Investigation of genome annotated metabolic pathways of *Desulfovibrio vulgaris* Hildenborough via high resolution mass spectrometry (Abstract 06-GM-A-4256-ASM)

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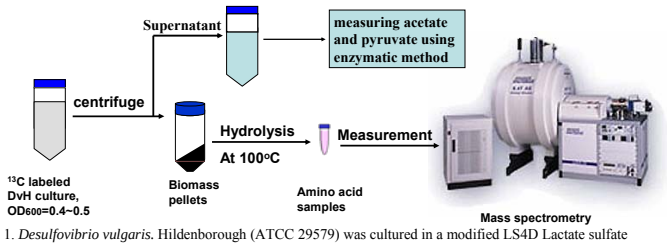
Introduction

Sulfate reducing bacteria such as *Desulfovibrio vulgaris* Hildenborough are ubiquitous in nature and play an important role in global sulfur cycling and the mineralization of organic matter. The ability of *D. vulgaris* to reduce heavy metals and radionucleotides to more insoluble forms provide a unique microbe-oriented solution for bioremediation. *D. vulgaris* also retains research interest due to its energy metabolism characteristics, which shows the potential to be used for hydrogen or methane production in either pure culture or mix cultures. The availability of an annotated genomic sequence for *D.vulgaris* makes it an ideal organism for investigation of SRB physiology. The recently published annotated genomic sequence of *D. vulgaris* contains some unresolved predictions, 1: although the TCA cycle lacks a typical α-ketoglutarate dehydrogenase, a ferredoxin-dependent α-ketoglutarate synthase homolog (EC 1.2.7.3) has been annotated to enable the (α-ketoglutarate → succinyl-CoA) reaction; 2: although several other δ -proteobacteria contain a citrate synthase, neither *D. vulgaris* nor the closely related G20, contain any gene homologous to a citrate synthase. However, these organisms are not autotrophic for amino acids typically derived from citrate and prior experiments have suggested the presence of an atypical synthase that enables the production of citrate; 3: while the annotation predicts pathways for respiration using sulfate and other terminal electron acceptors, whether the TCA cycle functions oxidatively or only reductively remains to be determined.

Metabolic flux analysis is an ideal *in vivo* tool to examine the metabolic pathways in organisms. Analysis of isotopomer distribution in the pathway metabolites (often amino acids) requires gas chromatography-mass spectrometry (GC/MS). However while GC/MS can be used to assign which fragment of a molecule is labeled, the labeled atom cannot be pin-pointed. To precisely locate the position of the isotopically labeled atom, a highly sensitive Fourier Transform Ion Cyclotron Resonance (FTICR) based mass spectroscopic technique was developed and is first demonstration of the use of this powerful technique in an isotopomer study.

Experimental method



1. *Desulfovibrio vulgaris*. Hildenborough (ATCC 29579) was cultured in a modified LS4D Lactate sulfate medium.
2. The seeds for all experiment were started from fresh frozen stock (non-labeled culture). Isotopic labeling experiments were run in triplicate using 99% [a-13C]-L-Lactate (Cambridge Isotope, USA) containing LS4D medium with a 1:10 inoculation volume. The culture in the exponential phase was sub-cultured using same labeled medium and inoculation volume. To remove the effect of non-labeled lactate from the initial culture, three subcultures were done to obtain the final sample.
3. Cell growth was monitored by measuring both optical density using absorbance at 600 nm (OD_{600}) and total protein concentration using the Bradford Protein Assay.
4. The concentration of lactate and acetate in the medium were measured using enzyme kits. Biomass constituents (carbohydrates, RNA, and DNA) were also measured.
5. Amino acids were hydrolyzed from biomass protein and its labeled pattern can be determined by GC/MS and FT-ICR.

Results 1: Biomass composition

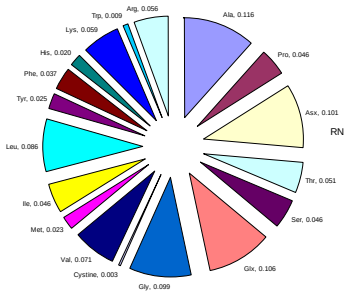


Figure 1: Mole fraction of protein-genetic amino acids in *D. vulgaris* Hildenborough

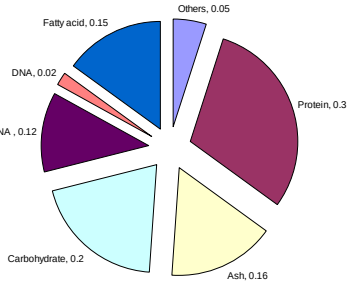


Figure 2: Biomass composition of *D. vulgaris* Hildenborough

Results 2: Growth kinetics

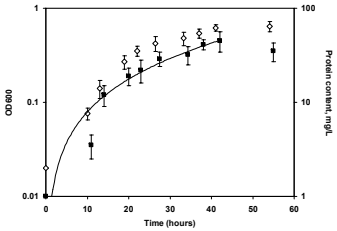


Figure 3. Growth curve in defined L-lactate medium. ◊: OD_{600}; ■: protein concentration; solid line: Model fitting.

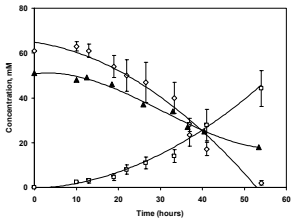


Figure 4: Lactate and sulfate consumption, acetate production curves in defined L-lactate medium. ◊: lactate; □: acetate; ▲: sulfate; solid line: model fitting

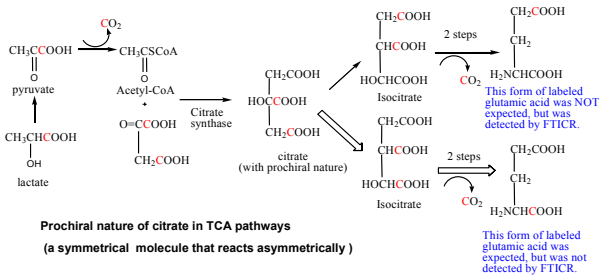
Table 1: Parameters of growth kinetics for *Desulfovibrio vulgaris* Hildenborough in lactate medium.

Growth condition	Yx/c g/mole	$Y_{P/C}$ g protein /mole	$Y_{SO4/Lac}$ mol/mol	$t_{1/2}$ hr*	μ_{max} hr^{-1}	K_s mM	End product, mol/mol lactate
Defined lactate medium	3.1 ± 0.9	0.89 ± 0.27	0.45 ± 0.09	8.2 ±2.0	0.14±0.01	28 ±2	acetate: 0.90± 0.05 alcohol: <0.002 pyruvate:~1mM succinate:~1mM
Lactate medium with yeast extract	5.3~6.8[1,2] 2.66~2.84[3]	0.74~0.79[3]	0.4~0.57[2,3]	2.7 ~4.3[1]	0.22~0.27[1,2]	29[2]	acetate: 0.97[1] ethanol: ~0.02[1] H_2: up to 0.5

*Based on OD_{600}
1. Traore et al., 1981; 2. Noguera et al., 1998; 3. Nagpal et al., 2002

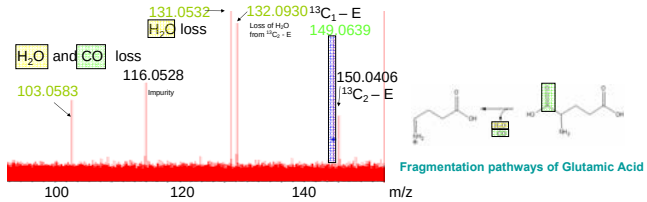
Results 3: FT-ICR MS Analysis of the TCA Cycle and atypical citrate synthase

After having a general picture of the central metabolism, specific reactions can be targeted to look at closely.



Prochiral nature of citrate in TCA pathways

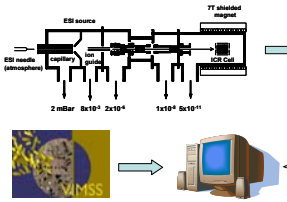
(a symmetrical molecule that reacts asymmetrically)



FTICR spectrum of glutamic acid shows that α-carboxyl group is not labeled with ^{13}C

Figure 5. The annotated genome sequence of *D. vulgaris* indicates that this strain may not have the citrate synthase gene. This study used ^{13}C lactate (labeled in the first position) as a single carbon source to investigate a potential citrate synthesis. The ^{13}C distribution data shows that the γ-carboxyl group of glutamate instead of α-carboxyl group is labeled with ^{13}C (as commonly expected in the TCA cycle), suggesting that there is a unique citrate synthase that may produce citrate with a different prochiral nature from that of other common bacteria.

Results 4: Metabolic Flux Analysis



Construction of pathway map based on genome annotation

Use isotopomer model to quantify the intracellular flux distribution

Table 2. Isotopomer distribution in amino acids

	Fragment	M0	M1	M2	M3
Serine	No loss	0.18	0.80	0.01	
	Loss of COOH	0.98	0.02	0	
Alanine	No loss	0.17	0.83		
	Loss of COOH	0.97	0.03		
Glutamate	No loss	0.21	0.76	0.03	
	Loss of COOH	0.23	0.77	0	
Asparate	No loss	0.05	0.27	0.68	
	Loss of COOH	0.20	0.80	0	
Histidine	No loss	0.20	0.58	0.08	0.13
	Loss of COOH	0.17	0.62	0.09	0.11
Phenylalanine	No loss	0.03	0.12	0.35	0.49
	Loss of COOH	0.08	0.32	0.56	0.03

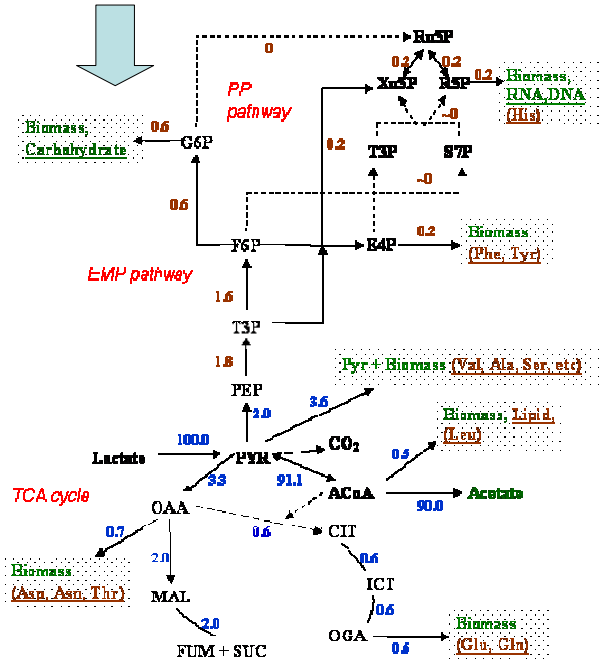


Figure 6. Flux distribution of carbon in *D. vulgaris* cultured in the minimal LS4D medium. Fluxes were estimated from measurements taken at middle log phase (30~40 hrs) and normalized by the averaged lactate uptake rate in log phase (= 1.5 mmol hr^{-1} L^{-1}). The dotted lines indicate reactions that are not supported by any annotated enzyme in the pathway.

Conclusions

The aim of the tracer experiments and isotopomer study were to examine the key metabolic pathways in *D. vulgaris* as well as the quantification of carbon flux through these pathways. A combination of GC and FTICR mass spectroscopic techniques were used to obtain complete isotopomer information in the metabolites for isotopomer analysis. Data from this study confirm several aspects of *D. vulgaris* metabolism, such as an incomplete pentose phosphate pathway and TCA cycle. However, the results of the TCA cycle analysis indicate that the predicted step for the ferredoxin-dependent α-ketoglutarate does not exist. Consistent with studies conducted in 1968 by Gottschalk, the FTICR isotopomer distribution data indicated the presence of an atypical R-citrate synthase. This study is the first documented use of the powerful FTICR mass spectroscopy for metabolite analysis using isotopically labeled biomass.

Acknowledgement

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