Metabolic Network Analysis and Metatranscriptomics of a Cosmopolitan and Streamlined Freshwater Lineage

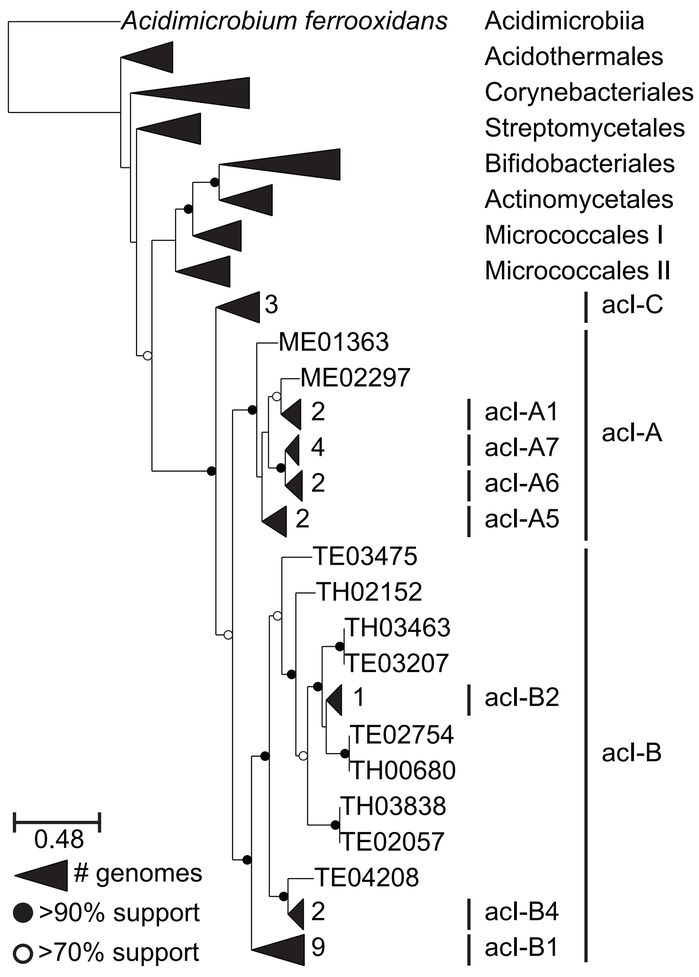
Joshua J. Hamilton[1], Sarahi L. Garcia[2], Matthew Bendall[3], Brittany S. Brown[1], Sarah L.R. Stevens[1], Stefan Bertilsson[2], Katrina T. Forest[1], Rex Malmstrom[3], Ramunas Stepanauskas[4], Susannah Tringe[2], Tanja Woyke[2], and Katherine D. McMahon[1]

[1]University of Wisconsin-Madison, [2]Uppsala University, [3]Department of Energy Joint Genome Institute, and [4]Bigelow Laboratory for Ocean Science

Introduction

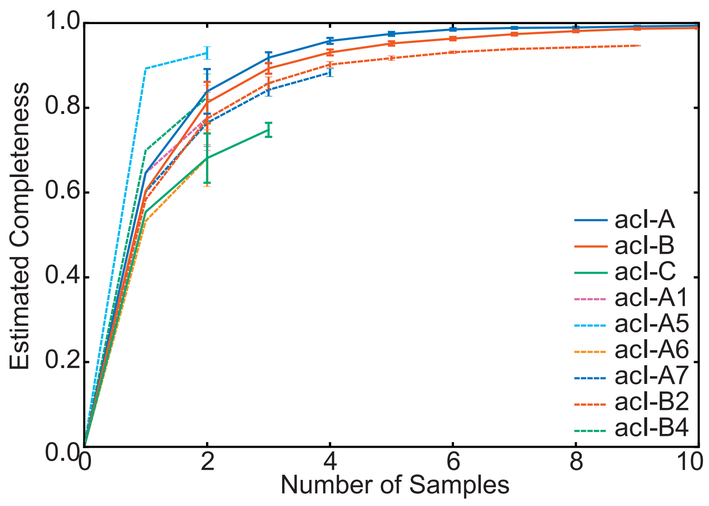
- Uncultured actinobacteria of the acI lineage are a dominant phylum in freshwater ecosystems
- Reference genome collection enables metabolic reconstruction of freshwater lineages
- A computational pipeline linking metabolic network reconstruction and metatranscriptomics provides insight into acI ecophysiology

Phylogeny



- acI form a monophyletic group within the class Actinobacteria
- 36 genomes across three previously defined clades (acI-A, -B, and –C)

Genome Completeness



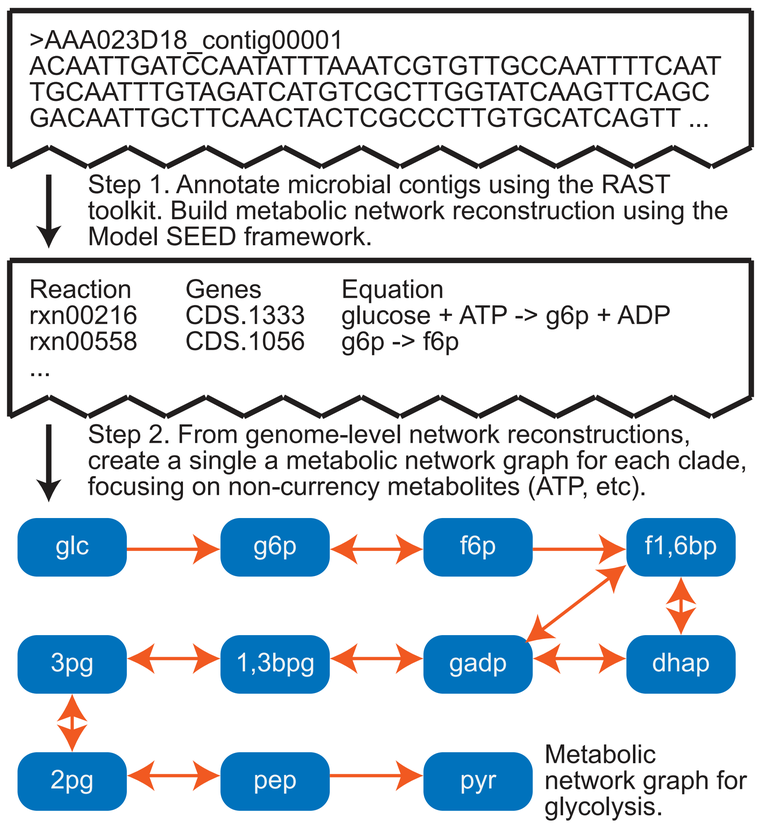
Estimated completeness of clade- and subclade-level population genomes based on single-copy marker genes.

Availability

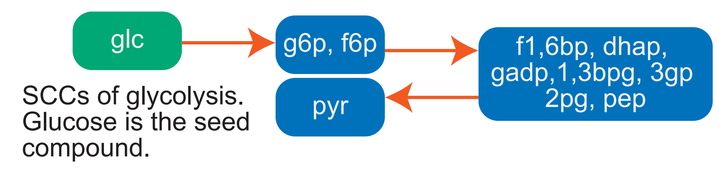
joshamilton

Code, example Jupyter notebooks, this poster, and more available on GitHub.

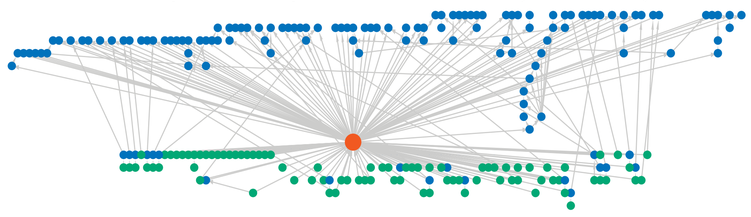
Metabolic Network Reconstruction



Step 3. Decompose the metabolic graph into its strongly connected components (SCCs), groups of compounds which are interconvertible. These graphs typically exhibit a "bow-tie" structure, with a single component containing many metabolites.



Step 4. For each clade, identify seed compounds, the minimal set of compounds from which all other compounds in the network can be synthesized. For a metabolic network represented as a directed graph, the seed set is the minimal set of nodes from which all other nodes can be reached. These nodes may represent auxotrophies or inputs to metabolism (see below).



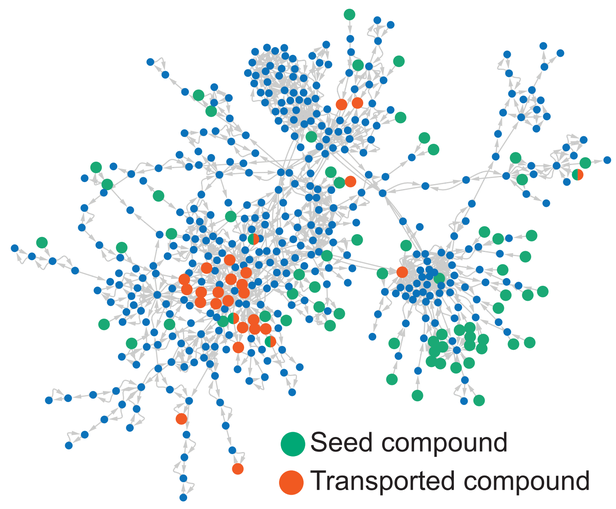
Metabolic network reconstruction for acI-A, showing its SCCs. Seed compounds are in green. The larges SCC (red circle) contains 60% of the metabolites.

Network Analysis and Metatranscriptomics

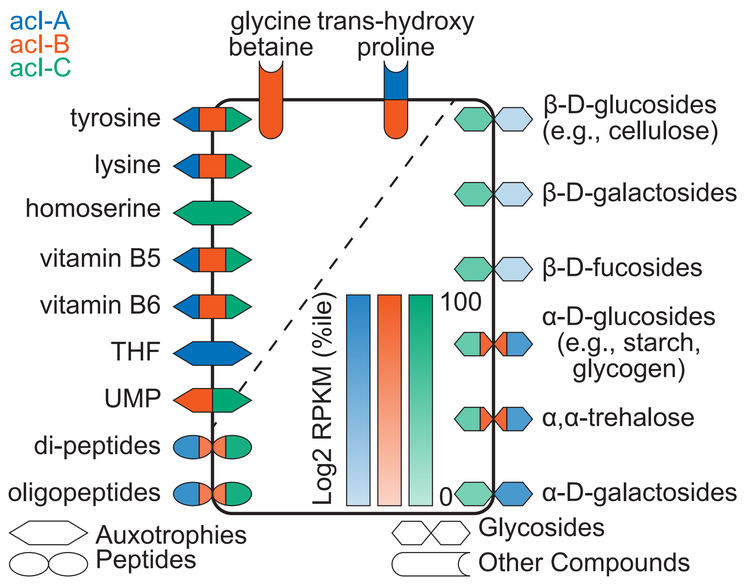
Network Curation and Read Mapping

- Curated list of seed compounds
- Re-annotated peptidases, glycoside hydrolases, and transporters
- Metatranscriptomes collected from Lake Mendota (Madison, WI) and mapped to reference acI genomes
- Gene expression calculated for orthologous gene clusters, as identified by OrthoMCL

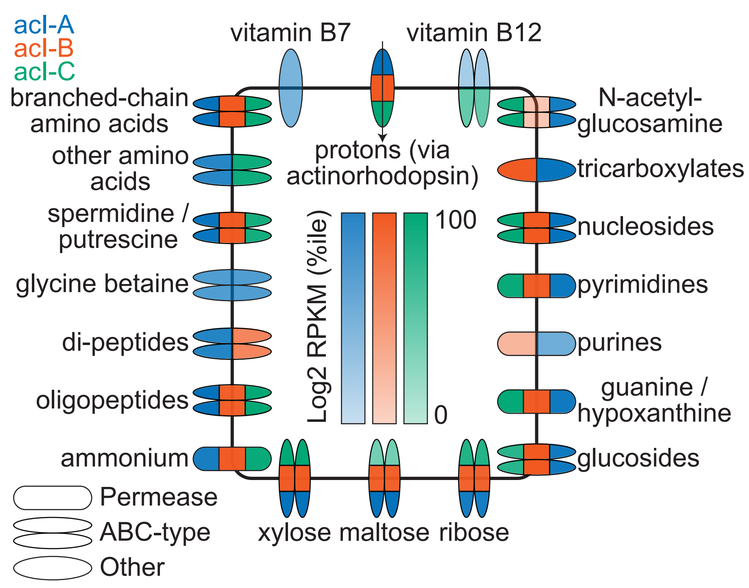
Metabolic Network of Clade acI-C



Curated Set of Seed Compounds



Expressed Transport Proteins



Conclusions

- Members of the acI lineage consume substrates near the center of their metabolic network, rather than the periphery
- Auxotrophies for vitamins and amino acids are consistent with acI from other lakes
- Broad substrate specificity may explain the ubiquity of the acI lineage

Acknowledgements

Bigelow Laboratory for Ocean Sciences
JGI Joint Genome Institute
Uppsala Universitet
USDA
LTER
NSF