Poster #K-059

Identification of amino acid synthesis pathways in *Desulfovibrio vulgaris* by isotopic labeling, metabolite analysis, and genome sequence analysis

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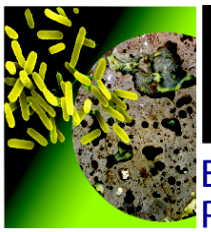
Virtual Institute for Microbial Stress & Survival (VIMSS)

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.



ESPP2

Environmental Stress Pathway Project

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Conclusions

We propose the following pathways:

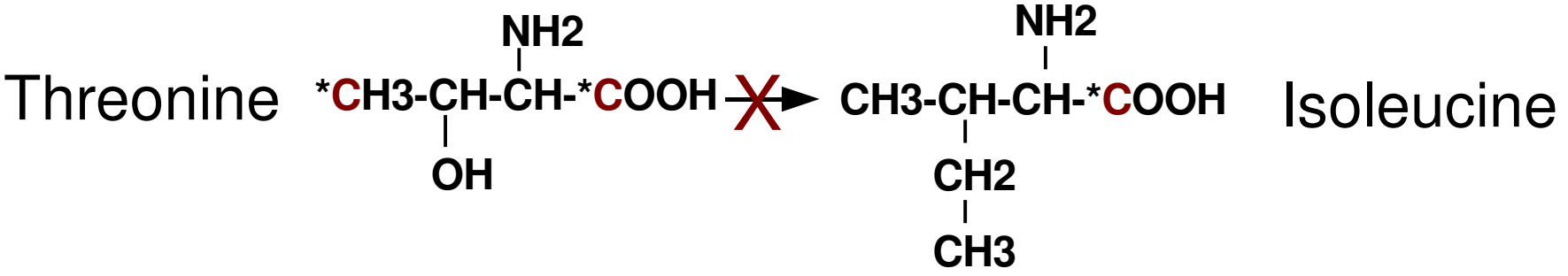
- Incomplete TCA cycle with an atypical Re-citrate synthase
  - role of DVU0398 verified in vitro and by knockout
- Synthesis of isoleucine via citramalate synthase
- Synthesis of methionine via O-succinyl-homoserine, cystathionine, and homocysteine, but the genes involved could not be identified
- Synthesis 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 α-ketoglutarate & succinate
    - labeling of glutamate confirms Re-citrate synthase
  - isoleucine does not originate from threonine + acteyl-CoA



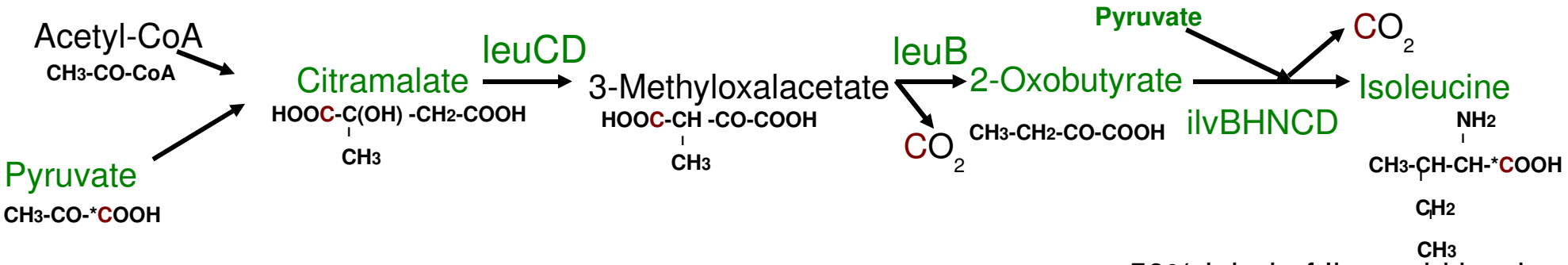
Y. Tang et al., J Bacteriol. 2007 189:940-9

DVU0398 is Re-Citrate Synthase

- Identified by proximity to aconitase in *Clostridia*
  - enzyme was recently characterized in *C. kluyveri* [1]
- In vitro, cleaves acteyl-CoA in presence of oxaloacetate
  - requires Mn^{2+} for activity
  - inactivated by O_2
- DVU0398 knockout requires α-ketoglutarate, glutamate, or glutamine to grow
  - confirms TCA cycle is broken at α-ketoglutarate dehydr.

1. F. Li et al, J Bacteriol 2007:4299-304

Predicted Citramalate Synthase DVU1914



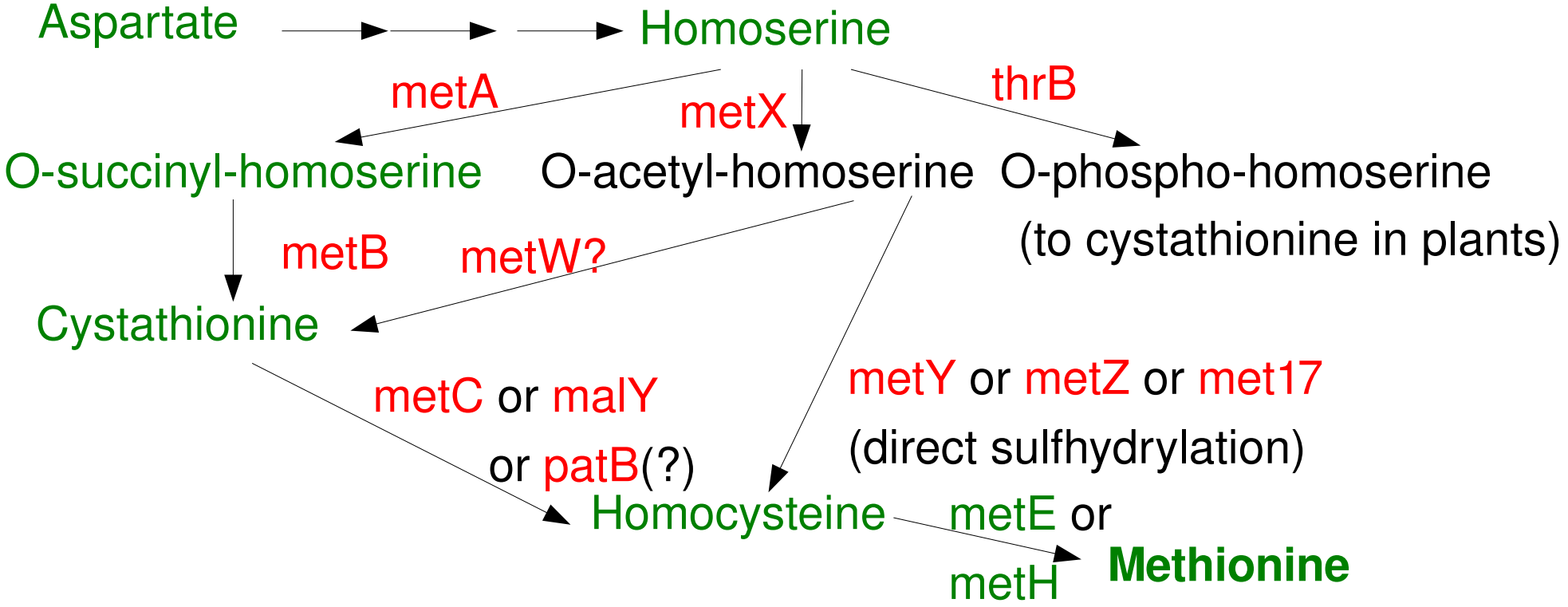
50% label of Ile could be due to reversible decarboxylation

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)

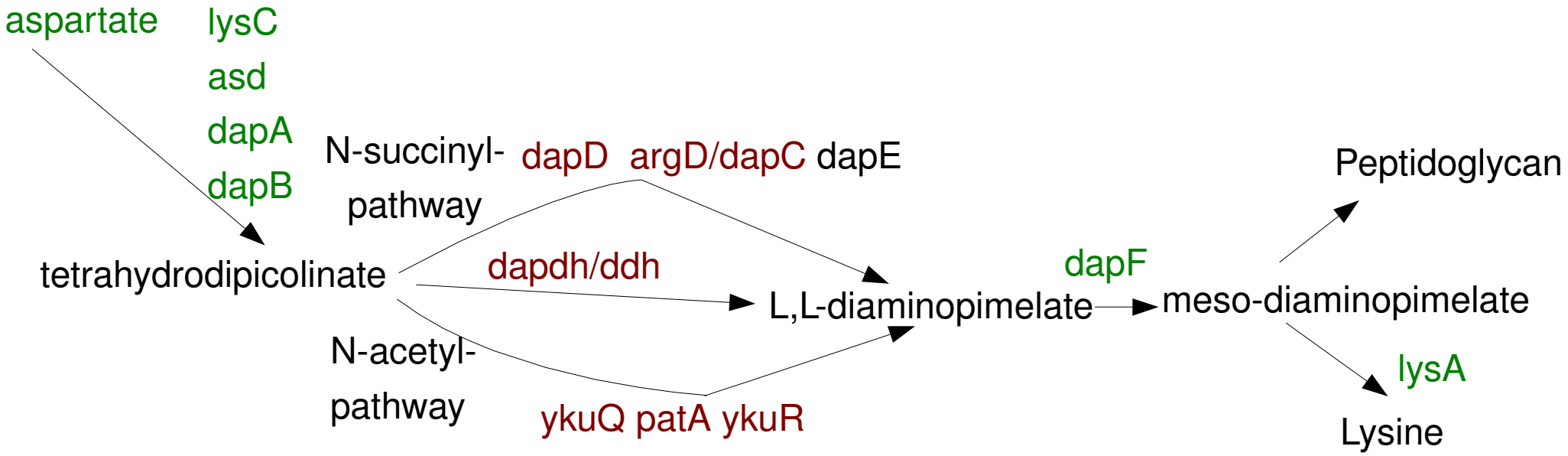
Methionine Synthesis Mystery

- 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)



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