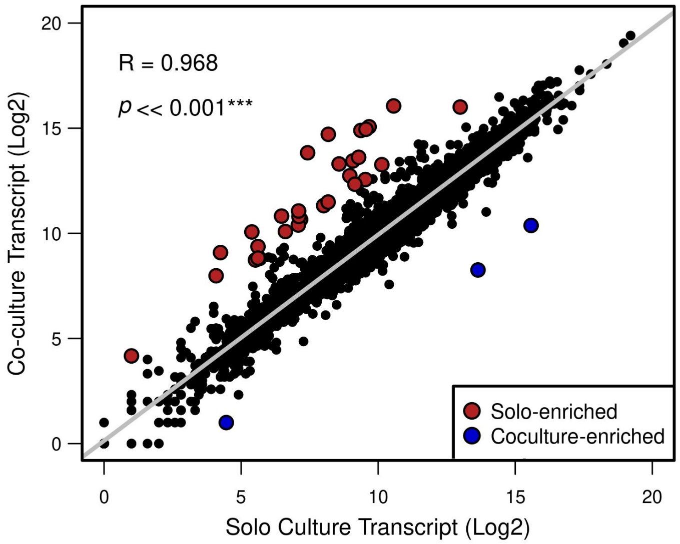
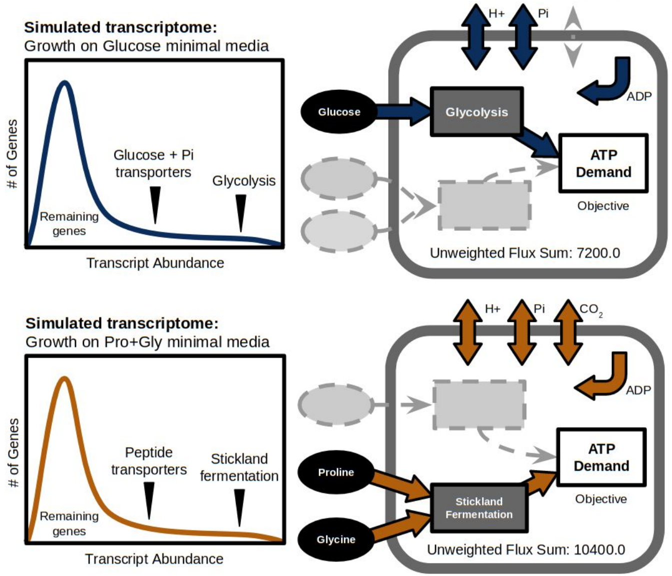
Co-culture GENRE Analysis

8-6-20

Contextualizing By Relying on Cell Economy





- Genome-scale metabolic network reconstructions
  - Collection of hypotheses to understand possible metabolism
- My approach to data integration focuses on evolution
  - Transcriptomes only account for <50% of protein
  - However, this action is still an investment for cells
  - Maximizes economy with respect to mRNA abundances

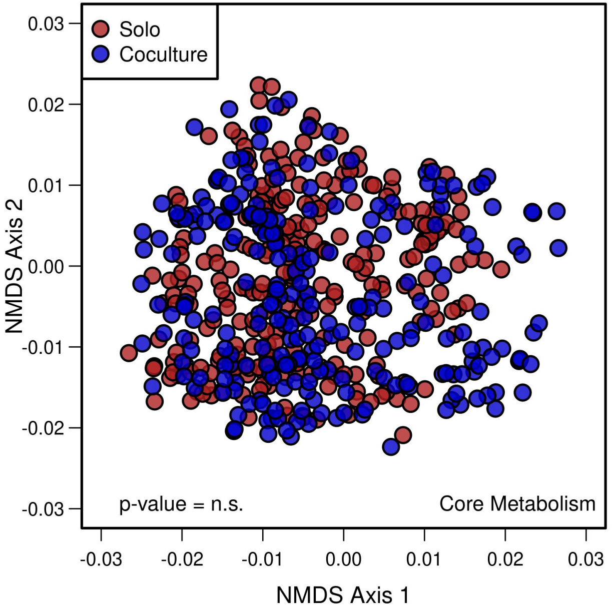
PLOS COMPUTATIONAL BIOLOGY

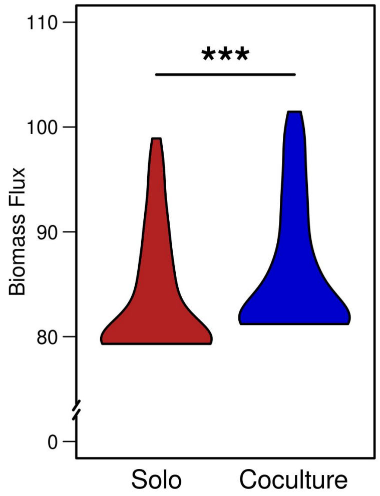
Transcriptome-guided parsimonious flux analysis improves predictions with metabolic networks in complex environments

Matthew L. Jenior[1], Thomas J. Moutinho, Jr.[1], Bonnie V. Dougherty[1], Jason A. Papin[1,2,3]*

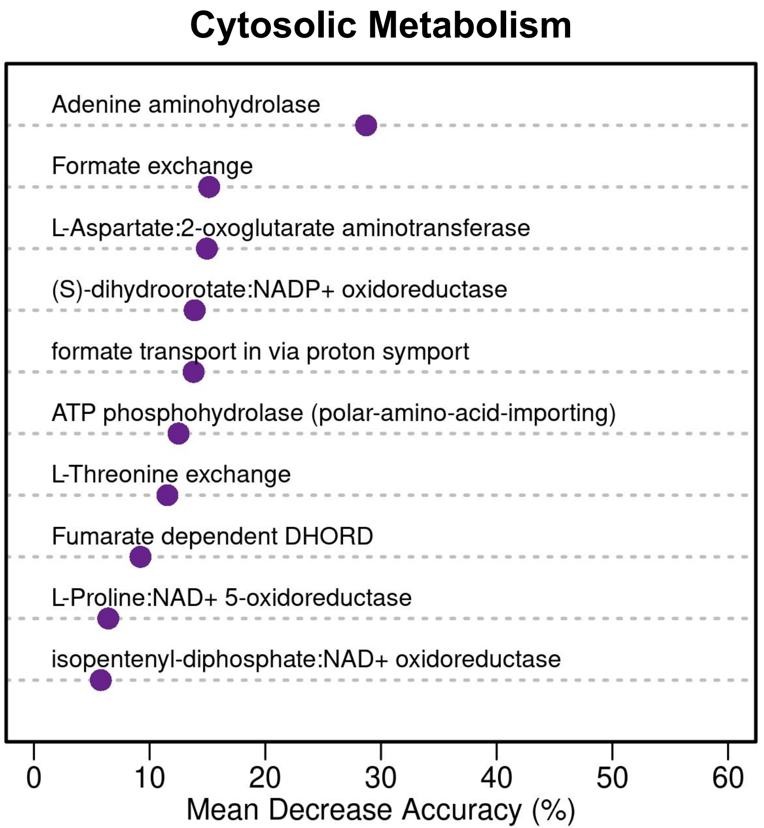
General Growth Simulation Results

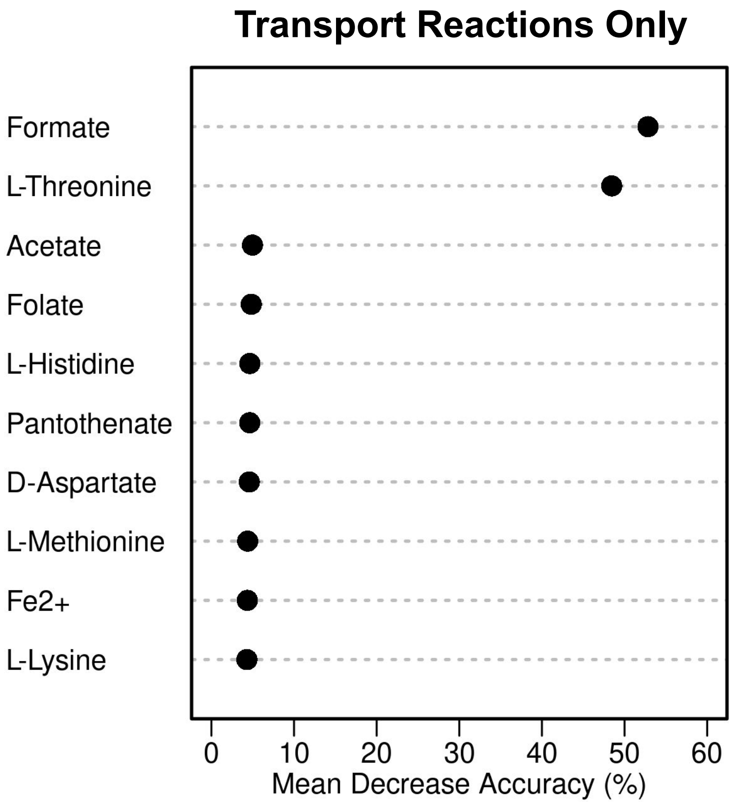
- Not a radical change in core metabolism
  - Focusing on shared reactions across groups
  - Flux sampling assesses all possibilities
  - Unsupervised machine learning
- Co-culture *C. difficile* growth enhanced
  - Not common in my analyses with moderate changes in overall transcriptome
- Distinct essential genes for growth:
  - Solo: Phosphate butyryltransferase (EC 2.3.1.19)
  - Co-culture: Deoxyribose-phosphate aldolase (EC 4.1.2.4)
- Indicates subtle shift in metabolic strategy





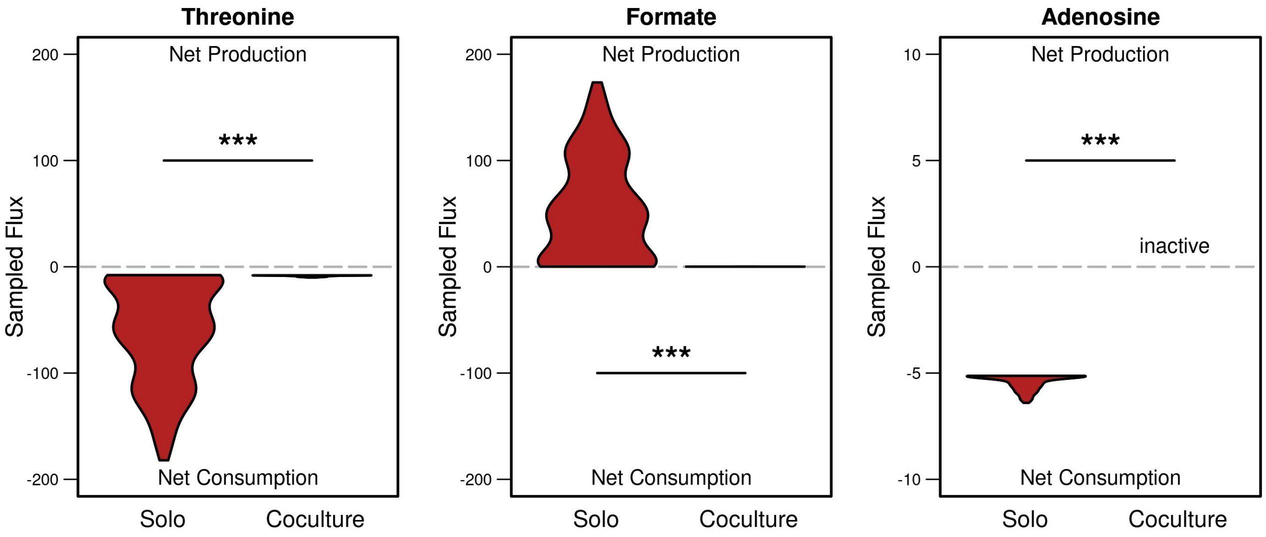
Random Forest Highlights Adenine and Threonine





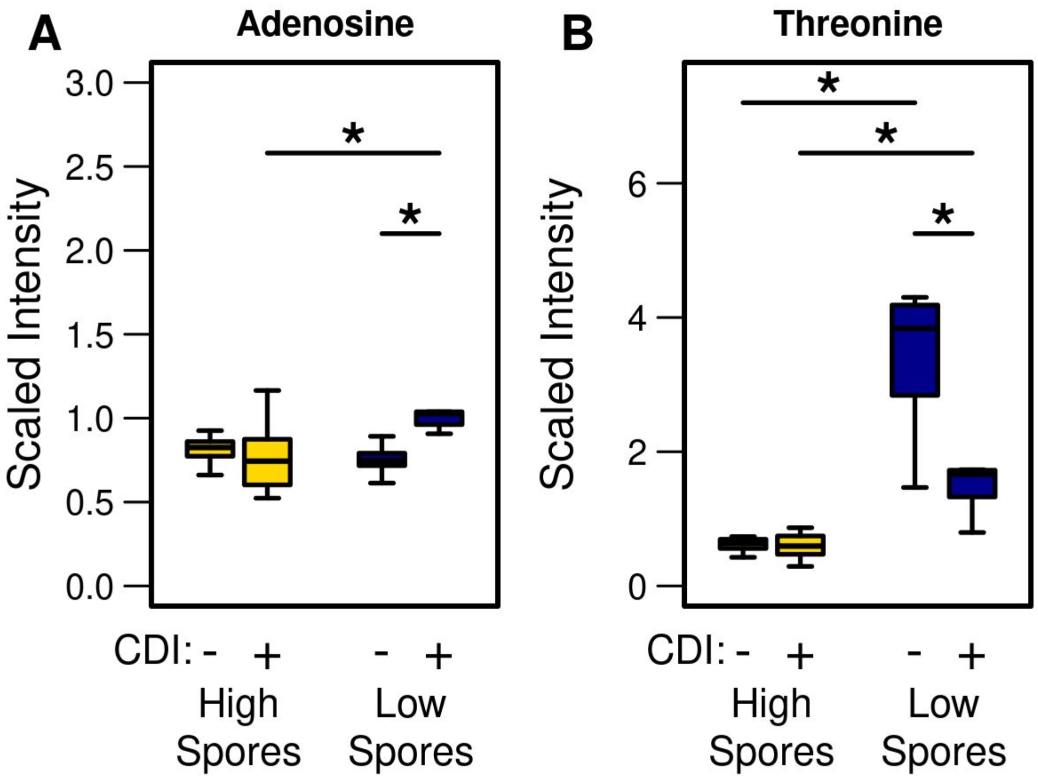
Threonine Fermentation Active in Solo Culture

- Exchange reactions interact with the edge of the system
  - Negative flux = import; Positive flux = export
- High degree of threonine fermentation predicted in solo culture
  - Not going to propionate fully, instead stopping at formate
- Significantly more cysteine, arginine, lysine, and methionine import in coculture
- Interestingly, adenosine is synthesized in the cytosol in co-culture
  - Major difference in extracellular topology



Links to Pathogenesis for Adenosine & Threonine

- Untargeted metabolomics from grad school (U of M)
- Cecal content from 18 hpi in two distinct antibiotic pretreatments
  - High sporulation: clindamycin
  - Low sporulation: cefoperazone
  - Very early for robust toxin measures
- Both results correlate with the increase virulence in co-culture
  - Enterococcus are clinda-resistant
  - Present at some abundance in colony



Data from Jenior ML, et al. (2017). *mSystems.*

Adenosine Alters Epithelium & Gm+ Virulence

Adenosine Deaminase Inhibition Prevents *Clostridium difficile* Toxin A-Induced Enteritis in Mice[∇]

Ana Flávia Torquato de Araújo Junqueira,[2] Adriana Abalen Martins Dias,[3] Mariana Lima Vale,[2] Graziela Machado Gruner Turco Spilborghs,[3] Aline Siqueira Bossa,[3] Bruno Bezerra Lima,[2] Alex Fiorini Carvalho,[3] Richard Littleton Guerrant,[4] Ronaldo Albuquerque Ribeiro,[2] and Gerly Anne Brito[1]*

RESEARCH ARTICLE

Extracellular adenosine modulates host-pathogen interactions through regulation of systemic metabolism during immune response in *Drosophila*

Adam Bajgar, Tomas Dolezal*

* also found in *Bacillus anthracis*

Staphylococcus aureus synthesizes adenosine to escape host immune responses

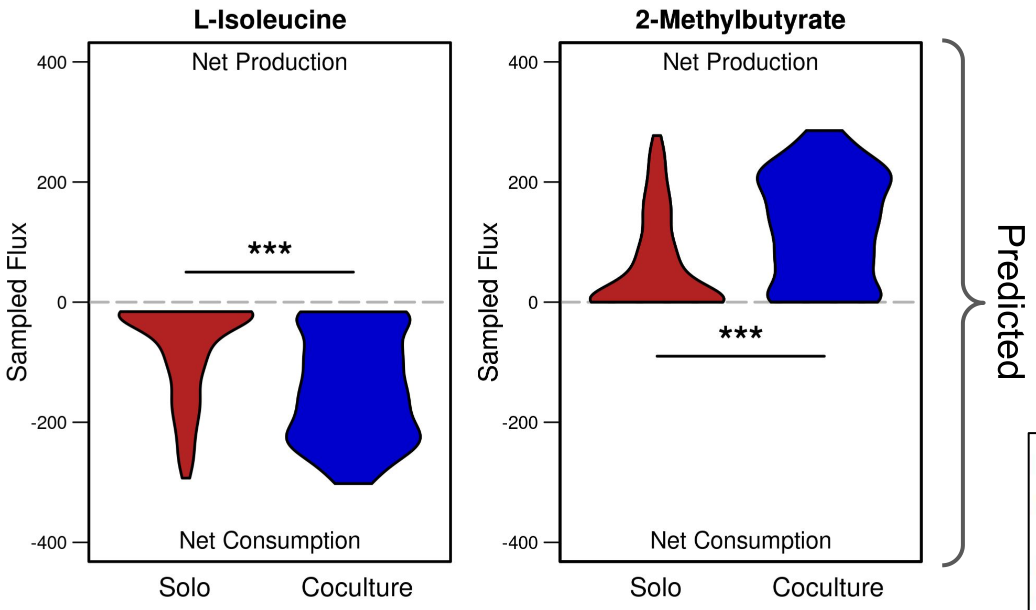
Vilasack Thammavongsa, Justin W. Kern, Dominique M. Missiakas, and Olaf Schneewind

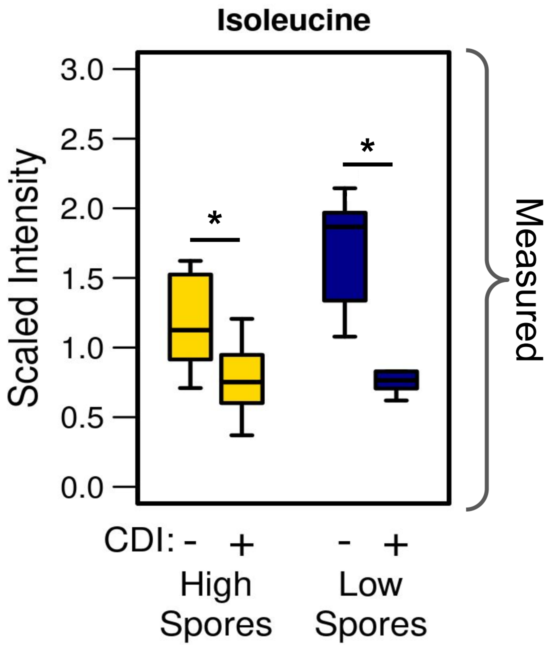
An adenosine triphosphate-independent proteasome activator contributes to the virulence of *Mycobacterium tuberculosis*

Jordan B. Jastrab[a], Tong Wang[b], J. Patrick Murphy[c], Lin Bai[b], Kuan Hu[b,d], Remco Merkx[e], Jessica Huang[f], Champak Chatterjee[f], Huib Ovaa[e], Steven P. Gygi[c], Huilin Li[b,d], and K. Heran Darwin[a,1]

Isoleucine Usage Increased in Co-culture

- Predicted import significantly higher in co-culture
  - Primarily used only for protein synthesis in solo culture
  - Concomitant increase in Stickland product in co-culture
- Previously linked to increased toxin synthesis



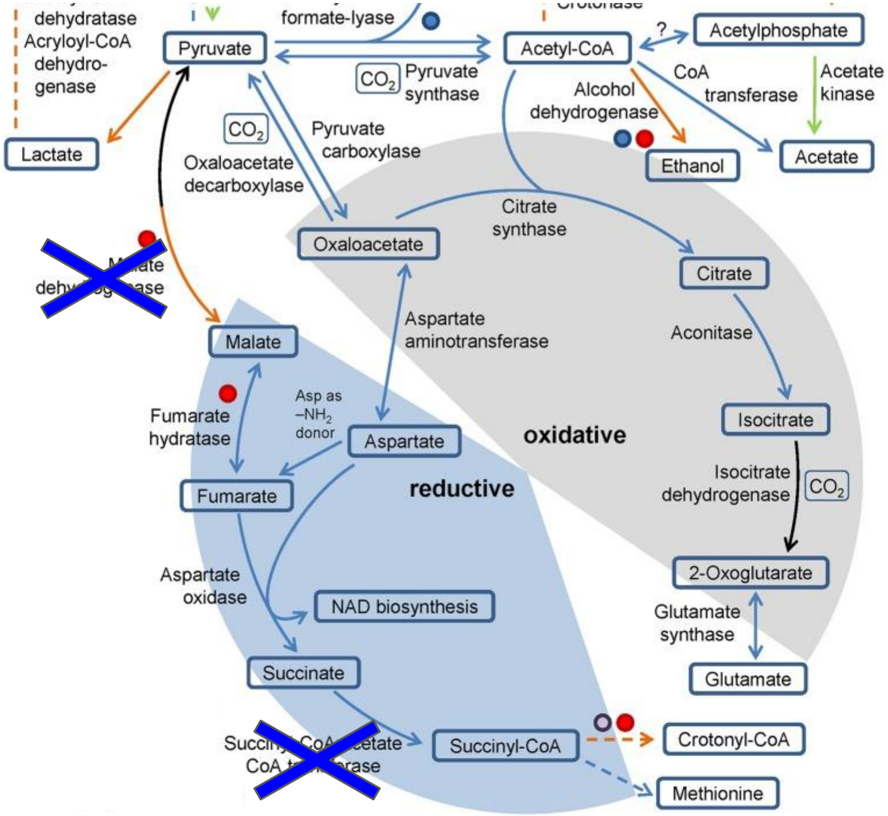


Effect of Isoleucine on Toxin Production by *Clostridium difficile* in a Defined Medium

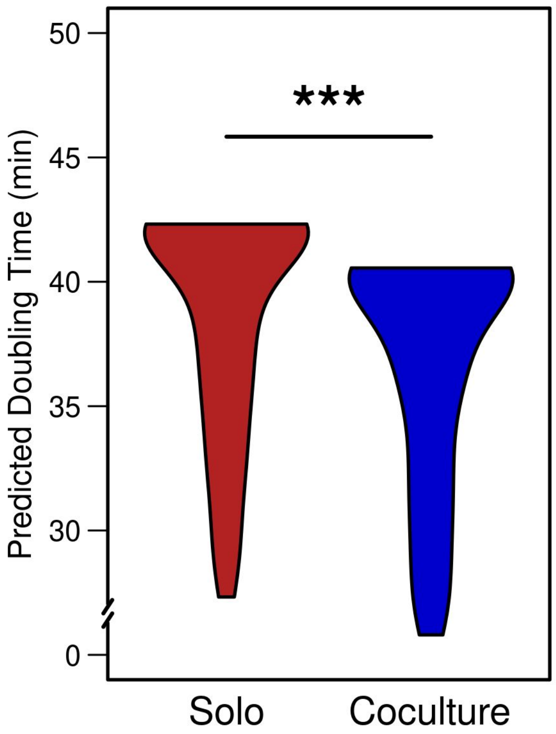
Daisuke Ikeda [1, 2], Tadahiro Karasawa [1], Kiyotaka Yamakawa [1], Ryuichiro Tanaka [3], Mikio Namiki [2], Shinichi Nakamura [1]

Topological Differences in Reactions

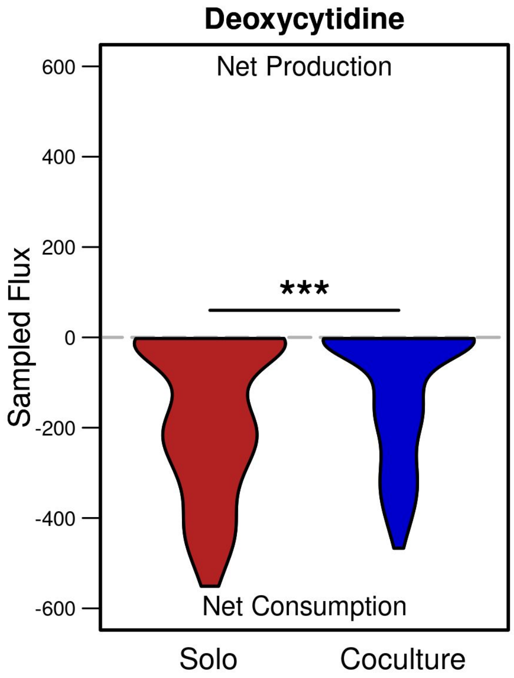
- Solo culture-only reactions:
  - Methyl-3-oxopropanoyl-CoA:pyruvate carboxyltransferase
  - L-threonine ammonia-lyase
  - Methylmalonyl-CoA isomerase
  - Succinyl-CoA synthase
  - Dimethyallyl diphosphate:NADP+ oxidoreductase
  - Adenosine hydrogen symport
  - Propanoyl-CoA:formate C-propanoyltransferase
  - Fumarate hydratase
  - sn-Glycerol-3-phosphate:NADP+ 2-oxidoreductase
  - L-Leucine:2-oxoglutarate aminotransferase
  - Malate reductase
- Coculture-only reactions:
  - sn-Glycerol-3-phosphate:NAD+ 2-oxidoreductase
  - L-Proline:NADP+ 5-oxidoreductase
  - Leucine aminotransferase
  - Adenosine:phosphate alpha-D-ribosyltransferase



Neumann-Schaal (2019). *Front Microbiol*.



50
45
40
35
30
0
Predicted Doubling Time (min)
***
Solo
Coculture



Deoxycytidine
Net Production
Net Consumption
Sampled Flux
600
400
200
0
-200
-400
-600
***
Solo
Coculture