE3) derivative	1.15 g/L with phenylalanine supplementation	Expression of feedback resistant aroF and tyrA allowing flux to enter 4-hydroxyphenylpyruvate. 4-hydroxyphenylpyruvate is subsequently decarboxylated and reduced	(Koma et al., 2012).
Phenyllactic acid	Antimicrobial and Polymer	BW25113(DE3) derivative	1 g/L	Expression of feedback resistant aroF and pheA allowing flux to enter phenylpyruvate. Phenylpyruvate is subsequently reduced by lactate dehydrogenase	(Koma et al., 2012).
Violacein	Antibiotic, antifungal, anticancer	BL21star	1.8 g/l	Transcriptional balancing of the five-gene violacein pathway was accomplished using a combinatorial method for identification of mutant T7 promoters	(Jones et al., 2015)
4-Hydroxyphenyllactic acid	Polymer	BW25113(DE3) derivative	1.47 g/L	Expression of feedback resistant aroF and tyrA allowing flux to enter 4-hydroxyphenylpyruvate. 4-Hydroxyphenylpyruvate is subsequently reduced by lactate dehydeorgenase	(Koma et al., 2012).
Phenylacetic acid	Pharmaceutical precursor	BW25113(DE3) derivative	1.2 g/L with tyrosine supplementation	Expression of feedback resistant aroF and pheA allowing flux to enter phenylpyruvate. Phenylpyruvate is subsequently decarboxylated and oxidized	(Koma et al., 2012).
4-Hydroxyphenylacetic acid	Pharmaceutical precursor	BW25113(DE3) derivative	930 mg/L with phenylalanine supplementation	Expression of feedback resistant aroF and tyrA allowing flux to enter 4-hydroxyphenylpyruvate. 4-hydroxyphenylpyruvate is subsequently decarboxylated and oxidized	(Koma et al., 2012).
salicylate 2-O-β-D-glucoside	anti-inflammatory agent	Co-culture of 2 strains based on BW25113	2.5 g/L	Split the pathway into two. One strain responsible for producing salicylate while the other converts it to salicylate 2-O-β-D-glucoside	(Ahmadi et al., 2016)
Succinic acid	Chemical feedstock	C derivative	86.5 g/L	Combined natural metabolic evolution based on succinic acid fermentation as the sole solution for NADH recycling under anaerobic conditions with rational metabolic engineering. Naturally evolved strain based on a simple C strain with Δ*ldhA*, Δ*adhE*, and Δ*ackA*. Subsequent deletions also were made to enhance flux. High titer achieved with batch fermentation	(Jantama et al., 2008).
		MG1655 derivative	7.28 g/L from acetate	Activation of glyoxylate pathway through deletion of *iclR*. Additional deletion of succinate dehydrogenase and malic enzyme with overexpression of citrate synthase	(Yang et al., 2016).
		MG1655 derivative	42.5 g/L from glycerol	Removal of native *pflB* and *ldhA*. Overexpression of PEP carboxykinase. Used two-stage fermentation where cell mass was accumulated during aerobic growth. Succinate production occurred during anaerobic incubation	(Li et al., 2017)
Glutaric acid	Polymer and plastics	WL3110 derivative	1.7 g/L from lysine and α-ketoglutarate	Lysine is first converted to 5-aminovalerate. Then 5-aminovalerate is converted to glutaric acid by transamination and oxidation	(Park et al., 2013).
Adipic acid	Polymer and plastics	MG1655 derivative carrying DE3 λ prophage	2.5 g/L	Reversal of β-oxidation acting on succinyl-CoA as the acyl primer. Iterative carbon extension allowed for biosynthesis of pimelic acid, suberic acid, and sebacic acid	(Cheong et al., 2016).
Malonic acid	Chemical feedstock	W3110 derivative	3.6 g/L	Constructed a synthetic pathway from which β-alanine, a natural metabolite involved in coenzyme A biosynthesis, is transaminated and oxidized	(Song et al., 2016).
Malic acid	Chemical feedstock	C derivative	34 g/L	Knock out of malic enzymes and fumarate reductase are accompanied by gene deletions made for succinate production	(Zhang et al., 2011).
Fumaric acid	Chemical feedstock	W3110 derivative	28.2 g/L	Aerobic bioreactor production. *iclR* removed to activate glyoxylate shunt. Fumarase genes, *arcA*, *aspA*, and *ptsG* were knocked out to prevent fumarate consumption and increase oxidative TCA flux. PEP carboxylase was overexpressed	(Song et al., 2013).

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Table 2 (*continued*)

Chemical	Uses	Base *E. coli* strain used	titer	Approach and notes	Reference
Muconic acid	Chemical feedstock	BW25113 derivative	390 mg/L	Construction of a novel pathway for muconate biosynthesis from anthraniliate, an intermediate in tryptophan biosynthesis	(Sun et al., 2013).
		ATCC 31884[b] derivative	1.5 g/L	Constructed a synthetic muconic acid production pathway utilizing the degradation of salicylate	(Lin et al., 2014).
		ATCC 31884[b] derivative	3.1 g/L	Co-expression of two individual pathways channeled the broad substrate pools into muconic acid biosynthesis	(Thompson et al., 2017)
		Co-colture of a K12 and a BL21(DE3) derivative.	4.7 g/L from xylose and glucose mixture	Each of the two strains specializes in utilization of glucose and xylose	(Zhang et al., 2015a).
		Co-colture of a K12 and a BL21(DE3) derivative.	2 g/L from glycerol	One strain is responsible for metabolizing glycerol to dehydroshikimate while the other converts it to muconic acid	(Zhang et al., 2015b).
Itaconic acid	Platform chemical	BW25113	43 g/L from glycerol	Removal of isocitrate decarboxylase streamlines flux to aconitate. Overexpression of cis-aconitate decarboxylase, aconitase, citrate synthase, and pyruvate carboxylase	(Chang et al., 2017).
		MG1655	47 g/L	Dynamic control of TCA cycle flux via temperature sensitive promoter	(Harder et al., 2018).
		W	3.57 g/L from acetate	W strain selected for higher acid tolerance. First to achieve itaconic acid production from acetate. Glyoxylate pathway was activated via deletion of *iclR* regulator	(Noh et al., 2017).
Mesaconic acid	Polymer applications	BW25113 derivative	6.96 g/L	Glutamate Is converted to methylaspartate via glutamate mutase. Methylaspartate is subsequently deaminated to mesaconic acid	(Wang and Zhang, 2015).
Citramalic acid	Chemical feedstock	MG1655 derivative	46.5 g/L	Expression of citramalate synthase using pyruvate with acetyl-CoA for citramalate production. Citrate synthase and acetate kinase were knocked out	(Wu and Eiteman, 2016).
Afzelechin	Pharmaceutical	Co-culture of 2 BL21star(DE3) derivative strains	41 mg/L from p-coumaric acid	Optimization of a co-culture strategy including induction time, inoculation ratios, temperature, and carbon source used for growth	(Jones et al., 2016).
Resveratrol	Neutraceutical	BW27784	2.3 g/L from p-coumaric acid	Assayed the efficiency of various stilbene synthases and assessed their expression in *E. coli*	(Lim et al., 2011).
		Co-culture of 2 W3110 derivative strains	23 mg/L from glycerol	Co-culture of two strains: one strain was optimized for *p*-coumaric acid biosynthesis, where the other designed to convert *p*-coumaric acid. An example of precursor independent resveratrol biosynthesis	(Camacho-Zaragoza et al., 2016).
Apigenin	Neutraceutical	BL21Star	415 μg/l	Expression of flavonoid 2-oxoglutarate-dependent dioxygenase-encoding flavone synthase from *Petroselinum crispum* was used as the basis for production of various flavones	(Leonard et al., 2006).
Genkwanin	Neutraceutical	BL21Star	208 μg/l		(Leonard et al., 2006)
Kaempferol	Neutraceutical	BL21Star	140 μg/l	Various Flavonols were produced using a functionally expressed P450 flavonoid 3',5'-hydroxylase (F3'5'H) fused with a P450 reductase	(Leonard et al., 2007)
Quercetin	Neutraceutical	BL21Star	20 μg/l		(Leonard et al., 2007)
Chondroitin	Pharmaceutical component	BL21star(DE3)	2.4 g/L	Expression of necessary enzymes from *E. coli* K4, a pathogenic strain which naturally contains fructosylated chondroitin in its capsular polysaccharide	(He et al., 2015).
Butanol	Biofuel	BW25113 derivative	30 g/L from glucose	Substitution of butyryl-CoA dehydrogenase with trans-enoyl-CoA reductase. Engineered a synthetic driving force by intracellular accumulation of NADH and acetyl-CoA	(Shen et al., 2011).
Isobutanol	Biofuel	BW25113 derivative	22 g/L	First demonstration of direct isobutanol production from engineered hosts. The same work also achieved production of diverse alcohols from corresponding α-keto acids	(Atsumi et al., 2008b).
		BW25113 derivative	50 g/L	Expression of ketoisovalerate decarboxylase, genes of valine biosynthesis, and alcohol dehydrogenase with fermentative genes and *pflB* knocked out. High titer achieved with in situ removal using gas stripping	(Baez et al., 2011).
2-methyl-butanol	Biofuel	BW25113 derivative	1.25 g/L	Overexpression of threonine biosynthesis and isoleucine biosynthesis with knockouts of competing pathways	(Cann and Liao, 2008).
3-methyl-butanol	Biofuel	BW25113 derivative	9.5 g/L	Isolated a mutant with enhanced leucine biosynthesis through chemical mutagenesis and selection on leucine analogue	(Connor et al., 2010a).

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Table 2 (*continued*)

Chemical	Uses	Base *E. coli* strain used	titer	Approach and notes	Reference
Pentanol	Biofuel	BW25113 derivative	4.3 g/L	Isolated a mutant of ketoisovalerate decarboxylase from saturated mutagenesis of a key residue V461. The resulting strain was specific for pentanol production. *In situ* removal by oleyl alcohol overlay increased the titer	(Chen et al., 2017).
Butyraldehyde	Chemical feedstock	BW25113	630 mg/L	Expression of mono-functional aldehyde dehydrogenase instead of the conventional bi-functional aldehyde/alcohol dehydrogenase	(Ku et al., 2017).
Isobutyraldehyde	Chemical feedstock	BW25113	35 g/L	Removal of endogenous aldehyde reductases with expression of the isobutanol production pathway without alcohol dehydrogenase. *In situ* removal of aldehyde was accomplished using gas stripping	(Rodriguez and Atsumi, 2012).
Propane	Biofuel	BL21 (DE3)	32 mg/L	Butyraldehyde is first produced by expression short chain thioesterase for butyryl-ACP. Butyraldehyde was then converted to propane via aldehyde deformylating oxygenase	(Kallio et al., 2014).
1,2-Propanediol	Polymer application	MG1655 derivative	5.6 g/L from glycerol	Expression of methylglyoxal synthase, glycerol dehydrogenase, ATP dependent dihydroxyacetone kinase, and aldehyde oxidoreductase yqhD. Lactate and acetate production pathways were disrupted	(Clomburg and Gonzalez, 2011a).
2,3-Butanediol	Polymer application	BW25113 derivative	92 g/L	Expression of acetolactate synthase, acetolactate decarboxylase, and secondary alcohol dehydrogenase. Showed the co-expression of NADH and NADPH utilizing alcohol dehydrogenases lead to significant reduction of byproduct acetoin	(Liang and Shen, 2017).
1,4-Butanediol	Chemical feedstock	MG1655 derivative	18 g/L	First demonstration of biological production of 1,4-butanediol. Synthesis via 4-hydroxybutyrate was selected as the best pathway through *in silico* prediction	(Yim et al., 2011).
		MG1655 derivative[c]	110 g/L	Identified more active enzymes by enzyme discovery and directed evolution, reduced enzyme product inhibition, balanced gene expression, eliminated the need of episomal expression and antibiotics resistance, used genes with constitutive promoters, increased resistance to phage, and eliminated competing host competing reactions	(Burgard et al., 2016).
		BW25113 derivative	12 g/L from xylose; 15.6 g/L from arabinose; 16.5 g/L from galacturonate	Designed a growth-based selection platform based on growth of a glutamate auxotroph to select a better gene cluster for xylose degradation to 2,5-dioxopentanoate, the precursor to butandial and 1,4 butanediol	(Tai et al., 2016).
Ethylene glycol	Plastics and lubricant	MG1655 derivative	20 g/L	Aldolase cleavage of xylose and xylonate degradation intermediate 2-dehydro− 3-deoxy-d-pentonate. Enzyme selection for best reduction of glycolaldheyde	(Alkim et al., 2015)
Isoprene	Synthetic rubber	BL21 derivative	24 g/L	Co-expression of both methyl-D-erythritol 4-phosphate and mevalonate pathways with isoprene synthase	(Yang et al., 2016).
Styrene	Plastics	ATCC 31884	260 mg/L	Over-expression of phenylalanine ammonia lyase from *Arabidopsis thaliana* and *trans*-cinnamate decarboxylase from *Saccharomyces cerevisiae* in an L-phenylalanine over-producing *Escherichia coli*	(McKenna and Nielsen, 2011).
p-hydroxystyrene	Plastics	ATCC 31884	400 mg/L	Expression of tyrosine/phenylalanine ammonia-lyase (PAL/TAL) converts tyrosine to p-hydroxycinnamic acid (pHCA) which is then converted to p-hydroxystyrene by pHCA decarboxylase	(Qi et al., 2007).
Butyrolactam	Polymer (Nylon)	W3110 derivative	54 g/L	Fed-batch cultivation of a strain expressing glutamate decarboxylase and *Clostridium propionicum* β-alanine CoA transferase	(Chae et al., 2017).
Valerolactam	Polymer (Nylon)	W3110 derivative	1.18 g/L	Deleted genes responsible for lysine degradation. Overexpressed phosphoenolpyruvate carboxylase with delta-aminovaleramidase and lysine 2-monooxygenase	(Chae et al., 2017).
Caprolactam	Polymer (Nylon)	BL21(DE3) derivative	79.6 μg/L from glycerol	Synthetic + 1 pathway for elongating α-ketoglutarate to α-ketopimelate. This is transaminated to aminopimelate followed by a decarboxylation, yielding 6-amino caproic acid that is converted to 6-amino caproate CoA thioester and spontaneously forms caprolactam	(Chae et al., 2017).

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Table 2 (*continued*)

Chemical	Uses	Base *E. coli* strain used	titer	Approach and notes	Reference
Amorphadiene	Pharmaceutical	DH10B	112 mg/L	First demonstration of amorphadiene biosynthesis using engineered *E. coli* with mevalonate pathway. Titer is provided as an estimate to account for evaporation	(Martin et al., 2003a)
		DH1	25 g/L	The mevalonate pathway was expressed with amorphadiene synthase. Identification of better HMG-CoA synthase and reductase with fermentation optimization significantly improved titer	(Tsuruta et al., 2009).
Taxadiene	Pharmaceutical	MG1655 derivative	1 g/L	Separating and optimizing the biosynthesis pathway into two modules: one using the methylerythritol-phosphate pathway for synthesis of isoprenoid precursor isopentenyl pyrophosphate and dimethylallyl pyrophosphate, while the other module converts isopentenyl pyrophosphate to taxadiene	(Ajikumar et al., 2010)
Thebaine	Pharmaceutical	BL21(DE3)	2.1 mg/L	Separated the synthesis of thebaine into four strains. Then a step wise production strategy was used for conversion of glycerol to dopamine, dopamine to (R,S)THP, (R,S)THP to R-reticuline, and (R)-reticuline to thebaine	(Nakagawa et al., 2016).
Hydrocodone	Pharmaceutical	BL21(DE3)	360 µg/L	Thebaine biosynthesis is coupled to thebaine 6-O-demethylase and morphinone reductase	(Nakagawa et al., 2016)

[a] unless otherwise specified, the carbon source used is glucose.
[b] phenylalanine overproducer and derivative of K-12.
[c] based on reports from Yim et al.(2011).

fermentation pathway requires carboxylation of PEP to form oxaloacetate (OAA) using PEP carboxylase, encoded by *ppc*. However, the carboxylation reaction catalyzed by Ppc also releases phosphate from PEP, which would otherwise yield 1 ATP molecule through conversion of PEP into pyruvate. Through evolution, the cell managed to conserve this ATP molecule through two adaptations. First, PEP carboxykinase, Pck, became the main enzyme responsible for the carboxylation reaction. Pck, normally used during gluconeogenesis in the decarboxylation direction, here produces ATP in the carboxylation of PEP. Furthermore, a mutation inactivated *ptsI,* part of the phosphotransferase system, which usually functions to phosphorylate glucose while simultaneously converting PEP into pyruvate. By doing so, glucokinase became the main reaction responsible for phosphorylation of glucose, preventing PEP conversion into pyruvate. This step is particularly important for succinate production as converting pyruvate back to PEP, required for flux to succinate, requires an expenditure of 2 ATP.

Several studies that sequenced genomes of evolved strains have noticed common mutations between strains of *E. coli* that have been evolved for enhanced growth within minimal media (Wannier et al., 2017). Most common to these are mutations within RNA polymerase subunits (RNAP), *rpoB* and *rpoC*, respectively, which alter regulation of a broad range of cellular processes. Small deletions in *rpoC* have been well characterized (Conrad et al., 2010) that confer systematic transcriptional changes including down-regulation of motility, acid resistance, fimbria, and curlin genes. These adaptive RNAP mutations are believed to enhance growth in minimal media by decreasing open complex longevity. This in turn reduces transcription from promoters with short-lived open complexes such as rRNA, and increases transcription of promoters that require longer engagement of RNAP. Interestingly, this mutation is also accompanied by a decreased growth rate in rich media, presumably caused by decreased transcription of ribosomal units. Observations of mutations within these RNA polymerase subunits has been reported elsewhere (Mundhada et al., 2017; LaCroix et al., 2015; Tenaillon et al., 2012; Sandberg et al., 2017; Wannier et al., 2017; Long et al., 2017; Sandberg et al., 2016).

The development of omics-tools have provided more in depth characterization of the evolved strains (Long et al., 2017; Sandberg et al., 2016; Long et al., 2018). After a directed evolution experiment, besides phenotypic characterization of the evolved strains, genotypic characterization will be performed via genomic sequencing and analyzing mutations. This can be followed by tracking the resulting transcriptional or enzyme activity changes. In the most ideal case, common mutations between different lines of evolution experiments can be identified, hence supporting the explanation of the novel phenotype (Long et al., 2018). In other cases, due to difficulty of identifying casual mutations, additional information may be required from other analysis methods, such as transcriptomics, proteomics, and metabolomics in order to establish a rational connection between phenotype and genotype (Conrad et al., 2010; Long et al., 2018). As one might expect, various genetic and transcriptional changes of a phenotype would also result in drastic metabolic flux shifts. Long et al. recently has reported that this may not be true (Long et al., 2017). They analyzed the metabolic flux of a previously-identified fast growing *E. coli* K-12 MG1655 strain that has a up to 1.6 fold increased growth rate in glucose minimal media by high-resolution 13C-metabolc flux analysis. To their surprise, the intracellular metabolic usage of the evolved strain actually altered very little from their wild type parental strain. The flux difference is much smaller compared to the difference observed between other *E. coli* wild type strains such as BL21 and BW25113. This work suggests that fluxomic analysis alone may not be sufficient in explaining the phenotypical changes. Henceforth, analyzing evolved strains with a combinatorial multi-omics approach will be beneficial for further understanding the underlying mechanisms for the observed phenotypical changes.

5. Chemicals production using *E. coli*

Being the best studied microorganism, *E. coli* often serves as the test bed for construction and validation of pathway design. As long as the pathway enzymes can be express in *E. coli*, almost any product can be produced by this organism. An important notion is the versatility of metabolism to be exploited for production of a large variety of compounds. While production of many chemicals has reached industrially relevant milestones, current explorations demonstrate the potential for new strategies to engineer *E. coli* to produce a larger variety of chemicals for industrial fermentation. Table 2 lists some of the products produced and their titers. Below, we discuss the development of *E. coli* cell factories for the production of selected products.

5.1. Flavor and fragrance

In many cases, microbial synthesis of flavor and fragrance can become economically competitive compared to chemical synthesis or extraction from plants. Flavor and fragrance compounds are primarily aromatic aldehydes and alcohols, esters, and terpenoids. Aromatic aldehydes and alcohols include vanillin and benzaldehyde (Kunjapur et al., 2014), cinnamaldehdye (Bang et al., 2016), and 2-phenylethanol (Kang et al., 2014). To produce these compounds, feedback resistant variants of 3-deoxy-D-arabinoheptulosonate 7-phosphate synthase coded by *aroG*, and chorismate mutase/prephenate dehydratase coded by *pheA*, were expressed to increase flux to the shikimate pathway and phenylalanine pathway, respectively. Genes coding for enzymes involved in the synthesis of other aromatic amino acids are deleted to channel carbon flux towards the desired product. The gene deletions resulted in amino acid auxotrophy and the required amino acids must be supplemented in the medium. Aldehydes, either as the end product or as the intermediate to form alcohols, are generally synthesized via two ways: decarboxylation of α-keto acid or activation of carboxylic acid to their corresponding CoA thioester followed by reduction.

Esters are naturally occurring scent molecules most prevalent in fruits, and are biologically synthesized through condensation of acyl-CoA and alcohol. This biosynthetic platform was engineered into *E. coli* to enable the synthesis of a wide array of different esters composed of C2 to C4 acyl-CoA and C2 – C14 alcohols (Rodriguez et al., 2014). Acetate esters of all alcohols tested were produced while propionate and butyrate esters were not. This is due to the condensation enzyme ATF1 (alcohol O-acyltransferase) from *Saccharomyces cerevisiae* which is more specific for acetyl-CoA as the acyl-donor.

Another class of fragrance molecule comprises terpenes and terpenoids. These compounds are naturally found in plants. In most cases, the synthesis of terpenoids involves in overexpression of either the mevalonate or the methyl-erythrotol-phosphate (MEP) pathway for the key intermediate isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). Subsequently, IPP and DMAPP condense into geranyl pyrophosphate (GPP) for the synthesis of monoterpene. GPP additionally can react with another IPP to form farnesyl pyrophosphate (FPP) for the synthesis of sesquiterpenes. The various terpenes are then produced from their corresponding pyrophosphate intermediate. Production of polyphenolic compounds, such as flavonoids, have also been produced in *E. coli*. Flavanoids are compounds that contain two benzene rings joined by a linear carbon chain, and have been used as neutraceuticals and food colorants. Their production has been investigated by multiple groups using a variety of techniques which have been reviewed recently (Chouhan et al., 2017; Pandey et al., 2016).

5.2. Di-acids

Compounds with two functional groups, such as di-acids, di-amines, and hydroxycarboxylic acids can be polymerized to make plastics. Many of these monomers can be produced using microbial fermentation. Succinic acid is one of the products in wild-type *E. coli* mixed acid

fermentation. Production of succinic acid by *E. coli* has a longer history than most of di-acids. These efforts are extensively reviewed elsewhere (Thakker et al., 2012; Cheng et al., 2013; Zhu and Tang, 2017). Because the most efficient pathway for succinate production is through the reductive branch of TCA cycle, most succinic acid production work would knock out the other pathways of mixed acid fermentation (Jantama et al., 2008), removal of PTS system for glucose uptake to conserve the PEP pool (Chatterjee et al., 2001), and overexpression of anaplerotic reactions (Millard et al., 1996; Stols et al., 1997). Upon deletion of other fermentation pathways, succinic acid biosynthesis becomes the major pathway for *E. coli* to recycle its NADH under anaerobic conditions. This property was used in combination with rational metabolic engineering to achieve metabolic evolution for more efficient succinic acid production (Jantama et al., 2008). This also led to the development of an efficient malic acid producer (Jantama et al., 2008) and the discovery that PEP carboxykinase can outperform PEP carboxylase as anaplerotic reaction due to co-production of ATP (Zhang et al., 2009). Adipic acid is a six-carbon dicarboxylic acid used in the production of nylon 66. As a non-natural metabolite, its biosynthesis required development of synthetic pathways. One synthetic pathway extends succinate by two carbons using acetyl-CoA to adipic acid through a pathway representing reverse β-oxidation (Fig. 3).

Other straight chain di-acids include malonic acid and glutaric acid. Glutaric acid, a five carbon straight chain carboxylic acid, is produced through three different pathways (Fig. 4). One pathway converts lysine to 5-aminovalerate followed by transamination and oxidation to convert amino group to carboxylic acid (Adkins et al., 2013). Another pathway focuses on removal the keto group of α-ketoglutarate. This synthetic pathway first reduces α-ketoglutarate to hydroxyglutarate, followed by activation to its corresponding CoA thioester. Subsequently, hydroxyglutaryl-CoA is dehydrated and reduced to yield glutaryl-CoA, which is then hydrolyzed to glutaric acid (Yu et al., 2017). This same study observed production of 28 mg/L of gluconate as a side product with a glutaric acid titer of 3.8 mg/L. The other pathway was recently constructed by extending α-ketoglutarate to α-ketoadipate using homocitrate synthase, homoaconitase, and homoisocitrate dehydrogenase from *Saccharomyces cerevisiae*, followed by decarboxylation and oxidation (Wang et al., 2017).

Unsaturated di-acids such as muconic acid and itaconic acid have attracted attention recently as chemical feedstocks. Muconic acid is a platform chemical for the production of adipic acid and terephthalic acid which are broadly used in polymers for making plastics and textiles. Muconic acid's biological precursor, catechol, can be formed via pathways derived from aromatic amino acid biosynthesis (Fig. 5). The shortest pathway involves conversion of 3-dehydroshikimate to muconic acid via three steps and was demonstrated in 1994 (Draths and Frost, 1994) with titer of around 2.4 g/L (16.8 mM). Subsequently, the same group further modified their *E. coli* strain and achieved 38.6 g/L in titer using fed-batch fermentation (Niu et al., 2002). Because catechol is a key intermediate involved in aerobic degradation of aromatic compounds, it can be additionally derived from various additional pathways.

Itaconic acid is a five carbon diacid containing a terminal olefin. Itaconic acid is produced from cis-aconitate through cis-aconitate decarboxylase. *E. coli* engineering for itaconic acid production centers on overexpression of cis-aconitate decarboxylase and aconitase with isocitrate dehydrogenase knocked out to prevent loss in aconitate flux (Chang et al., 2017; Okamoto et al., 2014).

5.3. Diols

Although several diols are natural to some microorganisms, using *E. coli* as a production host offers the advantage of easier engineering and cultivation. Microbial diol production has been extensively reviewed elsewhere (Jiang et al., 2014). Here we focus on the key development in non-natural diol production and improvements made to non-native diols in *E. coli*. Among the various diols, 1,3-PDO is the most successful case study, in which Dupont engineered a strain of *E. coli* that produces 1,3-PDO and commercialized it 2006 (Sabra et al., 2016). The biosynthesis of 1,3-PDO is achieved via dehydration of glycerol to 3-hydroxypropionaldehyde followed by reduction to 1,3-PDO. 1,2-Propanediol biosynthesis in *E. coli* has been achieved through engineering different pathways. One natural pathway in *E. coli* involves the expression of an aldolase to cleave phosphorylated pentoses, rhamnose and fucose, yielding lactaldheyde which is reduced to 1,2-propanediol (Bennett and San, 2001). Another pathway for 1,2-propanediol production relies on the conversion of dihydroxyacetone phosphate to methylgloxyal, which can then be converted to either acetol, R-lactaldehyde, or S-lactaldehyde through reduction. Following a second reduction this yields either R-1,2-propanediol or S-1,2-propanediol. To enhance titers of 1,2-propanediol from glycerol, ATP dependent kinase was used instead of the native PEP-dependent. The resulting titer was 5.6 g/L (Clomburg and Gonzalez, 2011b). The 1,2-propanediol pathway has also been further engineered to produce 1-propanol, an important chemical feedstock and biofuel (Jain et al., 2015).

2,3-Butanediol has three stereoisomers: R,R-, S,S-, and meso-. Meso-2,3-Butanediol production from *E. coli* has been enabled through expression of acetolactate synthase, acetolactate decarboxylase, and a secondary alcohol dehydrogenase (Ui et al., 1997) (Fig. 6). Upon characterization of several secondary alcohol dehydrogenases, enantiomerically pure R,R-2,3-butanediol was produced with titer up to 6.1 g/L (Yan et al., 2009). It is worth noting that due to expression of acetolactate decarboxylase, forming acetoin, R- chirality is formed, unless separate enzymes are used to reduce the diacetyl. S,S-2,3-butanediol can be produced as the major product if diacetyl is externally fed with expression of an S-specific alcohol dehydrogenase (Ui et al., 2004). One method to generate S,S-2,3-butanediol is by employing *E. coli* as a biocatalyst to recycle meso-2,3-butanediol and convert it to S,S-2,3-butanediol (J. et al., 2010). Alternatively, the 2,3-butanediol pathway can be reengineered without acetolactate decarboxylase, thereby obtaining in vivo diacetyl biosynthesis through non-enzymatic decarboxylation of acetolactate (Chu et al., 2015). The same study has shown that addition of Fe^{3+} aids decarboxylation and achieved enantiomerically pure S,S-2,3-butanediol production up to 2.2 g/L. In contrast, meso- and R,R-2,3-butanediol are produced at significantly higher titers through efforts in balancing and optimizing enzyme expression (Xu et al., 2014), induction levels (Ji et al., 2015), and reducing cofactor usage for reducing byproduct formation (Liang and Shen, 2017). Interestingly, while 2,3-butanediol is not a natural fermentation product, *E. coli* has been shown to contain all genes necessary for 2,3-

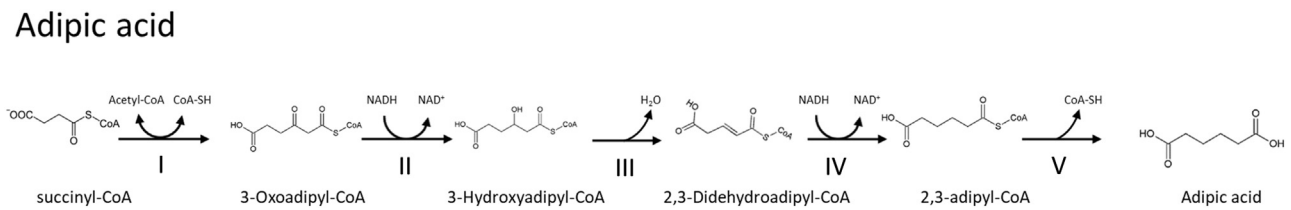

Fig. 3. Biosynthesis of adipic acid. The biosynthesis of adipic acid through β-oxidation (Cheong et al., 2016). I, β-ketoadipyl-CoA thiolase; II, 3-hydroxybutyryl-CoA dehydrogenase; III, crotonase; IV, trans-enoyl-CoA reductase; V, acyl-CoA thioesterases or transferases.

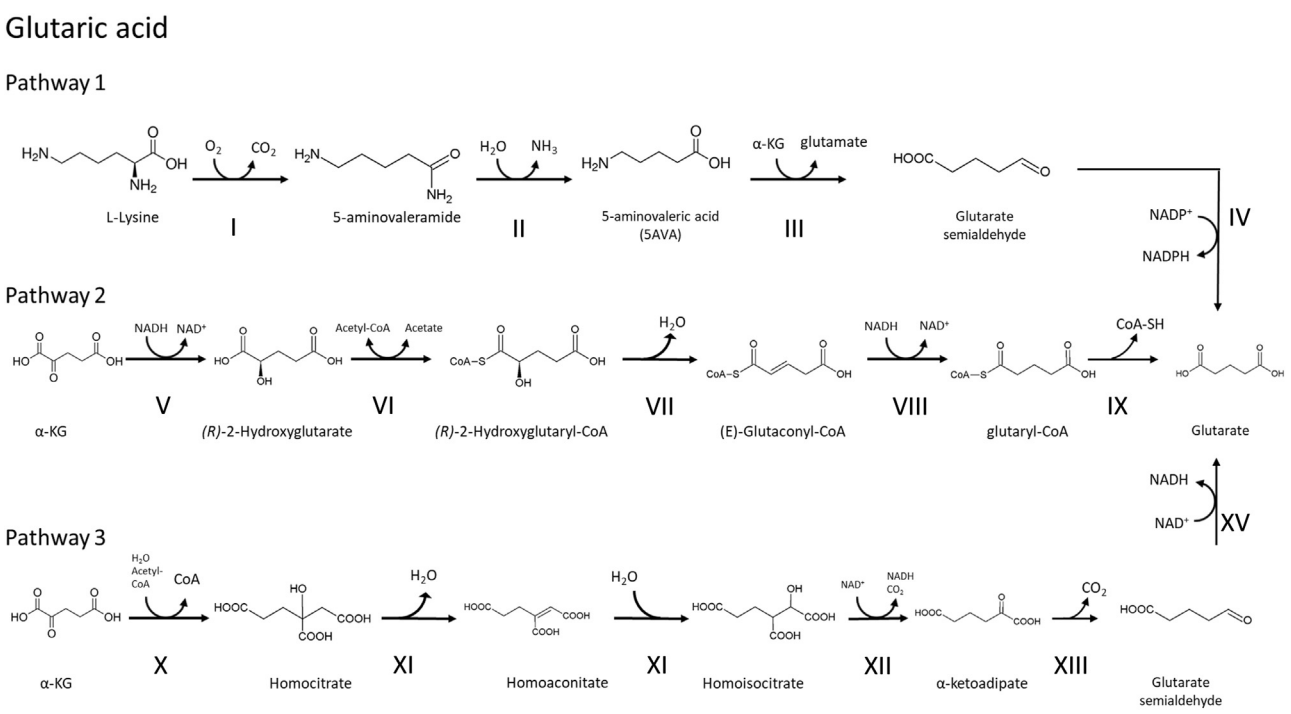

Fig. 4. Biosynthesis of glutaric acid. The biosynthesis of glutaric acid through lysine (Adkins et al., 2013), hydroxyglutarate (Yu et al., 2017), and homocitrate (Wang et al., 2017). I, lysine monooxygenase; II, 5-aminovaleramidase; III, 5-aminovalerate transaminase; IV, glutarate semialdehyde dehydrogenase; V,2-hydroxyglutarate dehydrogenase; VI, glutaconate CoA-transferase; VII, 2-hydroxyglutaryl-CoA dehydratase; VIII, enoyl trans-enoyl-CoA reductase; IX, thioesterase; X, homocitrate synthase ; XI, homoaconitase; XII, homoisocitrate dehydrogenase; XIII, α-keto acid decarboxylase.

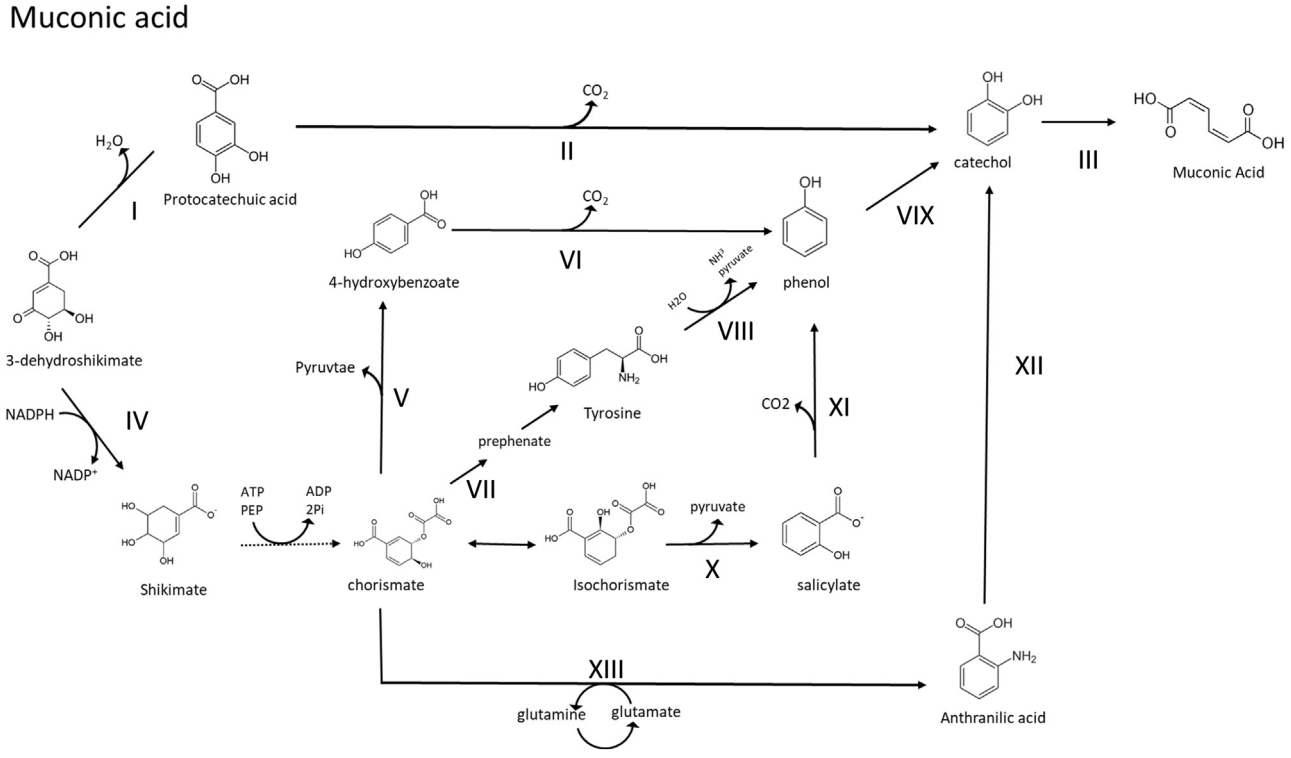

Fig. 5. Biosynthesis of muconic acid. The biosynthesis of muconic acid through 4-hydroxybenoate (Sun et al., 2013), anthranilate (Lin et al., 2014), and salicylate (Sengupta et al., 2015). I, DHS dehydratase; II, PCA decarboxylase; III, Catechol 1,2-dioxygenase; IV, Shikimate dehydrogenase; V, Chorismate pyruvate lyase; VI, 4-hydroxybenzoate decarboxylase; VII, fused chorismate mutase/prephenate dehydrogenase, VIII, tyrosine phenol lyase, VIX, phenol hydroxylase; X, isochorismate pyruvate lyase; XI, salicylate 1-monooxygenase; XII, Anthranilate 1,2-dioxygenase.

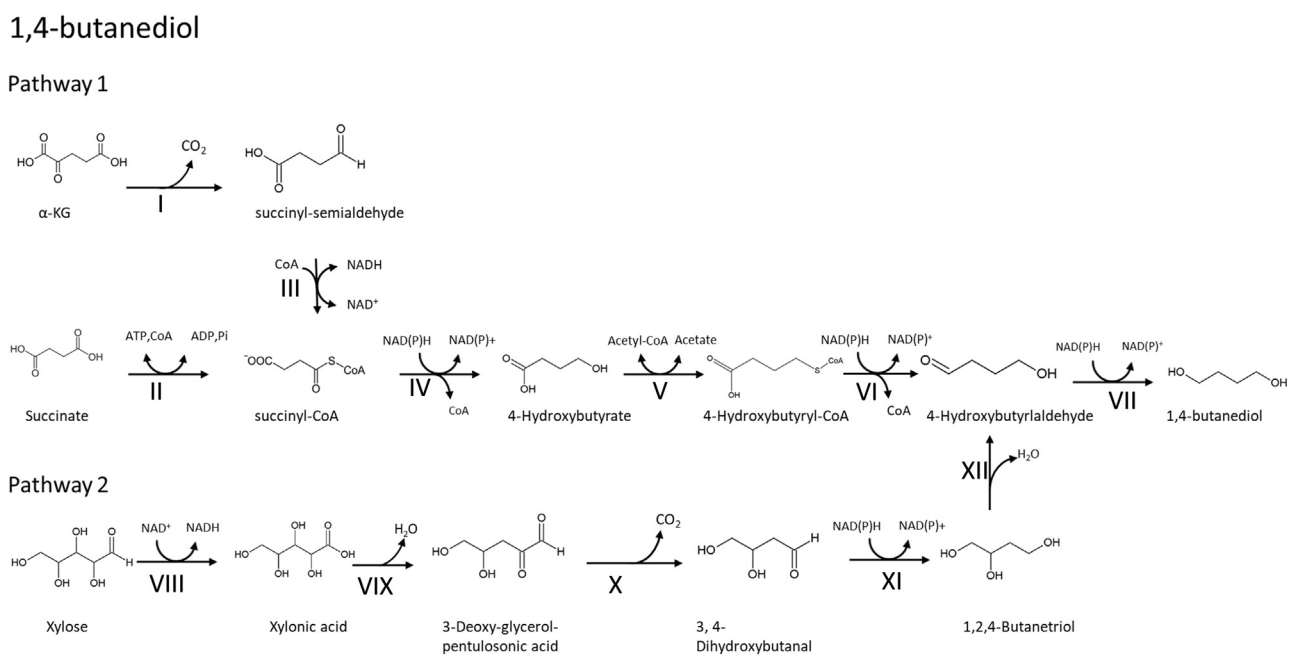

Fig. 6. Biosynthesis of 1,4-butanediol. The biosyntheisis of 1,4-butanediol through 4-hydroxybutyrate (Yim et al., 2011) and xylose (Wang et al., 2017). I, 2-oxoglutarate decarboxylase; II, succinyl-CoA synthetase; III, CoA-dependent succinate semialdehyde dehydrogenase; IV, 4-hydroxybutyrate dehydrogenase; V, 4-hydroxybutyryl-CoA transferase; VI, 4-hydroxybutyryl-CoA reductase; VII, alcohol dehydrogenase; VIII, xylose dehydrogenase; VIX, xylonate dehydratase; X, alpha-ketoisovalerate decarboxylase; XI, alcohol dehydrogenase; XII, diol dehydratase.

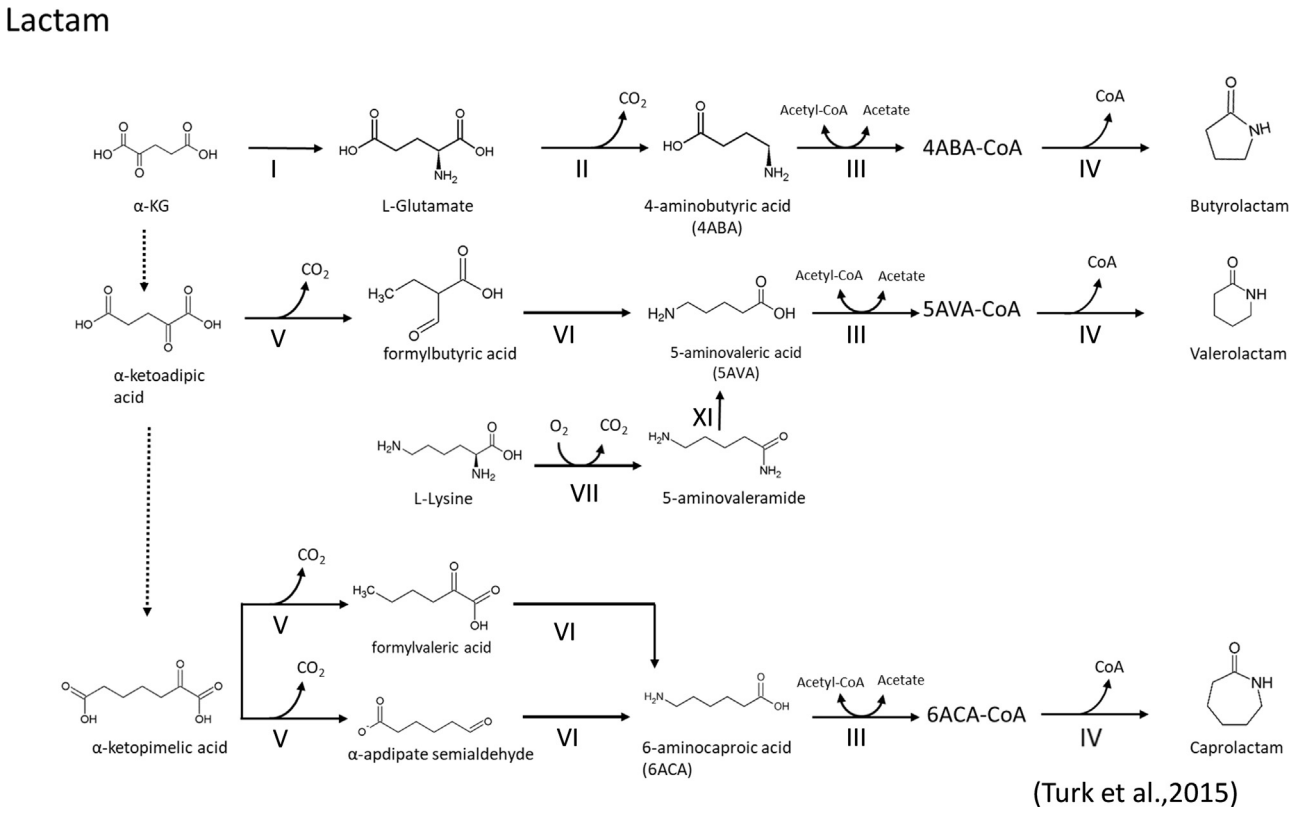

Fig. 7. Biosynthesis of lactams. The biosynthesis of Lactam production through various pathways (Turk et al., 2016; Zhang et al., 2017). I, Glutamate dehydrogenase; II, L-glutamate decarboxylase; III, β-alanine CoA transferase; IV, acyl-CoA lyase; V, branched chain α-ketoacid decarboxylase; VI, pyruvate transaminase, VII, L-lysine 2-monooxygenase.

butanediol production (Liang and Shen, 2017).

Compared to 2,3-butanediol, industrial interest is higher in 1,4-butanediol because it can serve as a platform chemical in the synthesis of other industrial chemicals such as tetrahydrofuran. Different from 2,3-butanediol and the propanediols, 1,4-butanediol is not natural to biological production. In 2011, Genomatica reported the biosynthesis of 1,4-butanediol from glucose by engineering the conversion of TCA cycle intermediates succinate and α-ketoglutarate to 1,4-butanediol through

4-hydroxybutyrate (Yim et al., 2011) with a titer of 18 g/L. This is a process which they subsequently commercialized. They systematically engineered their strain by identifying more active enzymes with enzyme discovery and directed evolution, reducing product inhibition of their enzymes, balancing gene expression, eliminating the need of episomal expression and antibiotic resistance for gene maintenance, expressing genes with constitutive promoters to eliminate costly inducers, increasing resistance to phage, and eliminating competing host reactions that divert carbon from product synthesis. As a result, the resulting titer of 1,4-butanediol produced reached over 110 g/L (Burgard et al., 2016).

Using the similar xylose degradation pathway for producing 1,4-butanediol, ethylene glycol is produced through an aldolase cleavage of the intermediate 2-dehydro-3-deoxy-d-pentonate to produce glycoaldehyde which is then reduced to ethylene glycol (Liu et al., 2013). In addition, ethylene glycol can also be produced from serine (Pereira et al., 2016).

5.4. Lactams

Lactams are used to synthesize nylon polyamide polymers. Recently, Lee and coworkers developed a platform for the direct production of lactams of C4 to C6 in length (Chae et al., 2017). In their work, lactams are formed via spontaneous intramolecular condensation of amino-acyl-CoA where an ω-amino group reacts with the carbonyl carbon. C4 to C6 ω-amino acids are produced via individual pathways. C4 ω-amino acid is formed through decarboxylation of glutamate. C5 ω-amino acid biosynthesis has been previously developed (Park et al., 2013) from lysine. C6 ω-amino acid biosynthesis has also been previously developed (Turk et al., 2016) that uses carbon chain elongation converting α-ketoglutarate to α-ketopimelate followed by decarboxylation and transamination to 6-aminocaproate. A generalized strategy is to use *E. coli* expressing an acyl-CoA ligase as a biological catalyst for converting various ω-amino acids into their corresponding acyl-CoA which would spontaneously become lactam (Zhang et al., 2017). The biosynthetic pathways for various lactams are listed in Fig. 7.

5.5. Olefins

Olefins are commonly used for production of plastics. Ethylene production in *E. coli* through the expression of an ethylene forming enzyme (Efe) or through the plant pathway using methionine has been studied and has recently been reviewed (Eckert et al., 2014). Therefore, we target our discussion on the larger olefins isoprene and styrene. 314 mg/L of isoprene has been produced by engineered *E. coli* expressing isoprene synthase from *Populus nigra* (black poplar) with additional overexpression of 5-phosphate (DXP) synthase and DXP reductoisomerase, representing key enzymes in the MEP pathway (Zhao et al., 2011). A different study using the mevalonate pathway for producing isoprene resulted in a significantly improved isoprene titer of 6.3 g/L (Yang et al., 2012). A shortened version of the mevalonate pathway was constructed in which mevalonate is directly converted to isoprene in two steps (Yang et al., 2016), however, with lower titer. Interestingly, a synergy exists between the MEP and mevalonate pathway as the co-expression of both pathways led to a significant increase in isoprene titer of 24 g/L (Yang et al., 2016).

5.6. Polyhydroxyalkanoates

E. coli has also been engineered to directly synthesize polyhydroxyalkanoates (PHA) with different monomer composition (Park et al., 2002). In particular, copolymers of lactic acid with various hydroxyacids have superior properties compared to polylactic acid (PLA) or PHA homopolymers and have been developed in *E. coli* (Jung et al., 2010). PLA and its co-polymers are non-natural bioplastics. The ability to synthesize PLA and co-polymers in vivo generally depends on the expression of a CoA transferase for activating lactate to lactoyl-CoA, and a mutant polyhydroxyalkanoate synthase (PhaC) (Yang et al., 2010; Taguchi et al., 2008). The development of lactate containing polymer biosynthesis has been reviewed (Yang et al., 2013). Recently, Lee and coworkers utilized mutant PhaC in conjunction with metabolic engineering for increased glycoyl-CoA biosynthesis and achieved direct one-step production of the FDA approved biocompatible and biodegradable polymer poly lactic-*co*-glycolic acid (PLGA) (Choi et al., 2016).

5.7. Biofuels

Short chain alcohols are a potential substitute for gasoline (Liao et al., 2016). While ethanol production is a matured technology, ethanol is hygroscopic and is lower in energy density compared to higher chain alcohols such as butanol. Butanol is a natural fermentation product from several *Clostridia* strains. However, owing to the strict anaerobic requirement and complex physiology of *Clostridia, E. coli* was investigated for potential of butanol production. Upon transfer of the *Clostridial* pathway into *E. coli*, low levels of butanol were observed (Atsumi et al., 2008; Inui et al., 2008). The difficulty in using the butanol pathway was subsequently solved by identifying a *trans*-enoyl-CoA reductase to replace the butyryl-CoA dehydrogenase complex that is responsible for a key reduction step in the butanol pathway (Shen et al., 2011; Bond-Watts et al., 2011). This resulted in a titer reaching 15–30 g/L. Subsequently, *E. coli* based butanol production was improved in several aspects: lowering of nutrient demand in the culture media by co-cultivation of a butyrate producing strain and a butyrate-to-butanol conversion strain (Saini et al., 2015), identification and mitigation of a CoA imbalance (Ohtake et al., 2017), construction of a self-inducible and stable strain by driving butanol biosynthetic genes under a promoter of native fermentative genes (Wen and Shen, 2016), development of a chromosomally stable butanol producing strain (Dong et al., 2017), and utilization of diverse substrates such as glycerol and xylose (Saini et al., 2017, 2016). The Clostridal butanol pathway was also expanded via chain elongation with acetyl-CoA to produce hexanol (Dekishima et al., 2011) and 2-pentanone (Lan et al., 2013). In a similar work, *E. coli* was engineered to produce 2-butanone with a titer of 1.3 g/L through elongation of propionyl-CoA with acetyl-CoA followed by thioester hydrolysis and decarboxylation (Srirangan et al., 2016). Intermediates of the *Clostridial* pathway have also been produced. The synthesis of butyraldehyde was achieved through replacing the bifunctional alcohol aldehyde dehydrogenase in the butanol pathway with a mono-functional aldehyde dehydrogenase (Ku et al., 2017). Butyraldehyde serves as a precursor to biological propane production (Kallio et al., 2014). The synthesis of crotonic acid and butyric acid was accomplished through expression of a thioesterase (Tseng et al., 2009; Baek et al., 2013b). Similarly, the production of S-3-hydrobutyrate was accomplished by expressing a partial *Clostridial* pathway up to the synthesis of 3-hydroxybutyryl-CoA and a thioesterase (Tseng et al., 2009). The reactions of the *Clostridial* butanol pathway represented the reversal of β-oxidation. Utilizing enzymes of β-oxidation, the iterative nature of Claisen condensation of acyl-CoA with acetyl-CoA enabled the synthesis of many higher alcohols such as hexanol and octanol, while butanol was produced with similar titer than the engineered *Clostridial* pathway (Dellomonaco et al., 2011). Using this pathway, the CoA-hydrolyzed products such as hydroxy acids, α-β unsaturated acids, and saturated carboxylic acids of the intermediates in the pathway were also produced (Clomburg et al., 2012; Kim et al., 2016).

Short chain alcohols can also be produced via decarboxylation and reduction of α-keto acids in the same manner as ethanol formation from pyruvate. The conversion of amino acids to corresponding α-keto acids within a pathway known as the Ehrlich pathway was proposed over a century ago. Owing to the interest in biofuel, this pathway regained interest in the early 21st century. Using a broad-substrate α-keto acid decarboxylase and a alcohol dehydrogenase, production of diverse

alcohols from their α-keto acid precursors was achieved (Atsumi et al., 2008a). Using the α-keto acid based pathway, 50 g/L of isobutanol was achieved from glucose (Baez et al., 2011). This pathway was also used to produce propanol and butanol (Shen and Liao, 2008), as well as several pentanols (Cann and Liao, 2008; Chen et al., 2017; Connor et al., 2010b). In particular, longer chain alcohol production was made possible through protein engineering of the α-keto acid decarboxylase and citramalate synthase which is responsible for chain elongation (Zhang et al., 2008; Marcheschi et al., 2012). Using the α-keto acid-based pathway, aldehydes such as isobutyraldehyde (Rodriguez and Atsumi, 2012, 2014) and carboxylic acids such as isobutyrate (Zhang et al., 2011; Xiong et al., 2015) have been produced with relatively high quantities of 35 g/L and 90 g/L, respectively.

Fatty acid esters and fatty alcohols are suitable diesel substitutes. The development of lipid-based biofuels was reviewed (d'Espaux et al., 2015). Native fatty acid biosynthesis is used for generation of fatty acyl-ACP. Fatty-acyl-ACP is usually hydrolyzed by thioesterase and re-activated to a corresponding CoA thioester that can either react with alcohols to form fatty acid esters (Steen et al., 2010; Nawabi et al., 2011; Sherkhanov et al., 2016) or be reduced to form fatty alcohols (Liu et al., 2013, 2016). Additionally, fatty acyl-CoA can be reduced to fatty aldehydes. Instead of further reduction, they can be deformylated to form alkanes. This strategy was used to produce alkanes in *E. coli* (Schirmer et al., 2010). Shorter alkanes have also been engineered for production in *E. coli* and are more suitable for gasoline fuels (Choi and Lee, 2013).

5.8. Amino acids

L-amino acids, such as lysine, threonine, methionine, tryptophan have been produced industrially primarily as animal feed, and partially for human nutritional supplements and pharmaceutical applications. Most of the amino acids are produced using microbial biosynthesis, except for methionine, which was produced exclusively by chemical synthesis until recently. Previously, many native amino acid producers have been identified in the genus of Brevibacterium and Corynebacterium. However, *E. coli*-based microbial processes have increasingly been developed and commercialized, particularly after the advent of metabolic engineering in the 1990s. This is because *E. coli* provides an excellent test-bed for practice industrial metabolic engineering, as the biosynthesis pathways and relevant physiology are best understood. Most of the biochemical features identified in other organisms can be transferred and reproduced in *E. coli*. Moreover, *E. coli* can grow at a temperature about 10 degrees higher than Corynebacterium, thus reducing the cost of cooling.

Methionine, one of the most important amino acids used as animal feed, provides a good example of the success of *E. coli* metabolic engineering. This compound is produced industrially at 600,000 tons/year. Previously, methionine was produced from petroleum-derived raw materials using chemical synthesis in a racemic mixture. Microbial production of L-methionine has recently been developed using *E. coli*. Methionine biosynthesis starts from aspartate and goes though the homoserine pathway. Then cysteine enters the pathway to provide the thiol group. Several patents have been granted for the microbial production of methionine. These include modifications that include the overexpression of pathway genes, deletion of competing genes, increase of glucose transport, and control of gene expression by inducible promoters (Dischert and Figge, 2016a, 2016b; Figge, 2016; Figge and Vasseur, 2017).

In producing amino acids, one of the first challenges is the feedback inhibition of the key biosynthetic enzyme by the product. This was solved in natural organisms by selecting feed-back resistant variants. Examples include L-lysine-resistant variants of dihydrodipicolinate synthase and aspartokinase for lysine production (Kojima et al., 2000), tyrosine-resistant and phenylalanine-resistant 3-deoxy-7-phosphoheptulonate synthase (Jossek et al., 2001; Patnaik and Liao, 1994b) for aromatic amino acid production, and leucine-resistant isopropylmalate synthase for branched chain amino acid production (Gusyatiner et al., 2002). Enzyme variants can be readily evolved, screened, and introduced into the desired strains. Eliminating competing pathways and degradation enzymes also represent important strategies. For example, eliminating lysine decarboxylase in *E. coli* improves lysine production (Kikuchi et al., 1998). Inactivating the *puuADRCBE* gene cluster, which codes for the putrescine degradation pathway, enhances L-arginine and L-ornithine (Gusyatiner et al., 2017).

Other strategies have been shown to aid in amino acid production. This includes enhancing glucose transport by modification of expression *of ptsG, sgrT, sgrS* or *dgsA* (Dischert and Figge (2016b), overexpressing supporting pathways (such as succinate dehydrogenase in methionine production (Figge, 2016)), increasing ATP production (Cheong et al., 2017), and controllable gene expression using temperature shifts (Figge and Vasseur, 2017).

As mentioned above, *E. coli* uses PTS for glucose transport, which also irreversibly converts PEP to pyruvate. However, PEP is a precursor to aromatic amino acids. Thus, the use of PTS reduces the maximum theoretical yields of aromatic compounds significantly (Patnaik and Liao, 1994b). A solution to this problem is either overexpression of PEP synthase to recycle pyruvate to PEP or to use an PTS-independent glucose transport system (Patnaik et al., 1995).

To date, all 20 L-amino acids can be produced using *E. coli*. Amino acid pathways are closely related to keto-acid derived alcohols, such as isobutanol (Atsumi et al., 2008a), 2-methyl butanol (Cann and Liao, 2008), and 3-methyl-butanol (Connor et al., 2010b). Thus, strains engineered for production of amino acids may have relevance for production of other compounds.

5.9. Pharmaceuticals

Insulin is a classic example of how *E. coli*-based biotechnology offers affordable medicine. Here we discuss some of the most important breakthroughs in metabolic engineering for production of pharmaceuticals and their precursors using *E. coli* as the production host. Amorphadiene, a precursor to anti-malaria drug, was produced by *E. coli* upon engineering of the mevalonate pathway with expression of amorphadiene synthase (Martin et al., 2003b). Although the initial titer was low, subsequent improvement steps, including the identification of better HMG-CoA synthase and reductase, and fermentation optimization, led to a significant increase of amorphadiene production to reach a titer of 25 g/L (Tsuruta et al., 2009). In another study, taxadiene, the precursor to taxol, was produced by *E. coli* expressing taxadiene synthase with mevalonate pathway and geranylgeranyl diphosphate synthase, however, with a low titer (Huang et al., 2001). In 2010, Stephanopoulos and coworkers engineered *E. coli* to produce taxadiene to around 1 g/L by separating its synthesis pathway into two modules: one using the MEP pathway for synthesis of isoprenoid precursor isopentenyl pyrophosphate and dimethylallyl pyrophosphate while the other module converts isopentenyl pyrophosphate to taxadiene (Ajikumar et al., 2010). The same study also took taxadiene further to taxadien-5α-ol by expression of a cytochrome P450. More recently, *E. coli* was used to synthesize opiates thebaine and hydrocodone which is derived from thebaine (Nakagawa et al., 2016). Due to the complexity of the pathway and enzymes, the biosynthesis of thebaine was separated into four strains, and the synthesis strategy follows a step-wise production. The first strain converts glycerol to dopamine. The second strain converts dopamine to (R,S)-Tetrahydropapaveroline ((R,S)THP). The third strain converts (R,S)THP to R-reticuline with a final strain converting R-reticuline to thebaine. The resulting titer was 2.1 mg/L. The same study took thebaine further by expressing thebaine 6-O-demethylase and morphinone reductase for producing hydrocodone, resulting in synthesis of 360 μg/L of hydrocodone. Production of linear hetero-polysaccharides called glycosaminoglycans (GAGs), which have a wide variety of biological roles, has also received attention.

Specifically, production of the anticoagulant heparin, has been accomplished using a variety of metabolic engineering techniques which have previously been reviewed (Badri et al., 2018; Suflita et al., 2015).

5.10. Co-culturing strategy allows compartmentalization of extensive pathways

Metabolic engineering often requires the overexpression of extensive pathway composed of numerous genes. In some cases, the number of genes required for a pathway exceeds ten or more. These extensive pathways can be separated into parts and implemented in individual strains. These strains are then optimized for the production of an intermediate permeable across the membrane. Subsequent co-culturing strategies are employed. This method was used in the production of anti-inflammatory agent salicylate 2-O-β-D-glucoside (Ahmadi et al., 2016), the platform chemical muconic acid (Zhang et al., 2015a), and the solvent butanol (Saini et al., 2015). This strategy also allows individual strains to have different host genetic backgrounds that favors the particular part of the pathway. The 15 genes responsible for producing antioxidant callistephin were split into 4 strains, each having a distinct chromosomal genotype such as phenylalanine overproduction and TCA cycle disruption (Jones et al., 2017). In another example, biosynthesis of resveratrol was split into two strains. One strain is responsible for forming p-coumaric acid, which requires host modification in aromatic amino acid biosynthesis. The other is responsible for extending it to resveratrol with malonyl-CoA, which requires little host modification and can focus on expression of necessary enzymes (Camacho-Zaragoza et al., 2016).

Co-culturing strategies relies on maintaining the proper composition of the culture. Further optimization such as induction level, temperature, and carbon source allows the development of computational models to support prediction of optimized cultivation strategies and has been used to improve the production of Afzelechin (Jones et al., 2016), and curcuminoids (Fang et al., 2017). In some cases, co-culturing of *E. coli* with other organisms is used for the production of complex molecules due to the requirement of special cellular environments and enzyme post-translational modification. Such an example is the synthetic consortium formed between a strain of taxadiene producing *E. coli* and *S. cerevisiae* strains expressing downstream complex enzymes used for converting taxadiene to acetylated diol, a paclitaxel precursor. Neither of the strains could produce this compound individually (Zhou et al., 2015).

6. Alternative carbon source utilization

Raw material cost constitutes a major part of manufacturing cost in microbial production of chemicals, except for high value compounds such as pharmaceuticals. Currently, most of the microbial production depends on glucose or sucrose as the main renewable carbon source. Since these sugars compete with food supply and their costs are significant compared to the non-renewable fossil raw materials, development of other renewable carbon sources that are economical and sufficiently abundant is crucial to the sustainability of microbial bioproduction. Examples of alternative carbon sources include non-food biomass, industrial or agricultural byproducts, or C1 carbons. Although most of these alternative carbon sources are still non-competitive, successful development in this area is expected to be impactful.

6.1. Xylose

Renewable non-food biomass, including agricultural and wood residues, mostly contain cellulose and hemicellulose (Grohmann and Bothast, 1997). D-xylose is the main component of hellicellulose, being ranked as the second most abundant sugar after glucose (Aristidou and Penttilä, 2000). Utilizing xylose as a sole carbon source and co-utilizing xylose and glucose (Neale et al., 1988) for production have long been investigated. After facile hydrolysis of hemicellulose, xylose can consist up to 70 − 80% of the resulting hydrolysates (Lee and Jeffries, 2011). As xylose uptake by *E. coli* is significantly slower than glucose, optimization has been accomplished by strategies including genetic engineering (Dien et al., 2003), co-culturing (Eiteman et al., 2009), implementing non-native uptake pathways (Cam et al., 2016; Huo et al., 2017) ameliorating catabolite repression (Lu et al., 2016), or fine tuning growth conditions. One of the recent reports is to utilize xylose as the sole carbon source to produce γ-aminobutyric acid with a titer or 3.95 g/L (Zhao et al., 2017).

6.2. Glycerol

Glycerol is a by-product in biodiesel production. It is inexpensive, abundant, and more reduced than glucose. Strains designed to use glycerol as a substrate must take into consideration the additional reducing equivalents provided compared to use of glucose as a substrate (Dharmadi et al., 2005). These reducing equivalents can be used to form redox-balanced production pathways for compounds such as ethanol or succinate (Yazdani and Gonzalez, 2008; Blankschien et al., 2010). However, it also must be noted that the excess reducing equivalents can have undesirable physiological consequences. Sufficient CO_2 concentrations are required for certain cell processes such as acetyl-CoA carboxylase used to produce the malonyl-CoA required for fatty acid biosynthesis. However, extra reducing equivalents formed from glycerol fermentation prevent CO_2 generation by dehydrogenases that require NAD+ . Fermentation of glycerol is only allowed by oxidation of formate into CO_2 via formate-hydrogen lyase under acidic conditions (Dharmadi et al., 2005). Many products have been reported from *E. coli* glycerol fermentation, including 1,2-propanediol (Clomburg and Gonzalez, 2011b), lactic acid (Mazumdar et al., 2010), α-farnesene (Wang et al., 2011), hydrogen (Tran et al., 2014), and 1-propanol (Jun Choi et al., 2012).

6.3. Alginate

Brown macroalgae (seaweed) contains abundant alginate, mannitol, and glucan. Because macroalgae do not require arable land, fertilizer, or fresh water for cultivation, and they do not contain lignin, these crops are attractive sources of carbon for microbial production. Glucan and mannitol can be utilized by microorganisms to produce ethanol (Roesijadi et al., 2010). However, alginate is difficult to digest. Alginate polysaccharide is a linear block copolymer of two uronic acids, β-D-mannuronate (M) and α-L-guluronate (G), arranged in varying sequences. Alginate transport and metabolism enzymes from *Vibrio splendidus* has been introduced into *E. coli* to allow extracellular depolymerization of alginate and its subsequent metabolism (Wargacki et al., 2012). A consolidated bioprocess achieved a 0.281 wt ethanol/weight dry microalgae (Wargacki et al., 2012).

6.4. Waste proteins

Waste protein from food, agriculture, and fermentation plants have been targeted as a potential feedstock (Huo et al., 2011; Choi et al., 2014). One of the major challenges presented is in the deamination of the amino acid to allow the carbon to be used as a source for further biosynthesis. Deamination is limited by thermodynamics and biological regulation that would otherwise use these protein sources for anabolic processes. Protein hydrolysates from *Saccharomyces cerevisiae, E. coli, Bacillus subtilis* and microalgae were utilized by a strain of *E. coli* engineered with an irreversible metabolic deamination process (Huo et al., 2011). With overexpression of exogenous transaminases and deamination cycles, free ammonia was generated and excreted from the cell (Huo et al., 2011). Ammonia re-uptake was blocked to provide a driving force to allow a one way shunt of nitrogen out of the cell. The process was used to produce 4 g/l of alcohols.

6.5. C1 compounds

C1 compounds have recently been considered as a carbon source due to the increased natural gas production and elevated emissions of bio-methane. Additionally, capturing and utilizing greenhouse gases such as methane and CO_2 in the atmosphere will ameliorate climate change. Moreover, C1 compounds including methanol and formate are intermediates of methane or CO_2 utilization, and can be generated by chemical or electrochemical methods from methane or CO_2, respectively. Although *E. coli* does not naturally assimilate C1 compounds, progress has been made in metabolic engineering to develop strains that can utilize these compounds.

In methylotrophic organisms, the first step of C1 assimilation in methylotrophic organisms is oxidation of methanol to formaldehyde. Incorporation of formaldehyde within central carbon metabolism occurs either through the ribulose monophosphate pathway (RuMP) (Brautaset and Jakobsen, 2007; Müller et al., 2015), the serine cycle in bacteria (Lidstrom et al., 1990; Smejkalova et al., 2010; Vorholt, 2002), or the xylulose monophosphate pathway (XuMP) in yeasts (Anthony, 1982). Methanol bioconversion has been reported using methylotrophic bacteria such as *Methylocystis parvus* for polysaccharides (Hou et al., 1978), *Methylobacterium* sp. for L-serine (Hagishita et al., 1996), and *Methylobacillus glycogenes* for L-lysine (Motoyama et al., 2001). Engineering the methanol-utilizing pathways into *E. coli* would be of great interest, as this will expand the range of raw material usable by *E. coli*. Reports have already demonstrated that engineering *E. coli* to assimilate methanol in central metabolism is feasible (Müller et al., 2015). Recently, *E. coli* has also been demonstrated to produce a small amount of nargenine along with methanol in rich medium (Whitaker et al., 2017) (Whitaker et al., 2016). Most of these efforts focused on incorporating the RuMP cycle into *E. coli* (Müller et al., 2015; Whitaker et al., 2017). The RuMP cycle first uses methanol dehydrogenase (coded by *mdh*) to convert methanol to formaldehyde, which reacts with Ru5P to form hexulose-6-phosphate by hexulose-6-phosphate synthase (Hps). This is then followed by 6-phospho-3-hexuloisomerase (Phi) to convert Hexulose 6-phosphate to fructose 6-phosphate to enter glycolysis (Bennett et al., 2018a; Price et al., 2016).. To date, methanol consumption by *E. coli* is still low and cannot support growth unless supplemented with rich medium such as yeast extract (Whitaker et al., 2017; Bennett et al., 2018b; Gonzalez et al., 2017).

Another important issue to note for methanol assimilation is its limitation of carbon yield in natural pathways. Methanol can be assimilated by various routes: the Rump pathway, serine pathway, or oxidation to CO_2 then followed by CO_2 fixation via Calvin–Benson-Bassham (CBB) pathway. Acetyl-CoA is a crucial central metabolite for growth and potential to serve as a common precursor. However, the yield of acetyl-CoA production is limited by either the decarboxylation step of pyruvate (resulting in 1/3 carbon loss), or the requirement of ATP as an input for both the serine and CBB pathways. A synthetic methanol condensation cycle (MCC) has been developed (Bogorad et al., 2014), which is capable of converting methanol to acetyl-CoA with 100% carbon conservation and without any ATP input. Constructing MCC in *E. coli* will enable higher carbon efficiency than other natural pathways.

Another C1 carbon, formate was also been investigated. A computationally designed synthetic pathway was proposed for formate condensation and assimilation in order to bypass complexity and challenging heterologous overexpression of natural occurring C1 assimilation pathways (Siegel et al., 2015). The synthetic pathway consisted of three steps: (i) aldehyde dehydrogenase from *Listeria monocytogenes,* along with native acetyl-CoA synthase, reduces formate into formaldehyde (ii) a computationally designed "formylase," derived from benzaldehyde lyase from *Pseudomonas biovar I*, forms dihydroxyacetone from the condensation of three formaldehyde (iii) Phosphorylation of dihydroxyacetone to dihydroxyacetone phosphate by dihydroxyacetone kinase from *S. cerevisiae*. Nevertheless, Siegel et al. were only able to demonstrate the synthetic cycle in vitro with verification by ^{13}C-formate labelling experiments. No growth with formate as sole carbon source was achieved, which may be due to insufficient formolase activity, or higher preference towards formation of glycoaldehyde from two molecules of formaldehyde (Poust et al., 2015). On the other hand, Zelcbuch et al. achieved co-utilization of formate and acetate under anaerobic condition (Bar-even, 2016). The authors took advantage of the reverse reaction of pyruvate-formate lyase (PflB) to condense acetyl-coA and formate to provide a source of pyruvate, while simultaneously knocking out glyoxylate shunt. Nevertheless, the strain could only grow to an OD 600 nm = 0.2 in 50 h, which suggests that further engineering is required.

7. Phage attack

Phage attach presents a significant risk for large scale fermentations of *E. coli* and other bacteria. Phage attack can lead to fermentation failure, or negatively impact product quantity and quality (de Melo et al., 2018; Samson and Moineau, 2013). Ongoing research is being performed to understand mechanisms that can be acquired to resist infection.

Several resistance mechanisms exist that protect bacteria from phage infection. The first is preventing phage absorption, which can occur either through blocking phage receptors (Foster, 2005; Pedruzzi et al., 1998), producing an extracellular matrix (Stirm, 1968; Perry et al., 2009), or producing competitive inhibitors (Destoumieux-Garzón et al., 2005). The second is preventing DNA entry using proteins called superinfection exclusion systems (Sie) (Lu et al., 1993), which phage DNA from entering into the host cell. The third is digestion of phage nucleic acids using restriction-modification (R-M) systems or CRISPR-Cas systems (Pingoud et al., 2005; Mcgrath et al., 1999; Raleigh and Wilson, 1986; Garneau et al., 2010). Most bacteria contain R-M systems that protect the cell against invading DNA. Unmethylated phage DNA that is recognized by the R-M system is likely to be degraded by restriction enzymes. However, to a smaller extent this DNA may be methylated to avoid restriction, and will lead to initiation of the phage lytic cycle. Several adaptive strategies between the phage and host exist to influence the fate of phage DNA (Krüger and Bickle, 1983). The fourth resistance mechanism is abortive systems, in which recognition of phage components induces mechanisms that lead to cell death (Snyder, 1995). This topic has previously been reviewed in detail (Labrie et al., 2010).

As previously discussed, CRISPR-Cas systems have gained attention for their potential in degradation of specific nucleotides (Fig. 2). CRISPR-Cas systems form a major component of phage immunity in many bacterial strains, and therefore may also be further engineered to improve resistance to phages throughout industrial fermentations. It was demonstrated that continuous rounds of bacteriophage exposure can be used to yield strains with enhanced bacteriophage immunity through acquisition of novel spacers (Barrangou et al., 2007; Deveau et al., 2008; Horvath and Barrangou, 2010). After four rounds of bacteriophage challenge, a strain of *Streptococcus thermophilis*, containing four CRISPR-Cas systems, accumulated several novel spacers within its genome (Barrangou et al., 2013). In this case, it was shown that establishing this CRISPR-encoded immunity does not make significant alterations to the rest of the genome, which is an important aspect for using this strategy on engineered strains.

A complex interplay exists between bacteriophage attack systems and bacterial immunity. CRISPR-Cas systems are known to be very effective when phages contain protospacer regions matching bacterial crRNA spacers (Semenova et al., 2011). However, phages may mutate to introduce mismatches between the crRNA spacer and the protospacer or PAM (Semenova et al., 2011; Deveau et al., 2010). These mutants may not be targetable by the host CRISPR-Cas system. In some cases, an almost perfect match of the crRNA is sufficient to induce nucleotide degradation (Westra et al., 2013). It was shown in *E. coli* that

acquisition of additional spacers could be induced by interaction of an I-E CRISPR-Cas system with partially matched protospacers (Datsenko et al., 2012). This process was named “primed CRISPR adaptation” and allows the host to regain interference against a mutant phage (Datsenko et al., 2012; Swarts et al., 2012). Beyond this, phages have adopted other mechanisms to improve efficacy of infection, including anti-CRISPR proteins that inactivate the host CRISPR-Cas system (Borges et al., 2017). Research is being done to further explore interaction between bacteria and bacteriophage to elucidate the resistance mechanisms (Borges et al., 2017; Strotskaya et al., 2017). Continued elucidation of these mechanisms will aid in the ability to rationally engineer systems to enhance phage immunity.

8. Conclusion

E. coli has long been used as a laboratory strain in molecular biology. It contributed significantly to the initial discovery of many important mechanisms in DNA replication, transcription, translation, and regulation at various levels. *E. coli* later become the workhorse for gene cloning and routine protein synthesis. After metabolic engineering for production of chemicals became a reality in early 1990s, many workers chose to use *E. coli* as a host, even though it presented some limitations that appeared challenging at the time. Others argued against the “*E. coli* syndrome”, which refers to the reliance on *E. coli,* and promoted the use of diverse organisms that are natively closer to the desired phenotype. Although work in other organisms have also advanced significantly, in many cases, the lack of efficient genetic tools and the scarce of knowledge in those unfamiliar organisms hindered the progress in metabolic engineering. In addition, alteration of an existing phenotype often faces resistance from the regulatory mechanism in the organism. Introducing an orthogonal phenotype to a familiar organism, such as *E. coli* with many genetic tools often turns out to make faster progress.

However, there remain some challenges for the use of *E. coli* as an industrial host. For example, it is still not possible to produce large structures (such as cellulosomes), to express complex proteins (such as methane monooxygenase) or to develop photosynthetic machinery in *E. coli*. Thus, *E. coli* still cannot utilize cellulosic biomass or methane, and cannot harvest sunlight to fix CO_2. Moreover, *E. coli* cannot grow under extreme conditions, such as low or high pH, high salt environments, or in temperatures greater than 45 degrees Celsius. These phenotypes may be useful for specific industrial applications. With the fast development in genome editing and systems analysis tools, coupled with rational metabolic design and evolution, it is not impractical to expect a dramatic increase in the range of phenotype that *E. coli* exhibits.

References

Feng, P., Weagant, S., Grant, M., Burkhardt, W., 2002. Bacteriological analytical Manual: enumeration of Escherichia coli and the Coliform bacteria. Bacteriol. Anal. Man (6), 1–13.

NIH, 2016. NIH GUIDELINES FOR RESEARCH INVOLVING RECOMBINANT OR SYNTHETIC NUCLEIC ACID MOLECULES,. [Online]. Available: ⟨https://osp.od.nih.gov/wp-content/uploads/NIH_Guidelines.html#_Appendix_C-II-A._Exceptions⟩.

M. M. Gusyatiner, Y. G. Rostova, M. Y. Kiryukhin, and A. Y. Romkina, “Method for producing an L-amino acid using a bacterium of the family Enterobacteriaceae having a disrupted putrescine degradation pathway,” 2017.

Shen, C.R., Lan, E.I., Dekishima, Y., Baez, A., Cho, K.M., Liao, J.C., 2011. Driving forces enable high-titer anaerobic 1-butanol synthesis in Escherichia coli. Appl. Environ. Microbiol. 77 (9), 2905–2915.

Ohtake, T., Pontrelli, S., Laviña, W.A., Liao, J.C., Putri, S.P., Fukusaki, E., 2017. Metabolomics-driven approach to solving a CoA imbalance for improved 1-butanol production in Escherichia coli (no. April). Metab. Eng. 41, 135–143.

H. Kojima, Y. Ogawa, K. Kawamura, and K. Sano, “Method of producing L-lysine by fermentation,” 2000.

Sabra, W., Groeger, C., Zeng, A.P., 2016. Microbial cell factories for diol production. Bioreact. Eng. Res. Ind. Appl. I Cell Factor. 155, 165–197.

Burgard, A., Burk, M.J., Osterhout, R., Van Dien, S., Yim, H., 2016. Development of a commercial scale process for production of 1,4-butanediol from sugar. Curr. Opin. Biotechnol. 42, 118–125.

Sanford, K., Chotani, G., Danielson, N., Zahn, J.A., 2016. Scaling up of renewable chemicals. Curr. Opin. Biotechnol. 38, 112–122.

Meyer, H., Schmidhalter, D.R., 2012. Microbial expression systems and manufacturing from a market and economic perspective. Innov. Biotechnol. 211–250.

Blattner, F.R., et al., 1997. The complete genome sequence of Escherichia coli K-12. Science (80-.) 277 (5331), 1453–1462.

NCBI, Genome Assembly and Annotation report. [Online]. Available: ⟨https://www.ncbi.nlm.nih.gov/genome/genomes/167⟩. (accessed 02-Feb-2018).

Baba, T., et al., 2006. Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection (2006.0008). Mol. Syst. Biol. 2 (2006.0008).

Studier, F.W., Moffatt, B.A., 1986. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. J. Mol. Biol. 189 (1), 113–130.

Phue, J.N., Sang, J.L., Trinh, L., Shiloach, J., 2008. Modified Escherichia coli B (BL21), a superior producer of plasmid DNA compared with Escherichia coli K (DH5$α$). Biotechnol. Bioeng. 101 (4), 831–836.

Novagen, ⟨http://www.novagen.com⟩.

Park, J.H., Jang, Y.S., Lee, J.W., Lee, S.Y., 2011. Escherichia coli W as a new platform strain for the enhanced production of L-Valine by systems metabolic engineering. Biotechnol. Bioeng. 108 (5), 1140–1147.

Prieto, M.A., Diaz, E., Garcia, J., 1996. Molecular characterization of the 4-hydroxyphenylacetate catabolic pathway of Escherichia coli W: engineering a mobile aromatic degradative cluster. Microbiology 178 (1), 111–120.

Ohta, K., Beall, D.S., Mejia, J.P., Shanmugam, K.T., Ingram, L., 1991. Genetic improvement of Escherichia coli for ethanol production: chromosomal integration of Zymomonas mobilis genes encoding pyruvate decarboxylase and alcohol dehydrogenase II. Appl. Environ. Microbiol. 57 (4), 893–900.

Jarboe, L.R., Grabar, T.B., Yomano, K.T., Shanmugan, L.P., Ingram, L.O., 2007. Development of Ethanologenic Bacteria. Springer.

Turner, P.C., et al., 2012. Optical mapping and sequencing of the Escherichia coli KO11 genome reveal extensive chromosomal rearrangements, and multiple tandem copies of the Zymomonas mobilis pdc and adhB genes. J. Ind. Microbiol. Biotechnol. 39 (4), 629–639.

Tao, F., Miao, J.Y., Shi, G.Y., Zhang, K.C., 2005. Ethanol fermentation by an acid-tolerant Zymomonas mobilis under non-sterilized condition. Process Biochem. 40 (1), 183–187.

Wernick, D.G., Pontrelli, S.P., Pollock, A.W., Liao, J.C., 2016. Sustainable biorefining in wastewater by engineered extreme alkaliphile Bacillus marmarensis. Sci. Rep. 6, 20224.

Hasunuma, T., Kondo, A., 2012. Consolidated bioprocessing and simultaneous saccharification and fermentation of lignocellulose to ethanol with thermotolerant yeast strains. Process Biochem. 47 (9), 1287–1294.

Bhalla, A., Bansal, N., Kumar, S., Bischoff, K.M., Sani, R.K., 2013. Improved lignocellulose conversion to biofuels with thermophilic bacteria and thermostable enzymes. Bioresour. Technol. 128, 751–759.

Olson, D.G., McBride, J.E., Joe Shaw, A., Lynd, L.R., 2012. Recent progress in consolidated bioprocessing. Curr. Opin. Biotechnol. 23 (3), 396–405.

de Melo, A.G., Levesque, S., Moineau, S., 2018. Phages as friends and enemies in food processing. Curr. Opin. Biotechnol. 49, 185–190.

Samson, J.E., Moineau, S., 2013. Bacteriophages in food fermentations: new frontiers in a continuous arms race. Annu. Rev. Food Sci. Technol. 4 (1), 347–368.

Pflüger-Grau, K., Görke, B., 2010. Regulatory roles of the bacterial nitrogen-related phosphotransferase system. Trends Microbiol. 18 (5), 205–214.

Liao, J.C., Hou, S., Chao, Y., 1996. Pathway analysis, engineering, and physiological considerations for redirecting central metabolism. Biotechnol. Bioeng. 52 (1), 129–140.

Blangy, D., Buc, H., Monod, J., 1968. Kinetics of the allosteric interactions of phosphofructokinase from Escherichia coli. J. Mol. Biol. 31 (1), 13–35.

Deutscher, J., Francke, C., Postma, P.W., 2006. How phosphotransferase system-related protein phosphorylation regulates carbohydrate metabolism in bacteria. Microbiol. Mol. Biol. Rev. 70 (4), 939–1031.

Crasnier-Mednansky, M., Park, M.C., Studley, W.K., Saier, M.H., 1997. Cra-mediated regulation of Escherichia coli adenylate cyclase. Microbiology 143 (Pt 3), 785–792.

Notley-mcrobb, L., Death, A., Ferenci, T., 2006. The relationship between external glucose concentration and cAMP levels inside. Biochem. J. 1997, 1909–1918.

Reddy, P., Kamireddi, M., 1998. Modulation of Escherichia coli adenylyl cyclase activity by catalytic-site mutants of protein IIA(Glc) of the phosphoenolpyruvate: sugar phosphotransferase system. J. Bacteriol. 180 (3), 732–736.

Steinsiek, S., Bettenbrock, K., 2012. Glucose transport in Escherichia coli mutant strains with defects in sugar transport systems. J. Bacteriol. 194 (21), 5897–5908.

Gabor, E., Göhler, A.K., Kosfeld, A., Staab, A., Kremling, A., Jahreis, K., 2011. The phosphoenolpyruvate-dependent glucose-phosphotransferase system from Escherichia coli K-12 as the center of a network regulating carbohydrate flux in the cell. Eur. J. Cell Biol. 90 (9), 711–720.

Negrete, A., Ng, W.I., Shiloach, J., 2010. Glucose uptake regulation in E. coli by the small RNA SgrS: comparative analysis of E. coli K (JM109 and MG1655) and E. coli B (BL21). Microb. Cell Fact. 9 (1), 75.

Negrete, A., Majdalani, N., Phue, J.N., Shiloach, J., 2013. Reducing acetate excretion from E. coli K-12 by over-expressing the small RNA SgrS. N. Biotechnol. 30 (2), 269–273.

Ross, J.A., Trussler, R.S., Black, M.D., McLellan, C.R., Haniford, D.B., 2014. Tn5 transposition in Escherichia coli is repressed by Hfq and activated by over-expression of the small non-coding RNA SgrS. Mob. DNA 5 (1), 1–16.

Vanderpool, C.K., Gottesman, S., 2007. The novel transcription factor SgrR coordinates the response to glucose-phosphate stress. J. Bacteriol. 189 (6), 2238–2248.

Chelsea, C.K.V., Lloyd, R., Park, Seongjin, Fei, Jingyi, 2017. The small protein SgrT controls transport activity of the glucose- specific phosphotransferase system. J. Bacteriol. 199 (11), 1–14.

Patnaik, R., Liao, J.C., 1994a. Engineering of Escherichia coli central metabolism for aromatic metabolite production with near theoretical yield . Engineering of Escherichia coli Central Metabolism for Aromatic Metabolite Production with Near Theoretical Yield. Appl. Enviromental Microbiol 60 (11), 3903–3908.

Hernández-Montalvo, V., Martínez, A., Hernández-Chavez, G., Bolivar, F., Valle, F., Gosset, G., 2003. Expression of galP and glk in a Escherichia coli PTS mutant restores glucose transport and increases glycolytic flux to fermentation products. Biotechnol. Bioeng. 83 (6), 687–694.

Bogorad, I.W., Lin, T.-S., Liao, J.C., 2013. Synthetic non-oxidative glycolysis enables complete carbon conservation. Nature 502 (7473), 693–697.

Lin, P.P. *et al.*, Construction and evolution of an Escherichia coli strain solely relying on non-oxidative glycolysis for sugar catabolism, Proceedings Natl. Acad. Sci.

Neidhardt, F.C., Ingraham, J.L., Schaechter, M., 1990. Physiology of the Bacterial Cell: A Molecular Approach. Sinauer Associates, Sunderland, MA.

Gunsalus, R.P., 1992. Control of electron flow in Escherichia coli: coordinated transcription of respiratory pathway genes. J. Bacteriol. 174 (22), 7069–7074.

Unden, G., Bongaerts, J., 1997a. Alternative respiratory pathways of *Escherichia coli*: energetics and transcriptional regulation in response to electron acceptors. Biochim. Biophys. Acta 1320 (3), 217–234.

Unden, G., Bongaerts, J., 1997b. Alternative respiratory pathways of *Escherichia coli*: energetics and transcriptional regulation in response to electron acceptors. Biochim. Biophys. Acta 1320 (3), 217–234.

Iuchi, S., Lin, E.C.C., 1995. Signal transduction in the Arc system for control of operons encoding aerobic respiratory enzymes. Two-Compon. Signal Transduct. 223–232.

Salmon, K.A., et al., 2005. Global gene expression profiling in *Escherichia coli* K12. J. Biol. Chem. 280 (15), 15084–15096.

Iuchi, S., Weiner, L., 1996. Cellular and molecular physiology of Escherichia coli in the adaptation to aerobic environments. J. Biochem. 120 (6), 1055–1063.

Alexeeva, S., Hellingwerf, K.J., Teixeira de Mattos, M.J., 2003. Requirement of ArcA for redox regulation in Escherichia coli under microaerobic but not anaerobic or aerobic conditions. J. Bacteriol. 185 (1), 204–209.

Kiley, P.J., Beinert, H., 2003. The role of Fe-S proteins in sensing and regulation in bacteria. Curr. Opin. Microbiol. 6 (2), 181–185.

Kang, Y., Weber, K.D., Qiu, Y., Kiley, P.J., Blattner, F.R., 2005. Genome-Wide expression analysis indicates that FNR of Escherichia coli K-12 regulates a large number of genes of unknown function genome-wide expression analysis indicates that FNR of Escherichia coli K-12 regulates a large number of genes of unknown Function. Society 187 (3), 1135–1160.

Koebmann, B.J., Westerhoff, H.V., Snoep, J.L., Nilsson, D., Jensen, P.R., 2002. The glycolytic flux in Escherichia coli is controlled by the demand for ATP. J. Bacteriol. 184 (14), 3909–3916.

Mark, R., Amsterdam, B., 1999. The steady-state internal redox state (NADH / NAD) reflects the external redox state and is correlated with catabolic adaptation in Escherichia coli. J. Bacteriol. 181 (8), 2351–2357.

Kim, Y., Ingram, L.O., Shanmugam, K.T., 2008. Dihydrolipoamide dehydrogenase mutation alters the NADH sensitivity of pyruvate dehydrogenase complex of Escherichia coli K-12. J. Bacteriol. 190 (11), 3851–3858.

Nitta, K., Laviña, W.A., Pontrelli, S., Liao, J.C., Putri, S.P., Fukusaki, E., 2017. Orthogonal partial least squares/projections to latent structures regression-based metabolomics approach for identification of gene targets for improvement of 1-butanol production in Escherichia coli. J. Biosci. Bioeng. 124 (5), 498–505.

Kim, H.J., Hou, B.K., Lee, S.G., Kim, J.S., Lee, D.W., Lee, S.J., 2013. Genome-wide analysis of redox reactions reveals metabolic engineering targets for d-lactate overproduction in Escherichia coli. Metab. Eng. 18, 44–52.

Baek, J.M., et al., 2013a. Butyrate Production in engineered Escherichia coli with synthetic scaffolds. Biotechnol. Bioeng. 110 (10), 2790–2794.

Lim, J.H., Seo, S.W., Kim, S.Y., Jung, G.Y., 2013. Refactoring redox cofactor regeneration for high-yield biocatalysis of glucose to butyric acid in Escherichia coli. Bioresour. Technol. 135, 568–573.

Sánchez, A.M., Bennett, G.N., San, K.Y., 2005. Novel pathway engineering design of the anaerobic central metabolic pathway in Escherichia coli to increase succinate yield and productivity. Metab. Eng. 7 (3), 229–239.

Zhu, X., Tan, Z., Xu, H., Chen, J., Tang, J., Zhang, X., 2014. Metabolic evolution of two reducing equivalent-conserving pathways for high-yield succinate production in Escherichia coli (no. July). Metab. Eng. 24, 87–96.

Dharmadi, Y., Murarka, A., Gonzalez, R., 2005. Anaerobic fermentation of glycerol by Escherichia coli: a new platform for metabolic engineering. Biotechnol. Bioeng. 94 (5), 821–829.

Siedler, S., Bringer, S., Bott, M., 2011. Increased NADPH availability in Escherichia coli: improvement of the product per glucose ratio in reductive whole-cell biotransformation. Appl. Microbiol. Biotechnol. 92 (5), 929–937.

Martínez, I., Zhu, J., Lin, H., Bennett, G.N., San, K.Y., 2008. Replacing Escherichia coli NAD-dependent glyceraldehyde 3-phosphate dehydrogenase (GAPDH) with a NADP-dependent enzyme from Clostridium acetobutylicum facilitates NADPH dependent pathways. Metab. Eng. 10 (6), 352–359.

Berríos-Rivera, S.J., Bennett, G.N., San, K.Y., 2002. Metabolic engineering of Escherichia coli: increase of NADH availability by overexpressing an NAD+-dependent formate dehydrogenase. Metab. Eng. 4 (3), 217–229.

Kim, Y., Ingram, L.O., Shanmugam, K.T., 2007. Construction of an Escherichia coli K-12 mutant for homoethanologenic fermentation of glucose or xylose without foreign genes. Appl. Environ. Microbiol. 73 (, 6), 1766–1771.

Yazdani, S. Shams, Gonzalez, R., 2008. Engineering Escherichia coli for the efficient conversion of glycerol to ethanol and co-products. Metab. Eng. 10 (6), 340–351.

Atsumi, S., Hanai, T., Liao, J.C., 2008a. Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. Nature 451 (7174), 86–89.

Brinkmann-Chen, S., et al., 2013. General approach to reversing ketol-acid reductoisomerase cofactor dependence from NADPH to NADH. Proc. Natl. Acad. Sci. 110 (27), 10946–10951.

Wada, K., Toya, Y., Banno, S., Yoshikawa, K., Matsuda, F., Shimizu, H., 2017. 13C-metabolic flux analysis for mevalonate-producing strain of Escherichia coli. J. Biosci. Bioeng. 123 (2), 177–182.

Lee, H.C., Kim, J.S., Jang, W., Kim, S.Y., 2010. High NADPH/NADP+ ratio improves thymidine production by a metabolically engineered Escherichia coli strain. J. Biotechnol. 149 (1–2), 24–32.

Rathnasingh, C., Raj, S.M., Lee, Y., Catherine, C., Ashok, S., Park, S., 2012. Production of 3-hydroxypropionic acid via malonyl-CoA pathway using recombinant Escherichia coli strains. J. Biotechnol. 157 (4), 633–640.

Chemler, J.A., Fowler, Z.L., McHugh, K.P., Koffas, M.A.G., 2010. Improving NADPH availability for natural product biosynthesis in Escherichia coli by metabolic engineering. Metab. Eng. 12 (2), 96–104.

Feklístov, A., Sharon, B.D., Darst, S.A., Gross, C.A., 2014. Bacterial sigma factors: aa historical, structural, and genomic perspective. Annu. Rev. Microbiol. 68 (1), 357–376.

Wade, J.T., Struhl, K., 2004. Association of RNA polymerase with transcribed regions in Escherichia coli. Proc. Natl. Acad. Sci. USA 2004.

Jishage, M., Iwata, A., Ueda, S., 1996. Regulation of RNA polymerase sigma subunit synthesis in Escherichia coli : intracellular levels of four species of sigma subunit under various growth conditions. J. Bacteriol. 178 (18), 5447–5451.

Rahman, M., Hasan, M.R., Oba, T., Shimizu, K., 2009. Effect of rpoS gene knockout on the metabolism of escherichia coli during exponential growth phase and early stationary phase based on gene expressions, enzyme activities and intracellular metabolite concentrations Mahbuba. Appl. Environ. Microbiol. 94 (3), 2705–2711.

Gaida, S.M., Al-Hinai, M.A., Indurthi, D.C., Nicolaou, S.A., Papoutsakis, E.T., 2013. Synthetic tolerance: three noncoding small RNAs, DsrA, ArcZ and RprA, acting supra-additively against acid stress. Nucleic Acids Res. 41 (18), 8726–8737.

Löffler, M., et al., 2017. Switching between nitrogen and glucose limitation: unraveling transcriptional dynamics in Escherichia coli (no. January). J. Biotechnol. 258, 2–12.

Alper, H., Moxley, J., Nevoigt, E., Fink, G.R., Stephanopoulos, G., 2006a. Engineering yeast transcription machinery for improved ethanol tolerance and Production. Science (80-) 314 (2006), 1565–1568.

Alper, H., Stephanopoulos, G., 2007. Global transcription machinery engineering: a new approach for improving cellular phenotype. Metab. Eng. 9 (3), 258–267.

Alper, H., Jin, Y.S., Moxley, J.F., Stephanopoulos, G., 2005. Identifying gene targets for the metabolic engineering of lycopene biosynthesis in Escherichia coli. Metab. Eng. 7 (3), 155–164.

Zhang, F., Qian, X., Si, H., Xu, G., Han, R., Ni, Y., 2015. Significantly improved solvent tolerance of Escherichia coli by global transcription machinery engineering. Microb. Cell Fact. 14 (1), 1–11.

Goldfarb, T., et al., 2015. BREX is a novel phage resistance system widespread in microbial genomes. EMBO J. 34 (2), 169–183.

Bettenbrock, K., Sauter, T., Jahreis, K., Kremling, A., Lengeler, J.W., Gilles, E.D., 2007. Correlation between growth rates, EIIACrr phosphorylation, and intracellular cyclic AMP levels in Escherichia coli K-12. J. Bacteriol. 189 (19), 6891–6900.

Qian, Z.G., Xia, X.X., Lee, S.Y., 2009. Metabolic engineering of Escherichia coli for the production of putrescine: a four carbon diamine. Biotechnol. Bioeng. 104 (4), 651–662.

Smith, K.M., Liao, J.C., 2011. An evolutionary strategy for isobutanol production strain development in Escherichia coli. Metab. Eng. 13 (6), 674–681.

Santos, C.N.S., Xiao, W., Stephanopoulos, G., 2012. Rational, combinatorial, and genomic approaches for engineering L-tyrosine production in Escherichia coli. Proc. Natl. Acad. Sci. USA 109 (34), 13538–13543.

Wang, Q., Zhang, Y., Yang, C., Xiong, H., Lin, Y., 2010. Acetylation of metabolic enzymes coordinates carbon source utilization and metabolic flux and metabolic flux (no. May). Science (80) 327, 1004–1007.

Brown, C.W., et al., 2017. Large-scale analysis of post-translational modifications in E. coli under glucose-limiting conditions. BMC Genom. 18 (1), 301.

Soares, N.C., Spat, P., Krug, K., Macek, B., 2013. Global dynamics of the Escherichia coli proteome and phosphoproteome during growth in minimal medium. J. Proteome Res. 12 (6), 2611–2621.

Zhang, J., et al., 2009. Lysine acetylation is a highly abundant and evolutionarily conserved modification in *Escherichia coli*. Mol. Cell. Proteom. 8 (2), 215–225.

Schmidt, A., et al., 2016. The quantitative and condition-dependent Escherichia coli proteome. Nat. Biotechnol. 34 (1), 104–110.

Hentchel, K.L., Escalante-Semerena, J.C., 2015. Acylation of biomolecules in prokaryotes: a widespread strategy for the control of biological function and metabolic Stress. Microbiol. Mol. Biol. Rev. 79 (3), 321–346.

Weinert, B.T., et al., 2013. Acetyl-phosphate is a critical determinant of lysine acetylation in E.coli. Mol. Cell 51 (2), 265–272.

Linda I, H., Bruno P, L., Alan J, W., 2010. Bacterial protein acetylation: the dawning of a new age. Mol. Microbiol. 77 (1), 15–21.

Thao, S., Escalante-Semerena, J.C., 2011. Control of protein function by reversible N-lysine acetylation in bacteria. Curr. Opin. Microbiol. 14 (2), 200–204.

Abouelfetouh, A., et al., 2015. The E. coli sirtuin CobB shows no preference for enzymatic and nonenzymatic lysine acetylation substrate sites. Microbiologyopen 4 (1), 66–83.

Castaño-Cerezo, S., Bernal, V., Blanco-Catalá, J., Iborra, J.L., Cánovas, M., 2011. cAMP-CRP co-ordinates the expression of the protein acetylation pathway with central metabolism in Escherichia coli. Mol. Microbiol 82 (5), 1110–1128.

Schilling, B., et al., 2015. Protein acetylation dynamics in response to carbon overflow in Escherichia coli. Mol. Microbiol. 98 (5), 847–863.

Garwin, J.L., Klages, a.L., Cronan, J.E., 1980. Structural, enzymatic, and genetic studies of ??-ketoacyl-acyl carrier protein synthases I and II of Escherichia coli. J. Biol. Chem. 255 (24), 11949–11956.

Dworkin, J., 2015. Ser/Thr phosphorylation as a regulatory mechanism in bacteria. Curr. Opin. Microbiol. 24, 47–52.
Vincent, C., Doublet, P., Grangeasse, C., Vaganay, E., Cozzone, A.J., Duclos, B., 1999. Cells of Escherichia coli contain a protein-tyrosine kinase, Wzc, and a phosphotyrosine-protein phosphatase, Wzb. J. Bacteriol. 181 (11), 3472–3477.
Sun, M., et al., 2016. Characterization of protein lysine propionylation in Escherichia coli: global profiling, dynamic change, and enzymatic regulation. J. Proteome Res. 15 (12), 4696–4708.
Colak, G., et al., 2013. Identification of lysine succinylation substrates and the succinylation regulatory enzyme CobB in *Escherichia coli*. Mol. Cell. Proteom. 12 (12), 3509–3520.
Wilkins, M.R., et al., 1999. High-throughput mass spectrometric discovery of protein post-translational modifications. J. Mol. Biol. 289 (3), 645–657.
Jarboe, L.R., Liu, P., Royce, L.A., 2011. Engineering inhibitor tolerance for the production of biorenewable fuels and chemicals. Curr. Opin. Chem. Eng. 1 (1), 38–42.
Nicolaou, S.A., Gaida, S.M., E. T. P. Ã, 2010. A comparative view of metabolite and substrate stress and tolerance in microbial bioprocessing: from biofuels and chemicals, to biocatalysis and bioremediation. Metab. Eng. 12 (4), 307–331.
Atsumi, S., et al., 2010. Evolution, genomic analysis, and reconstruction of isobutanol tolerance in Escherichia coli. Mol. Syst. Biol. 6 (449), 449.
Dunlop, M.J., 2011. Engineering microbes for tolerance to next-generation biofuels. Biotechnol. Biofuels 4, 1–9.
Royce, L.A., Liu, P., Stebbins, M.J., Hanson, B.C., Jarboe, L.R., 2013. The damaging effects of short chain fatty acids on Escherichia coli membranes. Appl. Microbiol Biotechnol. 97, 8317–8327.
Lian, J., Mckenna, R., Rover, M.R., Nielsen, D.R., Wen, Z., Jarboe, L.R., 2016. Production of biorenewable styrene: utilization of biomass - derived sugars and insights into toxicity. J. Ind. Microbiol. Biotechnol. 43 (5), 595–604.
Dunlop, M.J., et al., 2011. Engineering microbial biofuel tolerance and export using efflux pumps. Mol. Syst. Biol. 7 (487), 1–7.
Takatsuka, Y., Chen, C., Nikaido, H., 2010. Mechanism of recognition of compounds of diverse structures by the multidrug efflux pump AcrB of Escherichia coli. Proc. Natl. Acad. Sci. USA 107 (15), 6559–6565.
Rutherford, B.J., et al., 2010. Functional genomic study of exogenous n-butanol stress in Escherichia coli. Appl. Environ. Microbiol. 76 (6), 1935–1945.
Mundhada, H., Schneider, K., Christensen, H.B., Nielsen, A.T., 2016. Engineering of high yield production of L-serine in Escherichia coli. Biotechnol. Bioeng. 113 (4), 807–816.
Mundhada, H., et al., 2017. Increased production of L-serine in Escherichia coli through adaptive laboratory evolution (no. May 2016). Metab. Eng. 39, 141–150.
Royce, L.A., Yoon, J.M., Chen, Y., Rickenbach, E., Shanks, J.V., Jarboe, L.R., 2015. Evolution for exogenous octanoic acid tolerance improves carboxylic acid production and membrane integrity. Metab. Eng. 29, 180–188.
Jin, T., et al., 2017. Damage to the microbial cell membrane during pyrolytic sugar utilization and strategies for increasing resistance. J. Ind. Microbiol. Biotechnol. 44 (9), 1279–1292.
Tana, Z., et al., 2017. Engineering Escherichia coli membrane phospholipid head distribution improves tolerance and production of biorenewables (no. August). Metab. Eng. 44, 1–12.
Tan, Z., Shanks, J.V., Jarboe, L.R., 2016. Membrane engineering via trans unsaturated fatty acids production improves Escherichia coli robustness and production of bioren (no. February). Metab. Eng. 35, 105–113.
Schomburg, I., Chang, A., Hofmann, O., Ebeling, C., Ehrentreich, F., Schomburg, D., 2002. BRENDA: a resource for enzyme data and metabolic information. Trends Biochem. Sci. 27 (1), 54–56.
Keseler, I.M., et al., 2005. EcoCyc: a comprehensive database resource for Escherichia coli (no. Database issue). Nucleic Acids Res 33, 334–337.
Guzmán, G.I., Utrilla, J., Nurk, S., Brunk, E., Monk, J.M., Ebrahim, A., 2014. Model-driven discovery of underground metabolic functions in Escherichia coli. Proc. Natl. Acad. Sci. USA 112 (3), 929–934.
Notebaart, R.A., Kintses, B., Feist, A.M., Papp, B., 2018. Underground metabolism: network-level perspective and biotechnological potential. Curr. Opin. Biotechnol. 49, 108–114.
Linster, C.L., Van Schaftingen, E., Hanson, A.D., 2013. Metabolite damage and its repair or pre-emption. Nat. Chem. Biol. 9 (2), 72–80.
Richard, J.P., 1984. Acid-Base catalysis of the elimination and isomerization reactions of triose phosphates. J. Am. Chem. Soc. 106 (17), 4926–4936.
Richard, J.P., 1993. Mechanism for the formation of methylglyoxal from triosephosphates. Biochem. Soc. Trans. 21 (2), 171–174.
Richarme, G., Mihoub, M., Dairou, J., Chi Bui, L., Leger, T., Lamouri, A., 2015. Parkinsonism-associated protein DJ-1/park7 is a major protein deglycase that repairs methylglyoxal- and glyoxal-glycated cysteine, arginine, and lysine residues. J. Biol. Chem. 290 (3), 1885–1897.
Erb, T.J., Zarzycki, J., 2016. Biochemical and synthetic biology approaches to improve photosynthetic CO_2-fixation. Curr. Opin. Chem. Biol. 34, 72–79.
Walker, B.J., VanLoocke, A., Bernacchi, C.J., Ort, D.R., 2016. The costs of photorespiration to food production now and in the Future. Annu. Rev. Plant Biol. 67 (1), 107–129.
Sun, J., Jeffryes, J.G., Henry, C.S., Bruner, S.D., Hanson, A.D., 2017. Metabolite damage and repair in metabolic engineering design (no. October). Metab. Eng. 44, 150–159.
Abdallah, J., Mihoub, M., Gautier, V., Richarme, G., 2016. The DJ-1 superfamily members YhbO and YajL from Escherichia coli repair proteins from glycation by methylglyoxal and glyoxal. Biochem. Biophys. Res. Commun. 470 (2), 282–286.
Oppenheimer, N.J., Kaplan, N.O., 1974. Glyceraldehyde-3-phosphate dehydrogenase catalyzed hydration of the 5-6 double bond of reduced β-nicotinamide adenine dinucleotide (βNADH). Formation of β-6-hydroxy-1, 4, 5, 6-tetrahydronicotinamide adenine dinucleotide. Biochemistry 13 (23), 4685–4694.
Marbaix, A.Y., Noël, G., Detroux, A.M., Vertommen, D., Van Schaftingen, E., Linster, C.L., 2011. Extremely conserved ATP- or ADP-dependent enzymatic system for nicotinamide nucleotide. J. Biol. Chem. 286 (48), 41246–41252.
Peracchi, A., et al., 2017. Nit1 is a metabolite repair enzyme that hydrolyzes deaminated glutathione. Proc. Natl. Acad. Sci. USA 114 (16), E3233–E3242.
Stover, P., Schirchs, V., 1990. Serine hydroxymethyltransferase catalyzes the hydrolysis to 5,10-methenyltetrahydrofolate to 5-formyltetrahydrofolate. J. Biol. Chem. 265 (24), 14227–14233.
Park, C.B., Lee, S.B., Ryu, D.D.Y., 2001. L-pyroglutamate spontaneously formed from L -glutamate inhibits growth of the hyperthermophilic archaeon sulfolobus solfataricus. Appl. Environ. Microbiol. 67 (8), 3650–3654.
Monk, J.M., et al., 2016. Multi-omics Quantification of species variation of Escherichia coli links molecular features with strain phenotypes (238–251.e12). Cell Syst. 3 (3) (238–251.e12).
Chubukov, V., Gerosa, L., Kochanowski, K., Sauer, U., 2014. Coordination of microbial metabolism. Nat. Rev. Microbiol. 12 (5), 327–340.
Nichols, R.J., et al., 2011. Phenotypic landscape of a bacterial cell. Cell 144 (1), 143–156.
Sévin, D.C., Kuehne, A., Zamboni, N., Sauer, U., 2015. Biological insights through nontargeted metabolomics. Curr. Opin. Biotechnol. 34, 1–8.
Fuhrer, T., Zamboni, N., 2015. High-throughput discovery metabolomics. Curr. Opin. Biotechnol. 31, 73–78.
Kochanowski, K., Gerosa, L., Brunner, S.F., Christodoulou, D., Nikolaev, Y.V., Sauer, U., 2017. Few regulatory metabolites coordinate expression of central metabolic genes in *Escherichia coli*. Mol. Syst. Biol. 13 (1), 903.
Sévin, D.C., Fuhrer, T., Zamboni, N., Sauer, U., 2017. Nontargeted in vitro metabolomics for high-throughput identification of novel enzymes in Escherichia coli. Nat. Methods 14 (2), 187–194.
George, K.W., et al., 2014. Correlation analysis of targeted proteins and metabolites to assess and engineer microbial isopentenol production. Biotechnol. Bioeng. 111 (8), 1648–1658.
Haas, R., Zelezniak, A., Iacovacci, J., Kamrad, S., Townsend, S., Ralser, M., 2017. Designing and interpreting 'multi-omic' experiments that may change our understanding of biology (no. September). Curr. Opin. Syst. Biol. 6, 37–45.
Makrides, S.C., 1996. Strategies for achieving high-level expression of genes in Escherichia coli. Microbiol. Mol. Biol. Rev. 60 (3), 512–538.
Tyo, K.E., Alper, H.S., Stephanopoulos, G.N., 2007. Expanding the metabolic engineering toolbox: more options to engineer cells. Trends Biotechnol. 25 (3), 132–137.
Muyrers, J.P.P., Zhang, Y., Stewart, A.F., 2001. Techniques: recombinogenic engineering - new options for cloning and manipulating DNA. Trends Biochem. Sci. 26 (5), 325–331.
Andrianantoandro, E., Basu, S., Karig, D.K., Weiss, R., 2006. Synthetic biology: new engineering rules for an emerging discipline. Mol. Syst. Biol. 2, 1–14.
Datsenko, K. a., Wanner, B.L., 2000. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc. Natl. Acad. Sci. USA 97 (12), 6640–6645.
Heermann, R., Zeppenfeld, T., Jung, K., 2008. Simple generation of site-directed point mutations in the Escherichia coli chromosome using Red(R)/ET(R) Recombination. Microb. Cell Fact. 7, 14.
Kuhlman, T.E., Cox, E.C., 2010. Site-specific chromosomal integration of large synthetic constructs. Nucleic Acids Res. 38 (6).
Alper, H., Fischer, C., Nevoigt, E., Stephanopoulos, G., 2006b. Tuning genetic control through promoter engineering. Proc. Natl. Acad. Sci. USA 103 (36), 3006–3007.
Jiang, Y., Chen, B., Duan, C., Sun, B., Yang, J., Yang, S., 2015. Multigene editing in the Escherichia coli genome via the CRISPR-Cas9 system. Appl. Environ. Microbiol. 81 (7), 2506–2514.
Wang, H.H., et al., 2009. Programming cells by multiplex genome engineering and accelerated evolution. Nature 460 (7257), 894–898.
Ellis, H.M., Yu, D., DiTizio, T., Court, D.L., 2001. High efficiency mutagenesis, repair, and engineering of chromosomal DNA using single-stranded oligonucleotides. Proc. Natl. Acad. Sci. USA 98 (12), 6742–6746.
Erler, A., et al., 2009. Conformational adaptability of Redβ during DNa annealing and implications for its structural relationship with Rad52. J. Mol. Biol. 391 (3), 586–598.
Modrich, P., 1991. Mechanisms and biological effects of mismatch repair. Annu. Rev. Genet. 25.
Wang, H.H., Xu, G., Vonner, A.J., Church, G., 2011. Modified bases enable high-efficiency oligonucleotide-mediated allelic replacement via mismatch repair evasion. Nucleic Acids Res. 39 (16), 7336–7347.
Isaacs, F.J., et al., 2011. Precise manipulation of chromosomes in vivo enables genome-wide codon replacement (no. July). Science 333, 348–353.
Wang, H.H., Kim, H., Cong, L., Jeong, J., Bang, D., Church, G.M., 2012. Genome-scale promoter engineering by coselection MAGE. Nat. Methods 9 (6), 591–593.
Choi, K.R., Lee, S.Y., 2016. CRISPR technologies for bacterial systems: current achievements and future directions. Biotechnol. Adv. 34 (7), 1180–1209.
Bolotin, A., Quinquis, B., Sorokin, A., Ehrlich, S. Dusko, 2005. Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin. Microbiology 151 (8), 2551–2561.
Mojica, F.J.M., Díez-Villaseñor, C., García-Martínez, J., Soria, E., 2005. Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. J. Mol. Evol. 60 (2), 174–182.
Brouns, S.J.J., et al., 2006. Small CRISPR RNAs guide antiviral defense in prokaryotes stan (no. May). Science 311, 518–522.
Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J.A., Charpentier, E., 2012. A programmable Dual-RNA – guided DNA endonuclease in adaptice bacterial immunity (no. August). Science 337, 816–822.
Mougiakos, I., Bosma, E.F., Ganguly, J., Van Der Oost, J., Van Kranenburg, R., Kranenburg, R., 2018. Hijacking CRISPR-Cas for high-throughput bacterial metabolic

engineering: advances and prospects. Curr. Opin. Biotechnol. 50, 146–157.
Donohoue, P.D., Barrangou, R., May, A.P., 2017. Advances in industrial biotechnology using CRISPR-Cas systems. Trends Biotechnol. 36 (2).
Makarova, K.S., et al., 2011. Evolution and classification of the CRISPR-Cas systems. Nat. Rev. Microbiol. 9 (6), 467–477.
Jiang, W., Bikard, D., Cox, D., Zhang, F., Marraffini, L.A., 2013. RNA-guided editing of bacterial genomes using CRISPR-Cas systems. Nat. Biotechnol. 31 (3), 233–239.
Deltcheva, E., et al., 2011. CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. Nature 471 (7340), 602–607.
Sternberg, S.H., Redding, S., Jinek, M., Greene, E.C., Doudna, J.A., 2014. DNA interrogation by the CRISPR RNA-guided endonuclease Cas9. Nature 507 (7490), 62–67.
Cui, L., Bikard, D., 2016. Consequences of Cas9 cleavage in the chromosome of Escherichia coli. Nucleic Acids Res. 44 (9), 4243–4251.
Shuman, S., Glickman, M.S., 2007. Bacterial DNA repair by non-homologous end joining. Nat. Rev. Microbiol. 5 (11), 852–861.
Pyne, M.E., Moo-Young, M., Chung, D.A., Chou, C.P., 2015. Coupling the CRISPR/Cas9 system with lambda red recombineering enables simplified chromosomal gene replacement in Escherichia coli. Appl. Environ. Microbiol. 81 (15), 5103–5114.
Li, Y., et al., 2015. Metabolic engineering of Escherichia coli using CRISPR-Cas9 meditated genome editing. Metab. Eng. 31, 13–21.
Reisch, C.R., Prather, K.L.J., 2015. The no-SCAR (Scarless Cas9 Assisted Recombineering) system for genome editing in Escherichia coli (no. September). Sci. Rep. 5, 1–12.
Dong, H., et al., 2017. A systematically chromosomally engineered Escherichia coli efficiently produces butanol (no. November). Metab. Eng. 44, 284–292.
Wu, M.Y., Sung, L.Y., Li, H., Huang, C.H., Hu, Y.C., 2017. Combining CRISPR and CRISPRi systems for metabolic engineering of E. coli and 1,4-BDO biosynthesis. ACS Synth. Biol. 6 (12), 2350–2361.
Chung, M.E., et al., 2017. Enhanced integration of large DNA into E. coli chromosome by CRISPR/Cas9. Biotechnol. Bioeng. 114 (1), 172–183.
Alonso-Gutierrez, J., et al., 2017. Towards industrial production of isoprenoids in Escherichia coli: lessons learned from CRISPR-Cas9 based optimization of a chromosomally integrated mevalonate pathway. Biotechnol. Bioeng.
Zhu, X., et al., 2017. The CRISPR/Cas9-facilitated multiplex pathway optimization (CFPO) technique and its application to improve the Escherichia coli xylose utilization pathway (no. March). Metab. Eng. 43, 37–45.
Liang, L., et al., 2017. CRISPR Enabled trackable genome engineering for isopropanol production in Escherichia coli. Metab. Eng. 41, 1–10.
Caliando, B.J., Voigt, C.A., 2015. Targeted DNA degradation using a CRISPR device stably carried in the host genome (no. May). Nat. Commun. 6, 1–10.
Andersen, J.T., Schäfer, T., Jørgensen, P.L., Møller, S., 2001. Using inactivated microbial biomass as fertilizer: the fate of antibiotic resistance genes in the environment. Res. Microbiol. 152 (9), 823–833.
Parisien, A., Allain, B., Zhang, J., Mandeville, R., Lan, C.Q., 2008. Novel alternatives to antibiotics: bacteriophages, bacterial cell wall hydrolases, and antimicrobial peptides. J. Appl. Microbiol. 104 (1), 1–13.
Gomaa, A. a., Klumpe, H.E., Luo, M.L., Selle, K., Barrangou, R., Beisel, L., 2014. Programmable removal of bacterial strains by use of genome-targeting CRISPR/Cas systems. MBio 5 (1), e00928–13.
Bikard, D., Jiang, W., Samai, P., Hochschild, A., Zhang, F., Marraffini, L.A., 2013. Programmable repression and activation of bacterial gene expression using an engineered CRISPR-Cas system. Nucleic Acids Res. 41 (15), 7429–7437.
Nielsen, J., et al., 2014. Engineering synergy in biotechnology. Nat. Chem. Biol. 10 (5), 319–322.
Barrangou, R., et al., 2013. Genomic impact of CRISPR immunization against bacteriophages. Biochem. Soc. Trans. 41 (6), 1383–1391.
Qi, L.S., et al., 2013. Repurposing CRISPR as an RNA-γuided platform for sequence-specific control of gene expression. Cell 152 (5), 1173–1183.
Bikard, D., Hatoum-Aslan, A., Mucida, D., Marraffini, L.A., 2012. CRISPR interference can prevent natural transformation and virulence acquisition during in vivo bacterial infection. Cell Host Microbe 12 (2), 177–186.
Rath, D., Amlinger, L., Hoekzema, M., Devulapally, P.R., Lundgren, M., 2015. Efficient programmable gene silencing by Cascade. Nucleic Acids Res. 43 (1), 237–246.
Tamsir, A., Tabor, J.J., Voigt, C.A., 2011. Robust multicellular computing using genetically encoded NOR gates and chemical 'wiresg,'. Nature 469 (7329), 212–215.
Stanton, B.C., Nielsen, A.A.K., Tamsir, A., Clancy, K., Peterson, T., Voigt, C.A., 2014. Genomic mining of prokaryotic repressors for orthogonal logic gates. Nat. Chem. Biol. 10 (2), 99–105.
Moon, T.S., Lou, C., Tamsir, A., Stanton, B.C., Voigt, C.A., 2012. Genetic programs constructed from layered logic gates in single cells. Nature 491 (7423), 249–253.
Beerli, R.R., Barbas, C.F., 2002. Engineering polydactyl zinc-finger transcription factors. Nat. Biotechnol. 20 (2), 135–141.
Miller, J.C., et al., 2007. An improved zinc-finger nuclease architecture for highly specific genome editing. Nat. Biotechnol. 25 (7), 778–785.
Morbitzer, R., Romer, P., Boch, J., Lahaye, T., 2010. Regulation of selected genome loci using de novo-engineered transcription activator-like effector (TALE)-type transcription factors. Proc. Natl. Acad. Sci. USA 107 (50), 21617–21622.
Cress, B.F., et al., 2016. Rapid generation of CRISPR/dCas9-regulated, orthogonally repressible hybrid T7-lac promoters for modular, tuneable control of metabolic pathway fluxes in Escherichia coli. Nucleic Acids Res. 44 (9), 4472–4485.
Nielsen, A.A., Voigt, C.A., 2014. Multi-input CRISPR/Cas genetic circuits that interface host regulatory networks. Mol. Syst. Biol. 10 (11), 763.
Didovyk, A., Borek, B., Hasty, J., Tsimring, L., 2016. Orthogonal modular gene repression in Escherichia coli using engineered CRISPR/Cas9. ACS Synth. Biol. 5 (1), 81–88.
Farzadfard, F., Perli, S.D., Lu, T.K., 2013. Tunable and multifunctional eukaryotic transcription factors based on CRISPR/Cas. ACS Synth. Biol. 2 (10), 604–613.
Mathew, R., Chatterji, D., 2006. The evolving story of the omega subunit of bacterial RNA polymerase. Trends Microbiol. 14 (10), 450–455.
Kim, S.K., Seong, W., Han, G.H., Lee, D.H., Lee, S.G., 2017. CRISPR interference-guided multiplex repression of endogenous competing pathway genes for redirecting metabolic flux in Escherichia coli. Microb. Cell Fact. 16 (1), 1–15.
Cress, B.F., et al., 2017. CRISPRi-mediated metabolic engineering of E. coli for O-methylated anthocyanin production. Microb. Cell Fact. 16 (1), 1–14.
Liu, C.C., et al., 2012. An adaptor from translational to transcriptional control enables predictable assembly of complex regulation. Nat. Methods 9 (11), 1088–1094.
Rodrigo, G., Landrain, T.E., Jaramillo, A., 2012. De novo automated design of small RNA circuits for engineering synthetic riboregulation in living cells. Proc. Natl. Acad. Sci. USA 109 (38), 15271–15276.
Mutalik, V.K., Qi, L., Guimaraes, J.C., Lucks, J.B., Arkin, A.P., 2012. Rationally designed families of orthogonal RNA regulators of translation. Nat. Chem. Biol. 8 (5), 447–454.
Lucks, J.B., Qi, L., Mutalik, V.K., Wang, D., Arkin, A.P., 2011. Versatile RNA-sensing transcriptional regulators for engineering genetic networks. Proc. Natl. Acad. Sci. 108 (21), 8617–8622.
Chappell, J., Westbrook, A., Verosloff, M., Lucks, J.B., 2017. Computational design of small transcription activating RNAs for versatile and dynamic gene regulation. Nat. Commun. 8 (1051), 1–11.
Green, A.A., Silver, P.A., Collins, J.J., Yin, P., 2014. Toehold switches: de-novo-designed regulators of gene expression. Cell 159 (4), 925–939.
Na, D., Yoo, S.M., Chung, H., Park, H., Park, J.H., Lee, S.Y., 2013. Metabolic engineering of Escherichia coli using synthetic small regulatory RNAs. Nat. Biotechnol. 31 (2), 170–174.
Lee, Y.J., Moon, T.S., 2018. Design rules of synthetic non-coding RNAs in bacteria, *Methods*, 2018 Jan 5.
Vigar, J.R.J., Wieden, H.J., 2017. Engineering bacterial translation initiation — do we have all the tools we need? Biochim. Biophys. Acta - Gen. Subj. 1861 (11), 3060–3069.
MacDonald, I.C., Deans, T.L., 2016. Tools and applications in synthetic biology. Adv. Drug Deliv. Rev. 105, 20–34.
Qi, H., Li, B.Z., Zhang, W.Q., Liu, D., Yuan, Y.J., 2015. Modularization of genetic elements promotes synthetic metabolic engineering. Biotechnol. Adv. 33 (7), 1412–1419.
Choi, Y.J., Lee, S.Y., 2013. Microbial production of short-chain alkanes. Nature 502 (7472), 571–574.
Chae, T.U., Kim, W.J., Choi, S., Park, S.J., Lee, S.Y., 2015. Metabolic engineering of Escherichia coli for the production of 1,3-diaminopropane, a three carbon diamine (no. March). Sci. Rep 5. pp. 1–13.
Kim, B., Park, H., Na, D., Lee, S.Y., 2014. Metabolic engineering of Escherichia coli for the production of phenol from glucose. Biotechnol. J. 9 (5), 621–629.
LaCroix, R.A., et al., 2015. Use of adaptive laboratory evolution to discover key mutations enabling rapid growth of Escherichia coli K-12 MG1655 on glucose minimal medium. Appl. Environ. Microbiol. 81 (1), 17–30.
Lee, C.H., Gilbertson, D.L., Novella, I.S., Huerta, R., Domingo, E., Holland, J.J., 1997. Negative effects of chemical mutagenesis on the adaptive behavior of vesicular stomatitis virus. J. Virol. 71 (5), 3636–3640.
Antonovsky, N., et al., 2016. Sugar synthesis from CO_2 in Escherichia coli. Cell 166 (1), 115–125.
Chou, H.H., Keasling, J.D., 2013. Programming adaptive control to evolve increased metabolite production. Nat. Commun. 4, 2595.
Tenaillon, O., et al., 2012. The molecular diversity of adaptive convergence. Science 335 (6067), 457–462.
Hughes, B.S., Cullum, A.J., Bennett, A.F., 2007. Evolutionary adaptation to environmental ph in experimental lineages of Escherichia coli. Evolution 61 (7), 1725–1734.
Dhar, R., Sägesser, R., Weikert, C., Yuan, J., Wagner, A., 2011. Adaptation of Saccharomyces cerevisiae to saline stress through laboratory evolution. J. Evol. Biol. 24 (5), 1135–1153.
Almario, M.P., Reyes, L.H., Kao, K.C., 2013. Evolutionary engineering of Saccharomyces cerevisiae for enhanced tolerance to hydrolysates of lignocellulosic biomass. Biotechnol. Bioeng. 110 (10), 2616–2623.
Sandberg, T., Lloyd, C., Palsson, B., Feist, A., 2017. Laboratory evolution to alternating substrate environments yields distinct phenotypic and genetic adaptive strategies. Appl. Environ. Microbiol. 83 (13), 1–15.
Argyros, D.A., et al., 2011. High ethanol titers from cellulose by using metabolically engineered thermophilic, anaerobic microbes. Appl. Environ. Microbiol. 77 (23), 8288–8294.
Burgard, A.P., Pharkya, P., Maranas, C.D., 2003. OptKnock : A Bilevel Programming Framework for Identifying Gene Knockout Strategies for Microbial Strain Optimization. Biotechnol Bioeng.
Fong, S.S., et al., 2005. In silico design and adaptive evolution of Escherichia coli for production of lactic acid. Biotechnol. Bioeng. 91 (5), 643–648.
Reyes, L.H., Gomez, J.M., Kao, K.C., 2014. Improving carotenoids production in yeast via adaptive laboratory evolution. Metab. Eng. 21, 26–33.
Liu, S.-D., Wu, Y.-N., Wang, T.-M., Zhang, C., Xing, X.-H., 2017. Maltose utilization as a novel selection strategy for continuous evolution of microbes with enhanced metabolite Production. ACS Synth. Biol., p. acssynbio 7b00247.
Zhang, X., Jantama, K., Moore, J.C., Jarboe, L.R., Shanmugam, K.T., Ingram, L.O., 2009. Metabolic evolution of energy-conserving pathways for succinate production in Escherichia coli. Proc. Natl. Acad. Sci. USA 106 (48), 20180–20185.
Wannier, T.M., Kunjapur, A.M., Rice, D.P., McDonald, M.J., Desai, M.M., Church, G.M., 2017. Long-term adaptive evolution of genomically recoded Escherichia coli. bioRxiv 162834.
Conrad, T.M., et al., 2010. RNA polymerase mutants found through adaptive evolution reprogram Escherichia coli for optimal growth in minimal media. Proc. Natl. Acad. Sci. USA 107 (47), 20500–20505.
Long, C.P., Gonzalez, J.E., Feist, A.M., Palsson, B.O., Antoniewicz, M.R., 2017. Fast

growth phenotype of E. coli K-12 from adaptive laboratory evolution does not require intracellular flux rewiring. Metab. Eng. 44 (August), 100–107.
Sandberg, T.E., Long, C.P., Gonzalez, J.E., Feist, A.M., Antoniewicz, M.R., Palsson, B.O., 2016. Evolution of E. coli on [U-13C]glucose reveals a negligible isotopic influence on metabolism and physiology. PLoS One 11 (3), 1–14.
Long, C.P., Gonzalez, J.E., Feist, A.M., Palsson, B.O., Antoniewicz, M.R., 2018. Dissecting the genetic and metabolic mechanisms of adaptation to the knockout of a major metabolic enzyme in *Escherichia coli*. Proc. Natl. Acad. Sci. 115 (1), 222–227.
Mirza, N., Crocoll, C., Olsen, C.E., Halkier, B.A., 2016. Engineering of methionine chain elongation part of glucoraphanin pathway in E. coli. Metab. Eng. 35, 31–37.
Zhang, J., Kao, E., Wang, G., Baidoo, E.E.K., Chen, M., Keasling, J.D., 2016. Metabolic engineering of Escherichia coli for the biosynthesis of 2-pyrrolidone (no. Supplement C). Metab. Eng. Commun. 3, 1–7.
Jones, J.A., et al., 2017. Complete Biosynthesis of Anthocyanins Using E. coli Polycultures. MBio 8 (3).
Pugh, S., McKenna, R., Halloum, I., Nielsen, D.R., 2015. Engineering Escherichia coli for renewable benzyl alcohol production (no. Supplement C). Metab. Eng. Commun. 2, 39–45.
Nybo, S.E., Saunders, J., McCormick, S.P., 2017. Metabolic engineering of Escherichia coli for production of valerenadiene. J. Biotechnol. 262, 60–66.
Kunjapur, A.M., Tarasova, Y., Prather, K.L.J., 2014. Synthesis and Accumulation of Aromatic Aldehydes in an Engineered Strain of Escherichia coli. J. Am. Chem. Soc. 136 (33), 11644–11654.
Bang, H.B., Lee, Y.H., Kim, S.C., Sung, C.K., Jeong, K.J., 2016. Metabolic engineering of Escherichia coli for the production of cinnamaldehyde. Microb. Cell Fact. 15.
Tai, Y.S., Xiong, M.Y., Zhang, K.C., 2015. Engineered biosynthesis of medium-chain esters in Escherichia coli. Metab. Eng. 27, 20–28.
Tashiro, Y., Desai, S.H., Atsumi, S., 2015. Two-dimensional isobutyl acetate production pathways to improve carbon yield. Nat. Commun. 6.
Rodriguez, G.M., Tashiro, Y., Atsumi, S., 2014. Expanding ester biosynthesis in Escherichia coli. Nat. Chem. Biol. 10 (4), 259.
Guo, D., Zhang, L., Pan, H., Li, X., 2017. Metabolic engineering of Escherichia coli for production of 2-Phenylethylacetate from L-phenylalanine. Microbiologyopen 6 (4).
Wang, P., Yang, X., Lin, B., Huang, J., Tao, Y., 2017. Cofactor self-sufficient whole-cell biocatalysts for the production of 2-phenylethanol. Metab. Eng. 44, 143–149.
Kang, Z., Zhang, C.Z., Du, G.C., Chen, J., 2014. Metabolic engineering of escherichia coli for production of 2-phenylethanol from renewable Glucose. Appl. Biochem. Biotechnol. 172 (4), 2012–2021.
Koma, D., Yamanaka, H., Moriyoshi, K., Ohmoto, T., Sakai, K., 2012. Production of aromatic compounds by metabolically engineered Escherichia coli with an expanded shikimate pathway. Appl. Environ. Microbiol. 78 (17), 6203–6216.
Alonso-Gutierrez, J., et al., 2013. Metabolic engineering of Escherichia coli for limonene and perillyl alcohol production. Metab. Eng. 19, 33–41.
Kim, E.M., Eom, J.H., Um, Y., Kim, Y., Woo, H.M., 2015. Microbial synthesis of myrcene by metabolically engineered Escherichia coli. J. Agric. Food Chem. 63 (18), 4606–4612.
Wu, W., Liu, F., Davis, R.W., Engineering Escherichia coli for Production of Mixtures of Caryophyllene, Caryophyllene Alcohol, and their Stereoisomers as Potential Aviation Fuel Compounds, *Metab. Eng. Commun.*
Mendez-Perez, D., et al., 2017. Production of jet fuel precursor monoterpenoids from engineered Escherichia coli. Biotechnol. Bioeng. 114 (8), 1703–1712.
Liu, W., et al., 2016. Engineering Escherichia coli for high-yield geraniol production with biotransformation of geranyl acetate to geraniol under fed-batch culture. Biotechnol. Biofuels 9.
Yang, J.M., et al., 2013. Metabolic engineering of Escherichia coli for the biosynthesis of alpha-pinene. Biotechnol. Biofuels 6.
Jones, J.A., et al., 2015. EPathOptimize: a combinatorial approach for transcriptional balancing of metabolic pathways. Sci. Rep. 5, 1–10.
Ahmadi, M.K., Fang, L., Moscatello, N., Pfeifer, B.A., 2016. E-coli metabolic engineering for gram scale production of a plant-based anti-inflammatory agent. Metab. Eng. 38, 382–388.
Jantama, K., et al., 2008. Combining metabolic engineering and metabolic evolution to develop nonrecombinant strains of Escherichia coli C that produce succinate and malate. Biotechnol. Bioeng. 99 (5), 1140–1153.
Yang, J.M., Nie, Q.J., Liu, H., Xian, M., Liu, H.Z., 2016. A novel MVA-mediated pathway for isoprene production in engineered E-coli. BMC Biotechnol. 16.
Li, Q., Huang, B., Wu, H., Li, Z.M., Ye, Q., 2017. Efficient anaerobic production of succinate from glycerol in engineered Escherichia coli by using dual carbon sources and limiting oxygen supply in preceding aerobic culture. Bioresour. Technol. 231, 75–84.
Park, S.J., et al., 2013. Metabolic engineering of Escherichia coli for the production of 5-aminovalerate and glutarate as C5 platform chemicals. Metab. Eng. 16, 42–47.
Cheong, S., Clomburg, J.M., Gonzalez, R., 2016. Energy- and carbon-efficient synthesis of functionalized small molecules in bacteria using non-decarboxylative Claisen condensation reactions. Nat. Biotechnol. 34 (5), 556–561.
Song, C.W., Kim, J.W., Cho, I.J., Lee, S.Y., 2016. Metabolic engineering of Escherichia coli for the production of 3-hydroxypropionic acid and malonic acid through beta-alanine route. ACS Synth. Biol. 5 (11), 1256–1263.
Zhang, X., Wang, X., Shanmugam, K.T., Ingram, L.O., 2011. L-Malate Production by metabolically engineered Escherichia coli. Appl. Environ. Microbiol. 77 (2), 427–434.
Song, C.W., Kim, D.I., Choi, S., Jang, J.W., Lee, S.Y., 2013. Metabolic engineering of Escherichia coli for the production of fumaric acid. Biotechnol. Bioeng. 110 (7), 2025–2034.
Sun, X.X., Lin, Y.H., Huang, Q., Yuan, Q.P., Yan, Y.J., 2013. A novel muconic acid biosynthesis approach by shunting tryptophan biosynthesis via anthranilate. Appl. Environ. Microbiol. 79 (13), 4024–4030.
Lin, Y.H., Sun, X.X., Yuan, Q.P., Yan, Y.J., 2014. Extending shikimate pathway for the production of muconic acid and its precursor salicylic acid in Escherichia coli. Metab. Eng. 23, 62–69.
Thompson, B., Pugh, S., Machas, M., Nielsen, D.R., 2017. Muconic acid production via alternative pathways and a synthetic metabolic funnel. ACS Synth. Biol.
Zhang, H.R., Pereira, B., Li, Z.J., Stephanopoulos, G., 2015a. Engineering Escherichia coli coculture systems for the production of biochemical products. Proc. Natl. Acad. Sci. USA 112 (27), 8266–8271.
Zhang, H.R., Li, Z.J., Pereira, B., Stephanopoulos, G., 2015b. Engineering E-coli-E-coli cocultures for production of muconic acid from glycerol. Microb. Cell Fact. 14.
Chang, P., Chen, G.S., Chu, H.Y., Lu, K.W., Shen, C.R., 2017. Engineering efficient production of itaconic acid from diverse substrates in Escherichia coli. J. Biotechnol. 249, 73–81.
Harder, B.J., Bettenbrock, K., Klamt, S., 2018. Temperature-dependent dynamic control of the TCA cycle increases volumetric productivity of itaconic acid production by Escherichia coli. Biotechnol. Bioeng. 115 (1), 156–164.
Noh, M.H., Lim, H.G., Woo, S.H., Song, J., Jung, G.Y., 2017. Production of itaconic acid from acetate by engineering acid-tolerant Escherichia coli W. Biotechnol. Bioeng.
Wang, J.Y., Zhang, K.C., 2015. Production of mesaconate in Escherichia coli by engineered glutamate mutase pathway. Metab. Eng. 30, 190–196.
Wu, X.H., Eiteman, M.A., 2016. Production of citramalate by metabolically engineered Escherichia coli. Biotechnol. Bioeng. 113 (12), 2670–2675.
Jones, J.A., et al., 2016. Experimental and computational optimization of an Escherichia coli co-culture for the efficient production of flavonoids. Metab. Eng. 35, 55–63.
Lim, C.G., Fowler, Z.L., Hueller, T., Schaffer, S., Koffas, M.A.G., 2011. High-yield resveratrol production in engineered Escherichia coli. Appl. Environ. Microbiol. 77 (10), 3451–3460.
Camacho-Zaragoza, J.M., et al., 2016. Engineering of a microbial coculture of Escherichia coli strains for the biosynthesis of resveratrol. Microb. Cell Fact. 15.
Leonard, E., Chemler, J., Kok, H.L., Koffas, M.A.G., 2006. Expression of a soluble flavone synthase allows the biosynthesis of phytoestrogen derivatives in Escherichia coli. Appl. Microbiol. Biotechnol. 70 (1), 85–91.
Leonard, E., Lim, K.H., Saw, P.N., Koffas, M. a.G., 2007. Engineering central metabolic pathways for high-level flavonoid production in Escherichia coli. Appl. Environ. Microbiol. 73 (12), 3877–3886.
He, W.Q., Fu, L., Li, G.Y., Jones, J.A., Linhardt, R.J., Koffas, M., 2015. Production of chondroitin in metabolically engineered E. coli. Metab. Eng. 27, 92–100.
Atsumi, S., Hanai, T., Liao, J.C., 2008b. Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels (86-U13). Nature 451 (7174) (86-U13).
Baez, A., Cho, K.-M., Liao, J.C., 2011. High-flux isobutanol production using engineered Escherichia coli: a bioreactor study with in situ product removal. Appl. Microbiol. Biotechnol. 90 (5), 1681–1690.
Cann, A.F., Liao, J.C., 2008. Production of 2-methyl-1-butanol in engineered Escherichia coli. Appl. Microbiol. Biotechnol. 81 (1), 89–98.
Connor, M.R., Cann, A.F., Liao, J.C., 2010a. 3-Methyl-1-butanol production in Escherichia coli: random mutagenesis and two-phase fermentation. Appl. Microbiol. Biotechnol. 86 (4), 1155–1164.
Chen, G.S., Siao, S.W., Shen, C.R., 2017. Saturated mutagenesis of ketoisovalerate decarboxylase V461 enabled specific synthesis of 1-pentanol via the ketoacid elongation cycle. Sci. Rep. 7.
Ku, J.T., Simanjuntak, W., Lan, E.I., 2017. Renewable synthesis of n-butyraldehyde from glucose by engineered Escherichia coli. Biotechnol. Biofuels 10, 291.
Rodriguez, G.M., Atsumi, S., 2012. Isobutyraldehyde production from Escherichia coli by removing aldehyde reductase activity. Microb. Cell Fact. 11.
Kallio, P., Pasztor, A., Thiel, K., Akhtar, M.K., Jones, P.R., 2014. An engineered pathway for the biosynthesis of renewable propane. Nat. Commun. 5.
Clomburg, J.M., Gonzalez, R., 2011a. Metabolic engineering of Escherichia coli for the production of 1,2-propanediol from glycerol. Biotechnol. Bioeng. 108 (4), 867–879.
Liang, K., Shen, C.R., 2017. Engineering cofactor flexibility enhanced 2,3-butanediol production in Escherichia coli. J. Ind. Microbiol. Biotechnol. 44 (12), 1605–1612.
Yim, H., et al., 2011. Metabolic engineering of Escherichia coli for direct production of 1,4-butanediol. Nat. Chem. Biol. 7 (7), 445–452.
Tai, Y.S., et al., 2016. Engineering nonphosphorylative metabolism to generate lignocellulose-derived products. Nat. Chem. Biol. 12 (4), 247.
Alkim, C., et al., 2015. Optimization of ethylene glycol production from (D)-xylose via a synthetic pathway implemented in Escherichia coli. Microb. Cell Fact. 14.
Yang, C., Gao, X., Jiang, Y., Sun, B.B., Gao, F., Yang, S., 2016. Synergy between methylerythritol phosphate pathway and mevalonate pathway for isoprene production in Escherichia coli. Metab. Eng. 37, 79–91.
McKenna, R., Nielsen, D.R., 2011. Styrene biosynthesis from glucose by engineered E. coli. Metab. Eng. 13 (5), 544–554.
Qi, W.W., et al., 2007. Functional expression of prokaryotic and eukaryotic genes in Escherichia coli for conversion of glucose to p-hydroxystyrene. Metab. Eng. 9 (3), 268–276.
Chae, T.U., Ko, Y.S., Hwang, K.S., Lee, S.Y., 2017. Metabolic engineering of Escherichia coli for the production of four-, five- and six-carbon lactams. Metab. Eng. 41, 82–91.
Martin, V.J.J., Pitera, D.J., Withers, S.T., Newman, J.D., Keasling, J.D., 2003a. Engineering a mevalonate pathway in Escherichia coli for production of terpenoids. Nat. Biotechnol. 21 (7), 796–802.
Tsuruta, H., et al., 2009. High-level production of Amorpha-4,11-Diene, a precursor of the antimalarial agent artemisinin, in Escherichia coli. PLoS One 4 (2).
Ajikumar, P.K., et al., 2010. Isoprenoid pathway optimization for taxol precursor overproduction in Escherichia coli. Sci. (80-.). 330 (6000), 70–74.
Nakagawa, A., et al., 2016. Total biosynthesis of opiates by stepwise fermentation using engineered Escherichia coli. Nat. Commun. 7.
Chouhan, S., Sharma, K., Zha, J., Guleria, S., Koffas, M.A.G., 2017. Recent advances in the recombinant biosynthesis of polyphenols (no. NOV). Front. Microbiol. 8, 1–16.

Pandey, R.P., Parajuli, P., Koffas, M.A.G., Sohng, J.K., 2016. Microbial production of natural and non-natural flavonoids: pathway engineering, directed evolution and systems/synthetic biology. Biotechnol. Adv. 34 (5), 634–662.
Thakker, C., Martinez, I., San, K.Y., Bennett, G.N., 2012. Succinate production in Escherichia coli. Biotechnol. J. 7 (2), 213–224.
Cheng, K.K., Wang, G.Y., Zeng, J., Zhang, J.A., 2013. Improved succinate production by metabolic engineering. Biomed. Res. Int. 2013, 538790.
Zhu, L.W., Tang, Y.J., 2017. Current advances of succinate biosynthesis in metabolically engineered Escherichia coli. Biotechnol. Adv. 35 (8), 1040–1048.
Jantama, K., Zhang, X., Moore, J.C., Shanmugam, K.T., Svoronos, S.A., Ingram, L.O., 2008. Eliminating side products and increasing succinate yields in engineered strains of Escherichia coli C. Biotechnol. Bioeng. 101 (5), 881–893.
Chatterjee, R., Millard, C.S., Champion, K., Clark, D.P., Donnelly, M.I., 2001. Mutation of the ptsG gene results in increased production of succinate in fermentation of glucose by Escherichia coli. Appl. Environ. Microbiol. 67 (1), 148–154.
Millard, C.S., Chao, Y.P., Liao, J.C., Donnelly, M.I., 1996. Enhanced production of succinic acid by overexpression of phosphoenolpyruvate carboxylase in Escherichia coli. Appl. Environ. Microbiol. 62 (5), 1808–1810.
Stols, L., Kulkarni, G., Harris, B.G., Donnelly, M.I., 1997. Expression of Ascaris suum malic enzyme in a mutant Escherichia coli allows production of succinic acid from glucose. Appl. Biochem. Biotechnol. 63–65, 153–158.
Adkins, J., Jordan, J., Nielsen, D.R., 2013. Engineering Escherichia coli for renewable production of the 5-carbon polyamide building-blocks 5-aminovalerate and glutarate. Biotechnol. Bioeng. 110 (6), 1726–1734.
Yu, J.L., Xia, X.X., Zhong, J.J., Qian, Z.G., 2017. A novel synthetic pathway for glutarate production in recombinant Escherichia coli. Process Biochem. 59, 167–171.
Wang, J., Wu, Y.F., Sun, X.X., Yuan, Q.P., Yan, Y.J., 2017. De novo biosynthesis of glutarate via alpha-keto acid carbon chain extension and decarboxylation pathway in Escherichia coli. ACS Synth. Biol. 6 (10), 1922–1930.
Draths, K.M., Frost, J.W., 1994. Environmentally compatible synthesis of adipic acid from D-Glucose. J. Am. Chem. Soc. 116 (1), 399–400.
Niu, W., Draths, K.M., Frost, J.W., 2002. Benzene-free synthesis of adipic acid. Biotechnol. Prog. 18 (2), 201–211.
Sengupta, S., Jonnalagadda, S., Goonewardena, L., Juturu, V., 2015. Metabolic engineering of a novel muconic acid biosynthesis pathway via 4-hydroxybenzoic acid in Escherichia coli. Appl. Environ. Microbiol. 81 (23), 8037–8043.
Okamoto, S., et al., 2014. Production of itaconic acid using metabolically engineered Escherichia coli. J. Gen. Appl. Microbiol. 60 (5), 191–197.
Jiang, Y.D., Liu, W., Zou, H.B., Cheng, T., Tian, N., Xian, M., 2014. Microbial production of short chain diols. Microb. Cell Fact. 13.
Bennett, G.N., San, K.Y., 2001. Microbial formation, biotechnological production and applications of 1,2-propanediol. Appl. Microbiol. Biotechnol. 55 (1), 1–9.
Clomburg, J.M., Gonzalez, R., 2011b. Metabolic engineering of Escherichia coli for the production of 1,2-propanediol from glycerol. Biotechnol. Bioeng. 108 (4), 867–879.
Jain, R., Sun, X., Yuan, Q., Yan, Y., 2015. Systematically engineering Escherichia coli for enhanced production of 1,2-propanediol and 1-propanol. ACS Synth. Biol. 4 (6), 746–756.
Ui, S., Okajima, Y., Mimura, A., Kanai, H., Kudo, T., 1997. Molecular generation of an Escherichia coli strain producing only the meso-isomer of 2,3-butanediol. J. Ferment. Bioeng. 84 (3), 185–189.
Yan, Y.J., Lee, C.C., Liao, J.C., 2009. Enantioselective synthesis of pure (R,R)-2,3-butanediol in Escherichia coli with stereospecific secondary alcohol dehydrogenases. Org. Biomol. Chem. 7 (19), 3914–3917.
Ui, S., et al., 2004. Production of L-2,3-butanediol by a new pathway constructed in Escherichia coli. Lett. Appl. Microbiol. 39 (6), 533–537.
J, Z., et al., 2010. A novel whole-cell biocatalyst with NAD(+) regeneration for production of chiral chemicals. PLoS One 5 (1).
Chu, H.P., et al., 2015. Metabolic engineering of Escherichia coli for production of (2S,3S)-butane-2,3-diol from glucose. Biotechnol. Biofuels 8.
Xu, Y., et al., 2014. Systematic metabolic engineering of Escherichia coli for high-yield production of fuel bio-chemical 2,3-butanediol. Metab. Eng. 23, 22–33.
Ji, X.J., Liu, L.G., Shen, M.Q., Nie, Z.K., Tong, Y.J., Huang, H., 2015. Constructing a synthetic metabolic pathway in Escherichia coli to produce the enantiomerically pure (R, R)-2,3-butanediol. Biotechnol. Bioeng. 112 (5), 1056–1059.
Liang, K.M., Shen, C.R., 2017. Selection of an endogenous 2,3-butanediol pathway in Escherichia coli by fermentative redox balance. Metab. Eng. 39, 181–191.
Wang, J., et al., 2017. Rational engineering of diol dehydratase enables 1,4-butanediol biosynthesis from xylose. Metab. Eng. 40, 148–156.
Liu, H.W., Ramos, K.R.M., Valdehuesa, K.N.G., Nisola, G.M., Lee, W.K., Chung, W.J., 2013. Biosynthesis of ethylene glycol in Escherichia coli. Appl. Microbiol. Biotechnol. 97 (8), 3409–3417.
Pereira, B., Zhang, H.R., De Mey, M., Lim, C.G., Li, Z.J., Stephanopoulos, G., 2016. Engineering a novel biosynthetic pathway in Escherichia coli for production of renewable ethylene glycol. Biotechnol. Bioeng. 113 (2), 376–383.
Turk, S.C., et al., 2016. Metabolic engineering toward sustainable production of nylon-6. ACS Synth. Biol. 5 (1), 65–73.
Zhang, J., Barajas, J.F., Burdu, M., Wang, G., Baidoo, E.E., Keasling, J.D., 2017. Application of an acyl-CoA ligase from streptomyces aizunensis for lactam biosynthesis. Acs Synth. Biol. 6 (5), 884–890.
Eckert, C., et al., 2014. Ethylene-forming enzyme and bioethylene production. Biotechnol. Biofuels 7.
Zhao, Y.R., et al., 2011. Biosynthesis of isoprene in Escherichia coli via methylerythritol phosphate (MEP) pathway. Appl. Microbiol. Biotechnol. 90 (6), 1915–1922.
Yang, J.M., et al., 2012. Enhancing production of bio-isoprene using Hybrid MVA pathway and isoprene synthase in E-coli. PLoS One 7 (4).
Park, S.J., Park, J.P., Lee, S.Y., 2002. Metabolic engineering of Escherichia coli for the production of medium-chain-length polyhydroxyalkanoates rich in specific monomers. FEMS Microbiol. Lett. 214 (2), 217–222.
Jung, Y.K., Kim, T.Y., Park, S.J., Lee, S.Y., 2010. Metabolic engineering of Escherichia coli for the production of polylactic acid and its copolymers. Biotechnol. Bioeng. 105 (1), 161–171.
Yang, T.H., et al., 2010. Biosynthesis of polylactic acid and its copolymers using evolved propionate CoA transferase and PHA synthase. Biotechnol. Bioeng. 105 (1), 150–160.
Taguchi, S., et al., 2008. A microbial factory for lactate-based polyesters using a lactate-polymerizing enzyme. Proc. Natl. Acad. Sci. USA. 105 (45), 17323–17327.
Yang, J.E., Choi, S.Y., Shin, J.H., Park, S.J., Lee, S.Y., 2013. Microbial production of lactate-containing polyesters. Microb. Biotechnol. 6 (6), 621–636.
Choi, S.Y., et al., 2016. One-step fermentative production of poly(lactate-co-glycolate) from carbohydrates in Escherichia coli (+). Nat. Biotechnol. 34 (4), 435 (+).
Liao, J.C., Mi, L., Pontrelli, S., Luo, S.S., 2016. Fuelling the future: microbial engineering for the production of sustainable biofuels. Nat. Rev. Microbiol. 14 (5), 288–304.
Atsumi, S., et al., 2008. Metabolic engineering of Escherichia coli for 1-butanol production. Metab. Eng. 10 (6), 305–311.
Inui, M., et al., 2008. Expression of Clostridium acetobutylicum butanol synthetic genes in Escherichia coli. Appl. Microbiol. Biotechnol. 77 (6), 1305–1316.
Bond-Watts, B.B., Bellerose, R.J., Chang, M.C.Y., 2011. Enzyme mechanism as a kinetic control element for designing synthetic biofuel pathways. Nat. Chem. Biol. 7 (4), 222–227.
Saini, M., Chen, M.H., Chung-Jen, C., Chao, Y.P., 2015. Potential production platform of n-butanol in Escherichia coli. Metab. Eng. 27, 76–82.
Wen, R.C., Shen, C.R., 2016. Self-regulated 1-butanol production in Escherichia coli based on the endogenous fermentative control. Biotechnol. Biofuels 9 (1), 267.
Dong, H.J., et al., 2017. A systematically chromosomally engineered Escherichia coli efficiently produces butanol. Metab. Eng. 44, 284–292.
Saini, M., Lin, L.J., Chiang, C.J., Chao, Y.P., 2017. Synthetic consortium of escherichia coli for n-butanol production by fermentation of the glucose-xylose mixture. J. Agric. Food Chem. 65 (46), 10040–10047.
Saini, M., Li, S.Y., Wang, Z.W., Chiang, C.J., Chao, Y.P., 2016. Systematic engineering of the central metabolism in Escherichia coli for effective production of n-butanol. Biotechnol. Biofuels 9, 69.
Dekishima, Y., Lan, E.I., Shen, C.R., Cho, K.M., Liao, J.C., 2011. Extending carbon chain length of 1-butanol pathway for 1-hexanol synthesis from glucose by engineered Escherichia coli. J. Am. Chem. Soc. 133 (30), 11399–11401.
Lan, E.I., Dekishima, Y., Chuang, D.S., Liao, J.C., 2013. Metabolic engineering of 2-pentanone synthesis in Escherichia coli. AICHE J. 59 (9), 3167–3175.
Srirangan, K., Liu, X.J., Akawi, L., Bruder, M., Moo-Young, M., Chou, C.P., 2016. Engineering Escherichia coli for microbial production of butanone. Appl. Environ. Microbiol. 82 (9), 2574–2584.
Tseng, H.C., Martin, C.H., Nielsen, D.R., Prather, K.L.J., 2009. Metabolic engineering of Escherichia coli for enhanced production of (R)- and (S)-3-hydroxybutyrate. Appl. Environ. Microbiol. 75 (10), 3137–3145.
Baek, J.M., et al., 2013b. Butyrate production in engineered Escherichia coli with synthetic scaffolds,"scaffolds. Biotechnol. Bioeng. 110 (10), 2790–2794.
Dellomonaco, C., Clomburg, J.M., Miller, E.N., Gonzalez, R., 2011. Engineered reversal of the β-oxidation cycle for the synthesis of fuels and chemicals. Nature 476 (7360), 355–359.
Clomburg, J.M., Vick, J.E., Blankschien, M.D., Rodriguez-Moya, M., Gonzalez, R., 2012. A synthetic biology approach to engineer a functional reversal of the beta-oxidation Cycle. ACS Synth. Biol. 1 (11), 541–554.
Kim, S., Cheong, S., Gonzalez, R., 2016. Engineering Escherichia coli for the synthesis of short- and medium-chain alpha,beta-unsaturated carboxylic acids. Metab. Eng. 36, 90–98.
Shen, C.R., Liao, J.C., 2008. Metabolic engineering of Escherichia coli for 1-butanol and 1-propanol production via the keto-acid pathways. Metab. Eng. 10 (6), 312–320.
Connor, M.R., Cann, A.F., Liao, J.C., 2010b. 3-Methyl-1-butanol production in Escherichia coli: random mutagenesis and two-phase fermentation. Appl. Microbiol. Biotechnol. 86 (4), 1155–1164.
Zhang, K., Sawaya, M.R., Eisenberg, D.S., Liao, J.C., 2008. Expanding metabolism for biosynthesis of nonnatural alcohols. Proc. Natl. Acad. Sci. USA. 105 (52), 20653–20658.
Marcheschi, R.J., et al., 2012. A synthetic recursive '+1' pathway for carbon chain elongation. ACS Chem. Biol. 7 (4), 689–697.
Rodriguez, G.M., Atsumi, S., 2014. Toward aldehyde and alkane production by removing aldehyde reductase activity in Escherichia coli. Metab. Eng. 25, 227–237.
Zhang, K.C., Woodruff, A.P., Xiong, M.Y., Zhou, J., Dhande, Y.K., 2011. A synthetic metabolic pathway for production of the platform chemical isobutyric acid. ChemSusChem 4 (8), 1068–1070.
Xiong, M., Yu, P., Wang, J., Zhang, K., 2015. Improving engineered Escherichia coli strains for high-level biosynthesis of isobutyrate. AIMS Bioeng. 2 (2), 60–74.
d'Espaux, L., Mendez-Perez, D., Li, R., Keasling, J.D., 2015. Synthetic biology for microbial production of lipid-based biofuels. Curr. Opin. Chem. Biol. 29, 58–65.
Steen, E.J., et al., 2010. Microbial production of fatty-acid-derived fuels and chemicals from plant biomass. Nature 463 (7280), 559–562.
Nawabi, P., Bauer, S., Kyrpides, N., Lykidis, A., 2011. Engineering Escherichia coli for biodiesel production utilizing a bacterial fatty acid methyltransferase. Appl. Environ. Microbiol. 77 (22), 8052–8061.
Sherkhanov, S., Korman, T.P., Clarke, S.G., Bowie, J.U., 2016. Production of FAME biodiesel in E. coli by direct methylation with an insect enzyme. Sci. Rep. 6.
Liu, A.Q., Tan, X.M., Yao, L., Lu, X.F., 2013. Fatty alcohol production in engineered E-coli expressing Marinobacter fatty acyl-CoA reductases. Appl. Microbiol. Biotechnol. 97 (15), 7061–7071.
Liu, Y.L., et al., 2016. High production of fatty alcohols in Escherichia coli with fatty acid

starvation. Microb. Cell Fact. 15.
Schirmer, A., Rude, mathew A., Li, X., Popova, E., Cardayre, S.B. Del, 2010. Microbial Biosynthesis of Alkanes (no. July). Sci. (80-.) 329, 559–562.
Dischert, W., Figge, R., 2016a. Recombinant microorganism for the fermentative production of methionine, U.S. Patent No. 9,506,093. 29 Nov.
Dischert, W., Figge, R., 2016b. Microorganism for methionine production with enhanced glucose import, U.S. Patent No. 9,506,092. 29 Nov.
Figge, R., 2016. Increasing methionine production by overexpressing succinate dehydrogenase, U.S. Patent No. 9,267,160. 23 Feb.
Figge, R., Vasseur, P., 2017. Use of inducible promoters in the production of methionine, U.S. Patent No. 9,732,364. 15 Aug.
Jossek, R., Bongaerts, J., Sprenger, G.A., 2001. Characterization of a new feedback-resistant 3-deoxy-d-arabino-heptulosonate 7-phosphate synthase AroF of Escherichia coli. FEMS Microbiol. Lett. 202 (1), 145–148.
Patnaik, R., Liao, J.C., 1994b. Engineering of Escherichia coli central metabolism for aromatic metabolite production with near theoretical yield. Appl. Environ. Microbiol. 60 (11), 3903–3908.
M. M. Gusyatiner, M. G. Lunts, Y. I. Kozlov, L. V Ivanovskaya, and E. B. Voroshilova, "DNA coding for mutant isopropylmalate synthase L-leucine-producing microorganism and method for producing L-leucine," 2002.
Kikuchi, Y., Suzuki, T., Kojima, H., 1998. Lysine decarboxylase gene and method of producing L-lysine, U.S. Patent No. 5,827,698. 27 Oct.
Cheong, K.Y., Lee, S.M., Hwang, Y.B., LEE, K.C., Lee, K.H., 2017. L-threonine and L-tryptophan producing bacteria strain and method of making same, U.S. Patent Application No. 15/465,881.
Patnaik, R., Spitzer, R.G., Liao, J.C., 1995. Pathway engineering for production of aromatics in Escherichia coli: confirmation of stoichiometric analysis by independent modulation of AroG, TktA, and Pps activities. Biotechnol. Bioeng. 46 (4), 361–370.
Martin, V.J.J., Pitera, D.J., Withers, S.T., Newman, J.D., Keasling, J.D., 2003b. Engineering a mevalonate pathway in Escherichia coli for production of terpenoids. Nat. Biotechnol. 21 (7), 796–802.
Huang, Q.L., Roessner, C.A., Croteau, R., Scott, A.I., 2001. Engineering Escherichia coli for the synthesis of taxadiene, a key intermediate in the biosynthesis of taxol. Bioorg. Med. Chem. 9 (9), 2237–2242.
Badri, A., Williams, A., Linhardt, R.J., Koffas, M.A., 2018. The road to animal-free glycosaminoglycan production: current efforts and bottlenecks. Curr. Opin. Biotechnol. 53, 85–92.
Suflita, M., Fu, L., He, W., Koffas, M., Linhardt, R.J., 2015. Heparin and related polysaccharides: synthesis using recombinant enzymes and metabolic engineering. Appl. Microbiol. Biotechnol. 99 (18), 7465–7479.
Fang, Z., Jones, J.A., Zhou, J., Koffas, M.A.G., 2017. Engineering Escherichia coli cocultures for production of curcuminoids from glucose. Biotecnol. J. 1700576, 1–8.
Zhou, K., Qiao, K.J., Edgar, S., Stephanopoulos, G., 2015. Distributing a metabolic pathway among a microbial consortium enhances production of natural products (377-U157). Nat. Biotechnol. 33 (4) (377-U157).
Grohmann, K., Bothast, R.J., 1997. Saccharification of corn fibre by combined treatment with dilute sulphuric acid and enzymes. Process Biochem. 32 (5), 405–415.
Aristidou, A., Penttilä, M., 2000. Metabolic engineering applications to renewable resource utilization. Curr. Opin. Biotechnol. 11 (2), 187–198.
Neale, A.D., Scopes, R.K., Kelly, J.M., 1988. Alcohol production from glucose and xylose using Escherichia-Coli containing zymomonas-mobilis genes. Appl. Microbiol. Biotechnol. 29 (2–3), 162–167.
Lee, J.W., Jeffries, T.W., 2011. Efficiencies of acid catalysts in the hydrolysis of lignocellulosic biomass over a range of combined severity factors. Bioresour. Technol. 102 (10), 5884–5890.
Dien, B.S., Cotta, M.A., Jeffries, T.W., 2003. Bacteria engineered for fuel ethanol production: current status. Appl. Microbiol. Biotechnol. 63 (3), 258–266.
Eiteman, M.A., Lee, S.A., Altman, R., Altman, E., 2009. A substrate-selective co-fermentation strategy with Escherichia coli produces lactate by simultaneously consuming xylose and glucose. Biotechnol. Bioeng. 102 (3), 822–827.
Cam, Y., et al., 2016. Engineering of a synthetic metabolic pathway for the assimilation of (d)-xylose into value-added chemicals. ACS Synth. Biol. 5 (7), 607–618.
Huo, G., et al., 2017. Metabolic engineering of an E. coli ndh knockout strain for PHB production from mixed glucose–xylose feedstock. J. Chem. Technol. Biotechnol. 92 (10), 2739–2745.
Lu, H., et al., 2016. Enhancement of D-lactic acid production from a mixed glucose and xylose substrate by the Escherichia coli strain JH15 devoid of the glucose effect. BMC Biotechnol. 16 (1), 1–10.
Zhao, A., Hu, X., Wang, X., 2017. Metabolic engineering of Escherichia coli to produce gamma-aminobutyric acid using xylose. Appl. Microbiol. Biotechnol. 101 (9), 3587–3603.
Blankschien, M.D., Clomburg, J., Gonzalez, R., 2010. Metabolic engineering of Escherichia coli for the production of succinate from glycerol. Metab. Eng. 12 (5), 409–419.
Mazumdar, S., Clomburg, J.M., Gonzalez, R., 2010. Escherichia coli strains engineered for homofermentative production of D-lactic acid from glycerol. Appl. Environ. Microbiol. 76 (13), 4327–4336.
Wang, C., et al., 2011. Metabolic engineering of Escherichia coli for α-farnesene production. Metab. Eng. 13 (6), 648–655.
Tran, K.T., Maeda, T., Wood, T.K., 2014. Metabolic engineering of Escherichia coli to enhance hydrogen production from glycerol. Appl. Microbiol. Biotechnol. 98 (10), 4757–4770.
Jun Choi, Y., Hwan Park, J., Yong Kim, T., Yup Lee, S., 2012. Metabolic engineering of Escherichia coli for the production of 1-propanol. Metab. Eng. 14 (5), 477–486.
Roesijadi, G., Jones, S.B., Snowden-Swan, L.J., Zhu, Y., 2010. Macroalgae as a Biomass Feedstock : A Preliminary Analysis. Department of Energy, United States.
Wargacki, A.J., et al., 2012. An engineered microbial platform for direct biofuel production from brown macroalgae (no. January). Sci. (80-.) 230502, 308–314.
Huo, Y.-X., et al., 2011. Conversion of proteins into biofuels by engineering nitrogen flux. Nat. Biotechnol. 29 (4), 346–351.
Choi, K.Y., Wernick, D.G., Tat, C. a., Liao, J.C., 2014. Consolidated conversion of protein waste into biofuels and ammonia using Bacillus subtilis. Metab. Eng. 23, 53–61.
Brautaset, T., Jakobsen, Ø.M., 2007. Bacillus methanolicus : a candidate for industrial production of amino acids from methanol at 50 °C. Appl. Microbiol. Biotechnol. 22–34.
Müller, J.E.N., et al., 2015. Engineering Escherichia coli for methanol conversion. Metab. Eng. 28, 190–201.
Lidstrom, M.E., Stirling, D.I., Corporation, C., 1990. "METHYLOTROPHS: genetics and COlll1nercial applications. Annu. Rev. Microbiol. 44, 27–58.
Smejkalova, H., Erb, T.J., Fuchs, G., 2010. Methanol Assimilation in Methylobacterium extorquens AM1: demonstration of All Enzymes and Their Regulation. PLoS One 5 (10).
Vorholt, J.A., 2002. Cofactor-dependent pathways of formaldehyde oxidation in methylotrophic bacteria. Arch. Microbiol. 239–249.
Anthony, C., 1982. Biochemistry of Methylotrophs. Academic Press.
Hou, C.T., Laskin, A.I., Patel, R.N., 1978. Growth and polysaccharide production by methylocystis parvus OBBP on methanol. Appl. Environ. Microbiol. 37 (5), 800–804.
Hagishita, T., Toyokazu, Y., Yoshikazu, I., Toshio, M., 1996. Efficient L-serine production from methanol and glycine by resting cells of Methylobacterium sp. strainMN43. Biosci. Biotechnol. Biochem. 60 (10), 1604–1607.
Motoyama, H., Yano, H., Terasaki, Y., Hakko, K., Co, K., 2001. Overproduction of L-lysine from methanol by methylobacillus glycogenes derivatives carrying a plasmid with a mutated dapA gene. Appl. Environ. Microbiol. 67 (7), 3064–3070.
Whitaker, W.B., et al., 2017. Engineering the biological conversion of methanol to specialty chemicals in Escherichia coli (no. June 2016) 39, 49–59.
Bennett, R.K., Steinberg, L.M., Chen, W., Papoutsakis, E.T., 2018a. Engineering the bioconversion of methane and methanol to fuels and chemicals in native and synthetic methylotrophs. Curr. Opin. Biotechnol. 50, 81–93.
Price, J.V., Chen, L., Whitaker, W.B., Papoutsakis, E., Chen, W., 2016. Scaffoldless engineered enzyme assembly for enhanced methanol utilization. Proc. Natl. Acad. Sci. USA 113 (45), 12691–12696.
Bennett, R.K., Gonzalez, J.E., Whitaker, W.B., Antoniewicz, M.R., Papoutsakis, E.T., 2018b. Expression of heterologous non-oxidative pentose phosphate pathway from Bacillus methanolicus and phosphoglucose isomerase deletion improves methanol assimilation and metabolite production by a synthetic Escherichia coli methylotroph (no. July 2017). Metab. Eng. 45, 75–85.
Gonzalez, J.E., Long, C.P., Antoniewicz, M.R., 2017. Comprehensive analysis of glucose and xylose metabolism in Escherichia coli under aerobic and anaerobic conditions by13C metabolic flux analysis (no. November 2016). Metab. Eng. 39, 9–18.
Bogorad, I.W., et al., 2014. Building carbon–carbon bonds using a biocatalytic methanol condensation cycle. Proc. Natl. Acad. Sci. USA 111 (45), 15928–15933.
Siegel, J.B., et al., 2015. Computational protein design enables a novel one-carbon assimilation pathway (201500545). Proc. Natl. Acad. Sci. USA (201500545).
Poust, S., Piety, J., Bar-even, A., Louw, C., Baker, D., 2015. Mechanistic analysis of an engineered enzyme that catalyzes the formose reaction. Chembiochemistry 1950–1954.
Bar-even, A., 2016. Format. Assim.: Metab. Archit. Nat. Synth. Pathw.
Foster, T.J., 2005. Immune evasion by staphylococci. Nat. Rev. Microbiol. 3 (12), 948–958.
Pedruzzi, I., Rosenbusch, J.P., Locher, K.P., 1998. Inactivation in vitro of the Escherichia coli outer membrane protein FhuA by a phage T5-encoded lipoprotein. FEMS Microbiol. Lett. 168 (1), 119–125.
Stirm, S., 1968. Escherichia coli K Bacteriophages. J. Virol. 2 (10), 1107–1114.
Perry, L.L., et al., 2009. Sequence analysis of Escherichia coli O157:H7 bacteriophage ??V10 and identification of a phage-encoded immunity protein that modifies the O157 antigen. FEMS Microbiol. Lett. 292 (2), 182–186.
Destoumieux-Garzón, D., et al., 2005. The iron–siderophore transporter FhuA is the receptor for the antimicrobial peptide microcin J25: role of the microcin Val ^{11} –Pro ^{16} β-hairpin region in the recognition mechanism. Biochem. J. 389 (3), 869–876.
Lu, M.-J., Stierhof, Y.-D., Henning, U., 1993. Location and unusual membrane topology of the immunity protein of the Escherichia coli phage T4. J. Virol. 67 (8), 4905–4913.
Pingoud, A., Fuxreiter, M., Pingoud, V., Wende, W., 2005. Type II restriction endonucleases: structure and mechanism. Cell. Mol. Life Sci. 62 (6), 685–707.
Mcgrath, S., Seegers, J.F.M.L., Fitzgerald, G.F., Grath, S.M.C., Seegers, J.O.S.F.M.L., 1999. Molecular characterization of a phage-encoded resistance system in lactococcus lactis molecular characterization of a phage-encoded resistance system in Lactococcus lactis. Appl. Environ. Microbiol. 65 (5), 1891–1899.
Raleigh, E.A., Wilson, G., 1986. Escherichia coli K-12 restricts DNA containing 5-methylcytosine. Proc. Natl. Acad. Sci. Usa. 83 (23), 9070–9074.
Garneau, J.E., et al., 2010. The CRISPR/cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature 468 (7320), 67–71.
Krüger, D.H., Bickle, T. a., 1983. Bacteriophage survival: multiple mechanisms for avoiding the deoxyribonucleic acid restriction systems of their hosts. Microbiol. Rev. 47 (3), 345–360.
Snyder, L., 1995. Phage-exclusion enzymes: a bonanza of biochemical and cell biology reagents? Mol. Microbiol. 15 (3), 415–420.
Labrie, S.J., Samson, J.E., Moineau, S., 2010. Bacteriophage resistance mechanisms. Nat. Rev. Microbiol. 8 (5), 317–327.
Barrangou, R., et al., 2007. CRISPR provides acquired resistance against viruses in prokaryotes (no. March). Science 315, 1709–1712.
Deveau, H., et al., 2008. Phage response to CRISPR-encoded resistance in Streptococcus thermophilus. J. Bacteriol. 190 (4), 1390–1400.

Horvath, P., Barrangou, R., 2010. CRISPR/Cas, the immune system of bacteria and archaea,"archaea. Science 327 (5962), 167–170.

Semenova, E., et al., 2011. Interference by clustered regularly interspaced short palindromic repeat (CRISPR) RNA is governed by a seed sequence. Proc. Natl. Acad. Sci. USA 108 (25), 10098–10103.

Deveau, H., Garneau, J.E., Moineau, S., 2010. CRISPR/Cas system and its role in phage-bacteria interactions. Annu. Rev. Microbiol. 64 (1), 475–493.

Westra, E.R., et al., 2013. Type I-E CRISPR-Cas systems discriminate target from non-target DNA through base pairing-independent PAM recognition. PLoS Genet. 9 (9).

Datsenko, K.A., Pougach, K., Tikhonov, A., Wanner, B.L., Severinov, K., Semenova, E., 2012. Molecular memory of prior infections activates the CRISPR/Cas adaptive bacterial immunity system (no. May). Nat. Commun. 3, 945–947.

Swarts, D.C., Mosterd, C., van Passel, M.W.J., Brouns, S.J.J., 2012. CRISPR interference directs strand specific spacer acquisition. PLoS One 7 (4), 1–7.

Borges, A.L., Davidson, A.R., Bondy-Denomy, J., 2017. The discovery, mechanisms, and evolutionary impact of Anti-CRISPRs (p. annurev-virology-101416-041616). Annu. Rev. Virol. 4 (1) (p. annurev-virology-101416-041616).

Strotskaya, A., et al., 2017. The action of Escherichia coli CRISPR-Cas system on lytic bacteriophages with different lifestyles and development strategies. Nucleic Acids Res. 45 (4), 1946–1957.