K 054

ESPP2 Environmental Stress Pathway Project

Metabolic Pathways in the pleomorphic metal-reducing organism *Desulfovibrio africanus* strain PCS

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DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT

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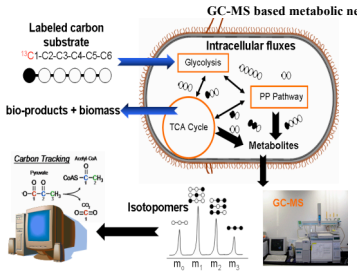
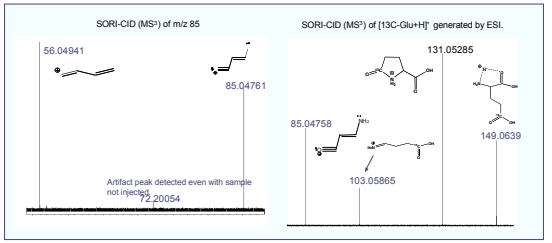
Abstract

Desulfovibrio africanus is a strict anaerobic sulfate-reducing organism that can also reduce toxic metals like Chromium (VI) to non-toxic Chromium (IV). While a lot of researchers have focused on studying the biochemistry, isolation and structure of metalloproteins in this organism, not much is known about the physiology of this organism. Previously, *Desulfovibrio africanus* strain PCS (99% similar to the type strain) was isolated from sediments in San Diego as a lactate oxidizing sulfate reducer. Using lactate as the sole electron donor, this organism could reduce almost 200mM Chromium(VI) even when subjected to stress by 50mM nitrate. Strain PCS appears as thin spiral shaped bacterium in mid log phase but morphs into spherical form later in the growth phase. It was observed that the spiral morphotype actively utilizes lactate while the spherical morphotype does not. Using ^{13}C-labeled lactate as a single carbon source, we investigated the metabolic pathways of lactate utilization in mid-log cells of strain PCS with sulfate as the terminal electron acceptor. The isotopomer analysis of proteinogenic amino acids was performed using both gas chromatography-mass spectrometry and Fourier transform-ion cyclotron resonance mass spectrometry. Based on the labeling pattern of 8 key metabolites alanine, histidine, serine, isoleucine, leucine, aspartate, succinate and glutamate, we observed several unique metabolic pathways in strain PCS. In this organism, a branched tricarboxylic acid cycle exists due to no activity of ketoglutarate dehydrogenase. Also, the lack of an oxidative functional pentose phosphate pathway was observed. The results predict presence of a Re-type citrate synthase, similar to the recently characterized citrate synthase of Clostridia, while isoleucine synthesis seems to be completely via citramalate pathway rather than via L-threonine dehydratase. The isotope labeling pattern of amino acids allowed a preliminary prediction of the *in vivo* metabolic pathways through central pathways especially useful for microbes whose genetic fingerprint have yet to be completely deciphered.

Isotopomer analysis of ^{13}C labeled amino acids

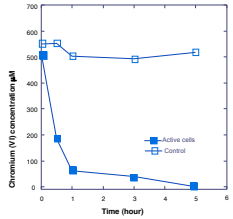


➢PCS is able to grow in anaerobic minimal medium, and therefore must contain complete amino acid and other necessary building block synthesis pathways. In order to further investigate central metabolic pathways, cells were grown in batch cultures and harvested in the exponential growth phase. The resulting isotopomer distributions in key amino acids directly derived from the precursors in central metabolism were used to profile the PCS strain's pathway.



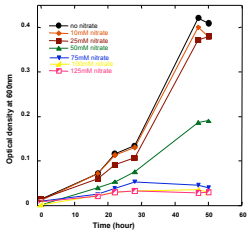
➢Citrate is a symmetrical molecule, but aconitase is known to be stereospecific for the pro-chiral structure of citrate, providing the stereochemical bias of the reaction. In a normal citrate synthase, the labeled carbon of β-carboxyl group of oxaloacetate (precursor of aspartic acid) is incorporated into the 1st carbon of 2-oxoglutarate (precursor of 2-oxoglutarate). However, via an *in vivo* non-radioactive ^{13}C tracer experiment, the results from the FT-ICR MS analysis of aspartic acid and glutamate suggest a unique carbon transition route, i.e., β-carboxyl group of oxaloacetate is incorporated into the 5th carbon of 2-oxoglutarate instead. Thus, the citrate synthase in PCS has an atypical stereochemical propensity.

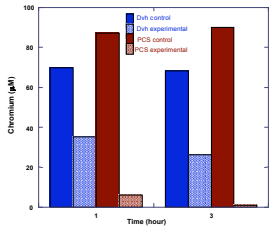
Cr (VI) reduction and morphology

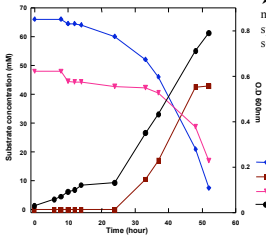


➢An active washed cell suspension of strain PCS enzymatically reduced 500 mM Chromium (VI) supplied as potassium chromate within 5 hours with lactate as the electron donor. No reduction occurred in parallel incubations without electron donor. Further, in the absence of cells, no abiotic reduction of Cr(VI) took place (data not shown).

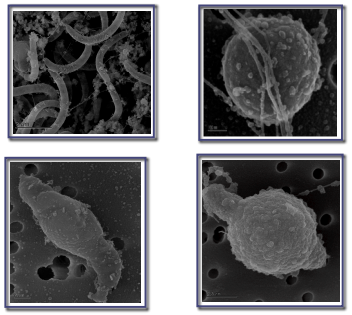
The nitrate MIC was determined to be 50mM. It was observed that even after being exposed to nitrate stress for 4 hrs, cell suspension of strain PCS reduced Cr(VI) quite efficiently. Strain PCS was able to reduce Cr(VI) faster than the model SRB, *D vulgaris* strain Hildenburough







➢Cells of strain PCS exhibits several morphotypes during its growth cycle. During midlog phase of growth, curved/spiral cells dominate, while during stationery phase, spherical cells are the dominant kind. Intermediary shaped cells as shown below are seen in different stages throughout the growth period.



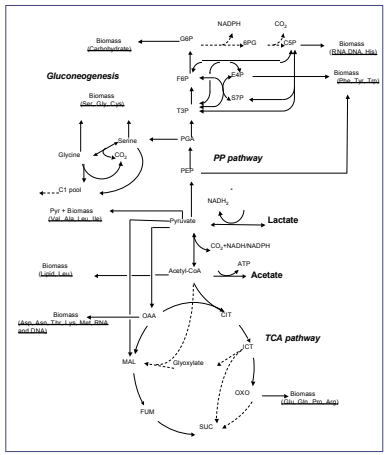
By further investigation and cell sorting by differential cetrifugation, it was possible to separate the 2 dominant morphotypes.Once inoculated into fresh media, cell density along with substrate utilization measurements revealed that the spiral morphotype actively utilizes lactate while the spherical morphotype does not. Lactate utilization corresponds to change in cell morphotype as revealed by microscopy (data not shown)

Measured fragment mass distributions for ^{13}C-labeled metabolites from strain PCS hydrolysates.

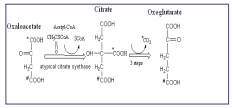
Amino acids (Precursors)	Fragment	M0	M1	M2	M3	C13 enriched position
Glycine (PEP)	No loss	0	0.99	0.01		carboxyl group
Serine (PEP)	No loss	0	0.99	0.01		carboxyl group
	Loss of COOH	0.99	0.01			
Alanine (Pyruvate)	No loss	0	0.99	0.01		carboxyl group
	Loss of COOH	0.97	0.01	0.02		
Leucine (=Isoleucine)* (Pyruvate/acetyl CoA)	Loss of COOH	0.95	0.03	0.01		carboxyl group
Glutamate (OXO)	No loss	0.08	0.89	0.03		C5 carboxyl group
	Loss of COOH	0.07	0.91	0.02		
Asparate (OAA)	No loss	0.01	0.06	0.93		C1 or C4 carboxyl group
	Loss of COOH	0.05	0.95			
Methionine (OAA)	No loss	0.02	0.07	0.86		carboxyl group
	Loss of COOH	0.03	0.87	0.04		
Histidine (C5P)	No loss	0.03	0.71	0.01	0.25	The carboxyl group is NOT enriched.
	Loss of COOH	0.05	0.65	0.02	0.25	
Phenylalanine (PEP+E4P)	No loss	0	0.01	0.10	0.82	The carboxyl group
	Loss of COOH	0.01	0.07	0.79	0.04	

➢Isotopomer analysis shows alanine and serine has same labeling pattern (99% labeling on 1st carbon), indicating that PGA is mostly from pyruvate. Over 93% of the aspartic acid is labeled on both carboxyl groups, while glutamate is only single labeled, suggesting the absence of flux through 2-oxoglutarate→succinate→ oxaloacetate or isocitrate→glyoxlate→ oxaloacetate; that is, carbon flow through a complete TCA cycle (including glyoxlate shunt) does not exist. In a branched TCA cycle, reactions (pyruvate+CO_2 → malate→ oxaloacetate, pyruvate+CO_2 → oxaloacetate, phospho*enol*pyruvate → oxaloacetate) may be present, as both carboxyl groups of oxaloacetate are labeled (i.e., α-carboxyl group is from pyruvate and β -carboxyl group is from $^{13}CO_2$).

➢T3P is from PGA and mostly labeled on its first position, so E4P is double labeled. This explains that most phenylalanine is triple-labeled, one ^{13}C in phenylalanine is from PEP and two ^{13}C is from E4P. The pathway T3P+F6P→E4P+C5P brings only one ^{13}C (1st carbon of T3P) into C5P (precursor of histidine). The pathway E4P+F6P→T3P+S7P→ C5P+C5P results in one single labeled C5P and one triple labeled C5P; while pathway T3P→F6P→G6P→6PG→C5P generates double labeled histidine. Therefore, since histidine's M2 ion is absent it is obvious the pentose phosphate pathway is also incomplete (missing G6P→C5P), similar to that in *Desulfovibrio vulgaris*.

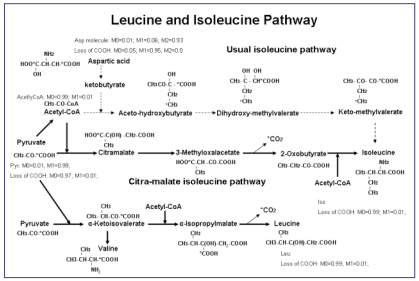


➢Pyruvate→ acetyl-CoA is a key reaction in PCS metabolism, and the first carbon of pyruvate (labeled) is lost in this step and thus leucine is mostly labeled on its carboxyl group (>95%). The labeling of leucine and isoleucine is similar. Thereby, the isoleucine and leucine share same precursors (pyruvate and acetylCoA)



The presence of an atypical citrate synthase has been also shown in several anaerobic bacteria, including *Desulfovibrio spp.* and *Clostridium kluyveri*, and was named (R)-citrate synthase.

Atypical citrate synthase similar to DvH



➢Pyruvate→ acetyl-CoA is a key reaction in metabolism. The first carbon of pyruvate (labeled) is lost in this step and thus leucine is mostly labeled on its carboxyl group (>95%).

The labeling of leucine and isoleucine is similar. Thereby, the isoleucine and leucine share same precursors (pyruvate and acetylCoA)

Conclusions

- *Desulfovibrio africanus* strain PCS isolated from Paleta Creek sediments (San Diego) is very efficient in reducing toxic metals like Cr(VI) to Cr(IV) under anaerobic conditions.
- Mass spectrometry techniques were used for predicting central metabolism in *Desulfovibrio* strain PCS when grown on lactate and sulfate as electron donor and acceptor respectively using ^{13}C-labeled carbon source.
- A branched tricarboxylic acid cycle exists due to no activity of ketoglutarate dehydrogenase.
- The lack of an oxidative functional pentose phosphate pathway was observed.
- The results predict presence of a Re-type citrate synthase.
- Isoleucine synthesis is via citramalate pathway rather than via L-threonine dehydratase. This result is similar to that observed in the model organism, *D vulgaris* strain *Hildenburough*

Acknowledgement

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