Growth Inhibition of *Desulfovibrio vulgaris* Hildenborough on Pyruvate Fermentation

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DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT

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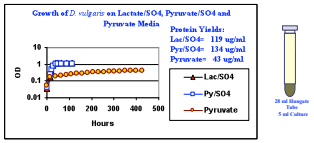
ABSTRACT

Desulfovibrio vulgaris, **a sulfate reducing bacterium, is known to grow well anaerobically on lactate or pyruvate using sulfate or sulfite as the electron acceptor (5 to 7 hours generation time at 32°C). *D. vulgaris* can also ferment pyruvate without an electron acceptor. Although all added pyruvate (60 mM) is consumed, growth of *D. vulgaris* by pyruvate fermentation is slow (a generation time over 100 hours) and the final protein yield is also low, about 1/3 that of pyruvate or lactate respiration. When pyruvate is oxidized to acetate, three additional products are made, electrons, protons, and CO_2. Hydrogen production, through the many hydrogenases of *D. vulgaris*, can serve as an electron sink, as can the production of formate, lactate, ethanol, or succinate. To understand the metabolic pathway of pyruvate fermentation, possible inhibition of pyruvate fermentative growth by metabolic end products was investigated and fermentation end products were analyzed by HPLC. We found that *D. vulgaris* pyruvate fermentation was very sensitive to added H_2 gas in a sealed growth tube. It also was sensitive to added CO_2 gas. Fermentative growth was slowed by the addition of $NaHCO_3$, Na_2CO_3, formate, lactate, or ethanol. With an inert gaseous headspace, growth by pyruvate fermentation was proportional to headspace volumes of the growth tube. Fermentation end products analyzed by HPLC revealed that when the fermentation was inhibited by added H_2 or CO_2, pyruvate oxidation was slowed proportionally.**

Twenty six deletion mutants of *D. vulgaris* were chosen for fermentation growth studies. Some mutants (12 of them) had no effect on pyruvate fermentation. Others (13 of them) either showed no growth [JW381 (*ΔnhaD-1*) and JW9021 (*ΔqmoABC*)] or slower growth on pyruvate fermentation than the wild type. One deletion mutant, JW385 (*ΔcycA*), showed better growth and larger final protein yields (2 fold or more) than the wild type when fermenting pyruvate.

RESULTS: Figure 1. Respiration and Fermentation



Growth Medium At 0 Time	Final Growth Hours	Final OD (600 nm)	Generation Time (Hours)	Protein (µg /ml)	% Protein	Lac* (mM)	Pyr (mM)	Acet (mM)	Form (mM)	OAA (mM)	Fum (mM)
Lactate/SO_4 (60/30 mM)	39	0.818	7	106	100%	13	2	61	0	1	0.09
Lactate/SO_4 (60/30 mM)	112	1.218	7	119	112%	0	0.5	62	0	0.6	0.1
Pyruvate/SO_4 (60/30 mM)	112	1.024	8	134	126%	0	0.3	45	0	0.4	0.06
Pyruvate (60 mM)	68	0.184	178	ND	---	1.7	23	17	3.2	3.1	0.04
Pyruvate (60 mM)	309	0.262	178	ND	---	3.2	15	27	0.9	2.5	0.03
Pyruvate (60 mM)	381	0.254	178	29	27%	4.3	8	34	0.7	0.3	0.03

*HPLC performed on Bio-Rad Aminex HPX-87H Column. The compounds were eluted using 30 mM H_2SO_4 at 53 C for 50 minutes. To identify compounds, their elution was compared to the standards which were detected by both Waters 484 UV absorbance Detector (210 nm) and Waters 410 Differential Refractometer. The column system was pumped using Shimadzu LC-10A pump with a 0.3 ml/min flow rate and 20 column pressure. Lac, Lactate; Pyr, Pyruvate; Acet, Acetate; Form, Formate; OAA, Oxaloacetate; and Fum, Fumarate.

Figure 2. Headspace and Pyruvate Fermentation

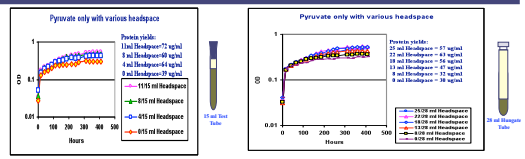
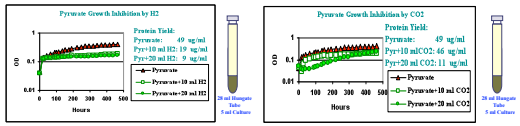


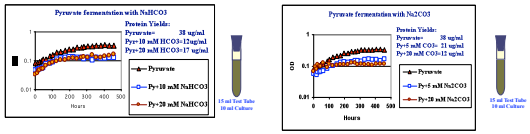
Figure 3. Inhibition of Fermentation by H_2 and CO_2



Growth Medium At 0 Time	Growth Hours	OD (600 nm)	Protein (ug/ml)	% Protein	Pyruvate (mM)	Acetate (mM)	Formate (mM)	Fumarate (mM)
Pyruvate (60 mM)	599	0.436	49	100%	0.4	34	2	0.04
Py (60 mM) +20 ml H_2	599	0.174	9	18%	>30	8	2	0.03
Py (60 mM), +20 ml CO_2	599	0.281	11	22%	>30	20	4	0.58

Analysis as for Figure 1.

Figure 4. Inhibition by $NaHCO_3$ or Na_2CO_3



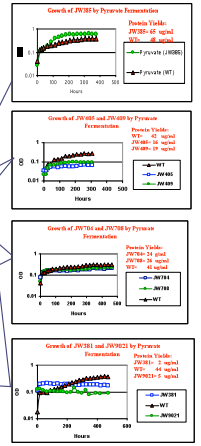
CONCLUSIONS

1. **Pyruvate fermentation of *D. vulgaris* was inhibited by H_2 or CO_2 in the headspace. Fermentation was decreased in yield by the addition of lactate, ethanol, formate, or succinate. H_2 appears to be the major electron sink for pyruvate fermentation.**
2. **Other biochemical steps that generated compounds acting as alternative electron sinks were pyruvate to malate, fumarate to succinate, and pyruvate to lactate or ethanol .**
3. **Remarkably, JW385 (*ΔcycA*; DVU3171; type 1 tetraheme cytochrome c_3 deletion mutant) grew faster by pyruvate fermentation than the wild type. Confirming this robust growth was the increase in protein yield by the mutant during fermentation (almost 2 fold).**
4. **JW9021 (*ΔqmoABC HP* mutant; electron carrier for adenosine 5' -phosphosulfate (APS) reduction) and JW381 (*ΔnhaD-1* mutant; Na+/H+ antiporter) did not ferment pyruvate at all in a sealed tube with a small headspace.**
5. **Other mutants decreased in pyruvate fermentation may be generally impaired in metabolism. They were JW380 (*ΔechA/mnhA*), JW405 (*ΔnorM-1*), JW409 (*ΔnorM-2*), JW704 (*ΔpspA*), JW707 (*Δfur*), JW708 (*ΔperR*), JW709 (*ΔzurR*), JW710 (*Δupp*), JW9005 (*Δpnp*), JW9019 (*Δldh*), and JW9023 (*Δnox*). These mutants are involved in energy metabolism, regulation, or metabolite transport.**

Figure 5. Effects of Gene Deletions on Pyruvate Fermentation

Strains	Deleted Gene(s)	DVU#	Gene description and relevant phenotypes	Relative protein yield
WT	None	NA	*Desufovibrio vulgaris* Hildenborough (ATCC29579, NCIMB8303)	100%
JW380	*echA/mnhA*	0434	NADH:ubiquinone oxidoreductase.	64%
JW381	*nhaD-1*	0027	Na+/H+ antiporter.	NG*
JW385	*cycA*	3171	Type 1 tetraheme cytochrome c_3. Respiration impaired.	162%
JW405	*norM-1*	1217	MATE efflux. Na+ driven mutidrug efflux pump.	41%
JW409	*norM-2*	2555	MATE efflux. Na+ driven mutidrug efflux pump.	51%
JW704	*pspA*	2988	Phage shock protein A. First gene of a 3 gene operon was deleted.	60%
JW707	*fur*	0942	Ferric up take regulator. A 2 gene operon with *pep* gene.	60%
JW708	*perR*	3095	Peroxide resposeive regulator.	63%
JW709	*zurR*	1340	Zinc up take regulator. A 4 gene operon, *zurABCR.*	66%
JW710	*upp*	1025	Uracil phosphoribosyl transferase.	71%
JW9005	*pnp*	0503	Polyribonucleotide nucleotidyltransferase.	61%
JW9019	*ldh*	0600	Putative L-lactate dehydrogenase.	68%
JW9021	*qmoABC Hp*	0848-0851	Quinone interacting oxidoreductase. No $SO_4^=$ respiration.	NG
JW9023	*nox*	3212	Pyridine nucleotide disulfide oxidoreductase.	71%

*NG, no growth



Deletion Strains Not Impaired in Pyruvate Fermentation

Mutant	Gene(s) deleted	DVU #	Gene annotation
JW2003	*hdrABC*	2399-2404	Hydrogenase and heterodisulfide reductase.
JW382	*nhaC-1*	0381	Na+/H+ antiporter.
JW383	*nhaC-2*	3108	Na+/H+ antiporter.
JW401	*cydA*	3271	Cytochrome d ubiquinol oxidase.
JW403	*rnfC*	2792	NADH:quinone oxidoreducatase.
JW407	*Cation efflux*	0164	Cation efflux. Co/Zn/Cd transporters.
JW411	*HP*	A0095	Hypothetical Protein.
JW413	*HP*	0303-0304	Hypothetical Protein.
JW415	*dhcA*	2791	Decaheme cytochrome *c*
JW9011	*hcpR*	2547	Transcriptional regulator; sulfate reduction
JW9025	*tatA/E*	1367	Twin arginine translocation protein. No formate respiration.
JW9027	*glgP*	2349	Carbohydrate phosphorylase.

ACKNOWLEDGEMENT

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