Folate auxotrophy in *Atribacterota* reveals extensive microbial cultivation problem

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Introduction

- B-vitamins are essential cofactors that played a central role in the origin and early evolution of life on Earth (1)
- While many microbes synthesize these vitamins de novo (prototrophy), others lack complete biosynthetic pathways and depend on environmental sources (auxotrophy), creating metabolic interdependencies within microbial communities (2)
- Understanding how vitamin auxotrophy is distributed across microbial lineages is therefore critical for interpreting ecosystem function and predicting metabolic strategies that could sustain life in nutrient-limited environments beyond Earth

Background

- Most bacterial phyla remain poorly cultivated, including *Atribacterota*, a widespread group of anaerobes implicated in syntrophic carbon and hydrogen metabolism (3)
- In this larger work, we isolated two novel *Atribacterota* species, *Caldatribacterium saccharofermentans* and *Caldatribacterium inferamans*
- Growth of both was only achieved following addition of yeast extract, suggesting vitamin dependence; prior genomic analyses indicated auxotrophy for multiple B-vitamins, including folate (4)
- LC/MS revealed that folate was inadvertently removed from standard vitamin solutions due to precipitation during filter-sterilization (5)
- This widely used sterilization method, dating back to at least 1977, may have limited cultivation efforts for decades (6)

Research Question:
How widespread is folate auxotrophy across the bacterial tree of life?

1. Monteverde et al., *Geobiology* 2017. doi:10.1111/gbi.12202
2. Sañudo-Wilhelmy et al., *PNAS* 2012. doi:10.1073/pnas.1208755109
3. Jiao et al., *Microbiome* 2024. doi:10.1186/s40168-024-01836-7
4. Dodsworth et al., *Nat Commun* 2013. doi:10.1038/ncomms2884
5. Wolin et al., *J Biol Chem* 1963. 238,8:2882-6
6. Zehnder et al., *Arch Microbiol* 1977. doi: 10.1007/BF00549357

Results

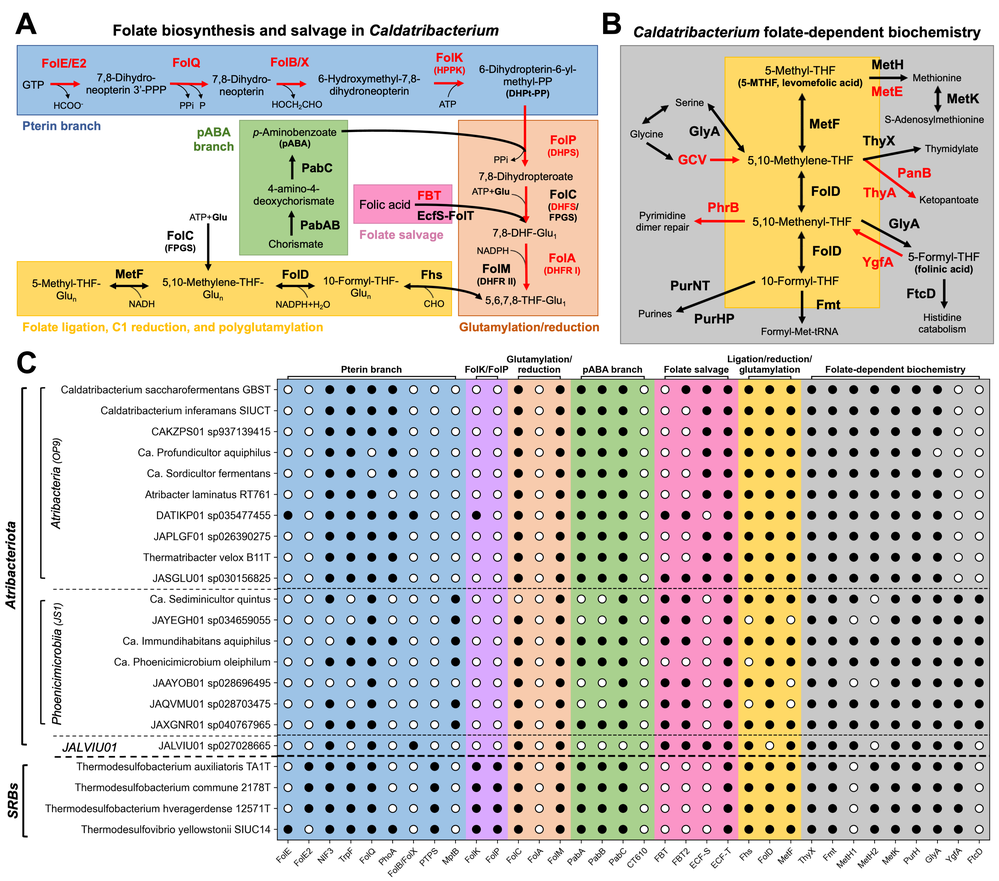

Figure 1. Folate metabolism in Atribacterota. A, Canonical folate biosynthetic pathway highlighting the different branches of the pathway. **B,** Folate-dependent biochemistry (grey) and reduction steps (yellow). Enzymes in black are encoded by the *Caldatribacterium* isolates, whereas those in red are absent. **C,** The presence (filled circle) or absence (open circle) of folate-related genes in all high-quality *Atribacterota* genomes, along with SRB used in experiments. Presence/absence was determined using the COG for each folate-related gene.

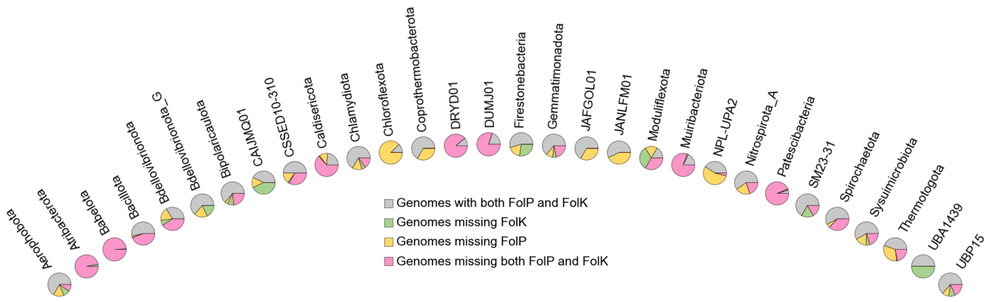

Figure 2. Bacterial phyla with extensive folate auxotrophy. Pie plots of bacterial phyla where ≥33% of all genomes were missing either FolK (required for pterin prototrophy), FolP (required for folate prototrophy), or both FolK and FolP. Presence/absence for FolK and FolP was determined by their respective COGs (COG0801 and COG0294). Each phylum is named per GTDB and the number of high-quality genomes analyzed follows in parentheses. 30 phyla are shown; 16 phyla with extensive auxotrophy were excluded from the figure due to having ≤5 high-quality genomes available in GTDB.

- ~35,000 genomes from the Web of Life 2 dataset supplemented with species representative genomes from the Genome Taxonomy Database (GTDB) were analyzed for folate biosynthesis and biochemistry genes **(Fig 1A, B)**
- Genomes were annotated using a pipeline within anvi'o: Prodigal > DIAMOND > Cluster of Orthologous Genes (COG) database
- FolK was identified as key for pterin prototrophy; FolP was identified as key for folate prototrophy
- The entire phylum of *Atribacterota* is auxotrophic for folate, and all representatives contain at least 1 of 2 salvage pathways
- Folate auxotrophy was predicted in ~29% of all bacterial genomes
- Extensive auxotrophy was predicted in 27% of bacterial phyla (≥33% of all genomes missing FolK and/or FolP)

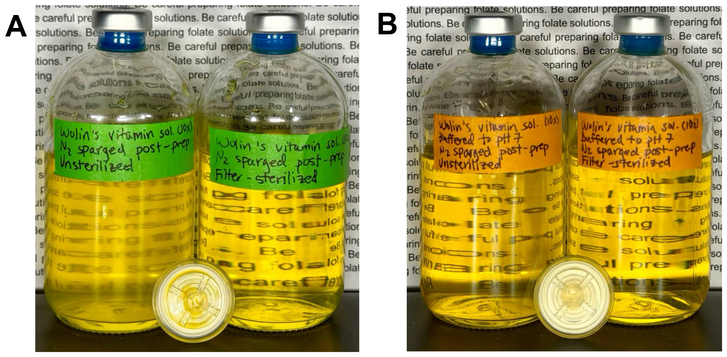

Figure 3. Removal of precipitated folate by filter-sterilization. A, Wolin's vitamin solution (10x) as prepared per Solution 5980 in MediaDive. **B,** Wolin's vitamin solution (10x) but prepared with anoxic H_2O under a stream of N_2, buffered and adjusted to pH 7. In both panels, the not sterilized solution is on the left and filter-sterilized solution on the right, and the used filter in-between. In **A,** visible precipitate is seen in the not sterilized solution and removed in the filter-sterilized version, as shown on the filter; in **B,** there is no visible precipitate.

Future Directions

- Expansion of this study to all species representative genomes of bacteria and archaea in GTDB (143,614 genomes as of current release) as well as to all B-vitamin biosynthesis pathway genes
- Using this dataset, training a graph neural network to model and predict patterns of prototrophy, auxotrophy, and metabolic interdependencies across the microbial tree of life

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