Flux analysis of anaerobic carbon metabolic pathways in *Shewanella oneidensis* MR-1 using isotopic metabolite labeling

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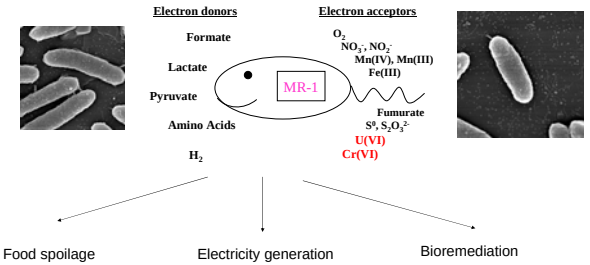
Abstract

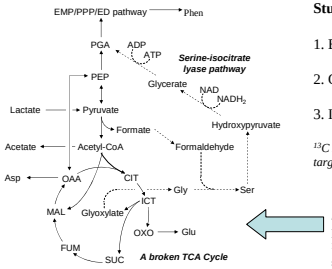
It has been proposed that during growth under anaerobic or oxygen-limited conditions *Shewanella oneidensis* MR-1 uses the serine-isocitrate lyase pathway common to many methylotrophic anaerobes, in which formaldehyde produced from pyruvate is condensed with glycine to form serine. The serine is then transformed through hydroxypyruvate and glycerate to enter central metabolism at phosphoglycerate. To examine its use of the serine-isocitrate lyase pathway under anaerobic conditions, we grew *S. oneidensis* MR-1 on [1-^{13}C] lactate as the sole carbon source with either trimethylamine N-oxide (TMAO) or fumarate as an electron acceptor. Analysis of cellular metabolites indicates that a large percentage (>70%) of lactate was partially oxidized to either acetate or pyruvate. The ^{13}C isotope distributions in amino acids and other key metabolites indicate that, under anaerobic conditions, although glyoxylate synthesized from isocitrate lyase reaction can be converted to glycine, a complete serine-isocitrate pathway is not present and serine/glycine is in fact oxidized via a highly reversible degradation pathway. The labeling data also suggest significant activity in the anaplerotic (malic enzyme and phospho*enol*pyruvate carboxylase) reactions. Although the tricarboxylic acid (TCA) cycle is often observed to be incomplete in many other anaerobes (absence of 2-oxoglutarate dehydrogenase activity), isotopic labeling supports the existence of a complete TCA cycle in *S. oneidensis* MR-1 under certain anaerobic condition, i.e., TMAO reducing condition. A flux distribution data according to our proposed pathway is estimated for both TMAO and fumarate reduction conditions.

Introduction

1. *Shewanella oneidensis* MR-1 has the versatile metabolism



2. *Shewanella oneidensis* MR-1 anaerobic carbon metabolism pathway proposed by Nealson and Scott (1994). They discovered a serine pathway in the carbon metabolism.



Study the metabolic pathway

1. Based on enzymatic activity in key reactions;

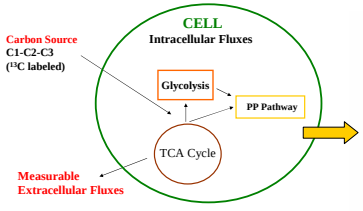
2. Genome annotation of pathway;

3. Isotopic signature in metabolites;

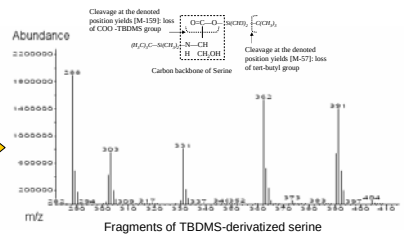
^{13}C Metabolic Flux Analysis can help to identify possible targets for genetic modifications.

***Shewanella oneidensis* MR-1 anaerobic pathway proposed before: the solid arrows denote the central metabolic pathways, and the dotted arrows denote the serine pathway.**

Method and Materials

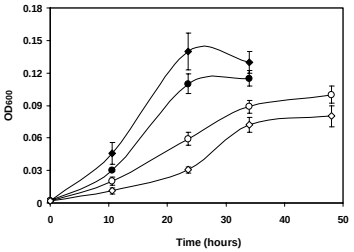


13C Isotopomer Analysis using GC-MS

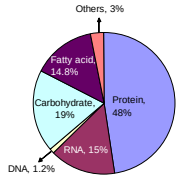


Fragments of TBDMS-derivatized serine

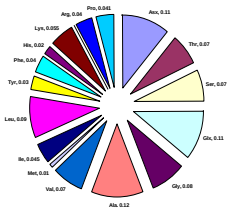
Results and Discussion



Anaerobic growth curves of MR-1 in the minimal 13C-L lactate medium



Cellular Biomass Determination



Molar fraction of total amino acids

Biomass Composition Analysis

Table: Measured concentrations of metabolites under TMAO or fumarate reduction conditions

	Lactate in the medium	Lactate (mM)	Glycine (mg/L)	Acetate (mM)	Pyruvate (mM)	Succinate (mM)	Cell (OD$_{600}$)	Dried biomass, g/L^{3}
30 mM TMAO[2]	20 mM	1.5±0.5	-	6.6±1.0	7.8±2.2	0.4±0.2	0.08±0.01	~0.06
	22 mM with 100 mg/L glycine	3.9±0.5	53±12	11.6±0.3	3.6±1.1	<0.1	0.11±0.01	~0.08
23 mM fumarate	21 mM	~0	-	13.2±0.5	2.6±1.3	22.0±1.3	0.10±0.01	~0.07
	20 mM with 100 mg/L glycine	~0	37±9	12.5±0.7	1.9 ±0.8	23.8±1.0	0.14±0.02	~0.10

Anaerobic Pathways of lactate metabolism in *S. oneidensis* MR-1 interpreted by isotopic labeling

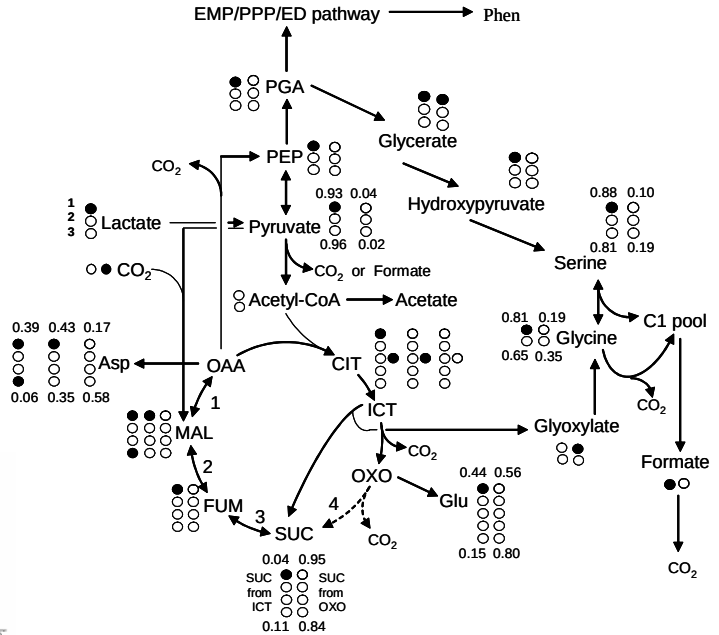
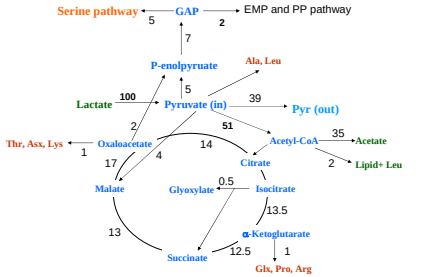


Table. Effect of non-labeled glycine or glyoxlate on mass fragment distribution [M-57: no loss] of key metabolites

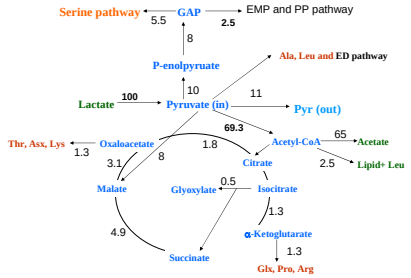
	Fragment	TMAO (Glycine)	Fumarate (Glycine)	Fumarate (Glyoxlate)
Glycine	M0	0.85	0.67	0.43
	M1	0.15	0.33	0.57
Serine	M0	0.37	0.38	0.17
	M1	0.63	0.61	0.80
	M2	0	0.01	0.02
Glutamate	M0	0.77	0.56	0.54
	M1	0.18	0.43	0.45
	M2	0.03	0.01	0.01
Aspartate	M0	0.55	0.17	0.20
	M1	0.33	0.40	0.41
	M2	0.10	0.43	0.38
Phenylalanine	M0	0.17	0.16	0.15
	M1	0.04	0.02	0.02
	M2	0.21	0.17	0.18
	M3	0.57	0.63	0.62

Preliminary Flux Analysis

Flux distribution (TMAO)



Flux distribution (Fumarate)



Conclusions

1. Isotopic labeling evidence refutes the existence of the serine pathway proposed in the figure. Carbon metabolism can be done via serine degradation pathway.

2. TCA cycle is complete cycle under TMAO reduction condition.

3. Majority flux is to acetate and pyruvate (>70%)

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