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TITLE: Genetic Evidence for Two Carbon Fixation Pathways (the Calvin-Benson-Bassham Cycle and the Reverse Tricarboxylic Acid Cycle) in Symbiotic and Free-Living Bacteria

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ABSTRACT:

Very few bacteria are able to fix carbon via both the reverse tricarboxylic acid (rTCA) and the Calvin-Benson-Bassham (CBB) cycles, such as symbiotic, sulfur-oxidizing bacteria that are the sole carbon source for the marine tubeworm *Riftia pachyptila*, the fastest-growing invertebrate. To date, the coexistence of these two carbon fixation pathways had not been found in a cultured bacterium and could thus not be studied in detail. Moreover, it was not clear if these two pathways were encoded in the same symbiont individual, or if two symbiont populations, each with one of the pathways, coexisted within tubeworms. With comparative genomics, we show that *Thioflavicoccus mobilis*, a cultured, free-living gammaproteobacterial sulfur oxidizer, possesses the genes for both carbon fixation pathways. Here, we also show that both the CBB and rTCA pathways are likely encoded in the genome of the sulfur-oxidizing symbiont of the tubeworm *Escarpia laminata* from deep-sea asphalt volcanoes in the Gulf of Mexico. Finally, we provide genomic and transcriptomic data suggesting a potential electron flow toward the rTCA cycle carboxylase 2-oxoglutarate:ferredoxin oxidoreductase, via a rare variant of NADH dehydrogenase/heterodisulfide reductase in the *E. laminata* symbiont. This electron-bifurcating complex, together with NAD(P)⁺ transhydrogenase and Na⁺ translocating Rnf membrane complexes, may improve the efficiency of the rTCA cycle in both the symbiotic and the free-living sulfur oxidizer. **IMPORTANCE** Primary production on Earth is dependent on autotrophic carbon fixation, which leads to the incorporation of carbon dioxide into biomass. Multiple metabolic pathways have been described for autotrophic carbon fixation, but most autotrophic organisms were assumed to have the genes for only one of these pathways. Our finding of a cultivable bacterium with two carbon fixation pathways in its genome, the rTCA and the CBB cycle, opens the possibility to study the potential benefits of having these two pathways and the interplay between them. Additionally, this will allow the investigation of the unusual and potentially very efficient mechanism of electron flow that could drive the rTCA cycle in these autotrophs. Such studies will deepen our understanding of carbon fixation pathways and could provide new avenues for optimizing carbon fixation in biotechnological applications.

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