Lawrence Berkeley National Laboratory
Recent Work

Title
Identification of Amino Acid Synthesis Pathways in Desulfovibrio vulgaris by Isotopic Labeling, Metabolite Analysis, and Genome Sequence Analysis

Permalink
https://escholarship.org/uc/item/4st9j6k9

Authors
Price, Morgan N.
Tang, Yinjie J.
Benke, Peter I.
et al.

Publication Date
2008-06-02
Identification of amino acid synthesis pathways in *Desulfovibrio vulgaris* by isotopic labeling, metabolite analysis, and genome sequence analysis

Morgan N. Price¹, Yinjie J. Tang¹, Peter I. Benke¹, Edward E. Baidoo¹, Swapnil R. Chhabra¹, On-Yi Fok¹, Samuel Myers¹, Chris J. Petzold¹, Paramvir S. Dehal¹, Aindrila Mukhopadhyay¹, Grant M. Zane², Judy. D. Wall², Jay D. Keasling¹, Adam P. Arkin¹

¹Physical Biosciences Division, Lawrence Berkeley National Lab
²Department of Biochemistry, University of Missouri—Columbia

Virtual Institute for Microbial Stress & Survival (VIMSS)
Acknowledgements

This work was part of the Virtual Institute for Microbial Stress and Survival (http://VIMSS.lbl.gov) supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics:GTL program through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy.

This document was prepared as an account of work sponsored by the United States Government. While this document is believed to contain correct information, neither the United States Government nor any agency thereof, nor The Regents of the University of California, nor any of their employees, makes any warranty, express or implied, or assumes any legal responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by its trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof, or The Regents of the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof or The Regents of the University of California.
Contact:

MorganNPrice@yahoo.com
Conclusions

We propose the following pathways:

- Incomplete TCA cycle with an atypical Re-citrate synthase
  - role of DVU0398 verified in vitro and by knockout
- Synthesis of isoleucine via citramalate synthase
- Synthesis of methionine via O-succinyl-homoserine, cystathionine, and homocysteine, but the genes involved could not be identified
- Synthesis of lysine via L,L-diaminopimelate aminotransferase
Background

- *D. vulgaris Hildenborough* (DvH) is a model sulfate-reducing bacterium (δ-Proteobacteria)
  - oxidizes lactate to acetate
- Gaps in annotated pathways
  - for synthesis of Ile, Met, Lys
  - but it grows in minimal media
- TCA cycle unclear
  - often incomplete in anaerobes
  - DvH known to have Re-citrate synthase (not annotated)
Isotopic Labeling Analysis

- Grow DvH on $1^{-13}$C lactate
- Analyze amino acids (GC/MS, FT-ICR/MS)
- Infer sources of amino acids & metabolic fluxes
  - TCA cycle ends at $\alpha$-ketoglutarate & succinate
  - labeling of glutamate confirms Re-citrate synthase
  - isoleucine does not originate from threonine + acetyl-CoA

DVU0398 is Re-Citrate Synthase

- Identified by proximity to aconitase in Clostridia
  - enzyme was recently characterized in C. kluyveri
- In vitro, cleaves acetyl-CoA in presence of oxaloacetate
  - requires Mn$^{2+}$ for activity
  - inactivated by O$_2$
- DVU0398 knockout requires $\alpha$-ketoglutarate, glutamate, or glutamine to grow
  - confirms TCA cycle is broken at $\alpha$-ketoglutarate dehydr.

DVU1914 is a member of the IPM synthase family

- includes leuA, homocitrate, citramalate, Re-citrate synthase
- close homologs of DVU1914 are often near leuA, ilvCDBH
  - trees suggest DVU2981 is correctly annotated as leuA
- >50% identical to recently identified citramalate synthase in *G. sulfurreducens* (C. Risso et al., J Bact 190:2266-74)
Methionine Synthesis Mystery

- No annotated genes to convert homoserine to homocysteine
- Metabolomics supports the trans-sulfhydrylation pathway
- Candidate genes identified, but knockouts still grow w/o external methionine ("metW", DVU3369; "patB"; DVU0171)

Aspartate → Homoserine

- metA
- metX
- thrB

O-succinyl-homoserine → O-acetyl-homoserine → O-phospho-homoserine (to cystathionine in plants)

Cystathionine

- metB
- metW?

- metC or malY
- or patB(?)

Homocysteine

- metY or metZ or met17 (direct sulfhydrylation)

Homocysteine → Methionine

- metE or metH
Diaminopimelate Synthesis via L,L-DAP aminotransferase

Standard bacterial pathways are absent

L,L-DAP aminotransferase recently identified in bacteria

DVU1655 has close homologs in proximity to dapF in other organisms
54% identical to dapL1 from Moorella thermoacetica
A. Hudson et al., J. Bact 2008, 190:3256-63