Folate auxotrophy in *Atribacterota* reveals extensive microbial cultivation problem

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B-vitamins are essential cofactors that played a central role in the origin and early evolution of life on Earth. While many microbes synthesize these vitamins de novo, others lack complete biosynthetic pathways and depend on environmental sources, creating metabolic interdependencies within microbial communities. 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. Most bacterial phyla remain poorly cultivated, including *Atribacterota*, a widespread group of anaerobes implicated in syntrophic carbon and hydrogen metabolism. Here, we report the isolation of two novel Atribacterota species, *Caldatribacterium saccharofermentans* and *Caldatribacterium inferamans*, which initially resisted cultivation in defined media. Growth was only achieved following addition of yeast extract, suggesting vitamin dependence. Prior genomic analyses indicated that *Caldatribacterium* is auxotrophic for multiple B-vitamins, including folate. Subsequent investigation via LC/MS revealed that folate was inadvertently removed from standard vitamin solutions due to precipitation during filter-sterilization, a widely used sterilization method dating back to at least 1977 that may have limited cultivation efforts for decades. To assess the broader significance of this phenomenon, we analyzed ~35,000 bacterial genomes spanning all 183 phyla. Folate auxotrophy, defined by absence of key biosynthetic genes (*folK* and *folP*), was predicted in ~29% of all genomes, with extensive auxotrophy (≥33% genomes) observed in 27% of bacterial phyla. These results suggest that folate limitation may be a widespread and previously underappreciated barrier to microbial cultivation. Together, these findings highlight the importance of vitamin availability in structuring microbial ecosystems and suggest that metabolic interdependencies driven by vitamin auxotrophy may be a fundamental feature of life in both terrestrial and extraterrestrial environments.