The Biology and Community Structure of CO_2-Reducing Acetogens in the Termite Hindgut

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Abstract

In the guts of wood-feeding termites, CO_2-reductive acetogenesis serves as the dominant sink for H_2 generated during the fermentation of wood polysaccharides. This activity can generate up to 1/3 of the acetate that powers the energy metabolism of the host insect. The gene for formyl-tetrahydrofolate synthetase (FTHFS), a key gene in the acetyl-CoA pathway, can be used as a genetic marker of acetogenic capability. The dominant FTHFS types in the guts of wood-feeding termites are known to cluster phylogenetically with those from acetogenic *Treponemes*. In this work, we present the discovery that the guts of wood-feeding roaches are also dominated by *Treponeme*-like sequences. Phylogenetic analysis of roach-derived FTHFS sequences reveals a cluster that forms a basal radiation of the termite *Treponeme* cluster. This suggests that the *Treponemes* found in roach guts represent an ancient divergence, present in the last common ancestor of these insects, rather than a modern lineage acquired by cross-species symbiont transfer. The FTHFS sequences present in the guts of higher termites were also examined. Wood-, palm-, and litter-feeding termites were found to be dominated by acetogenic *Treponemes*, while subterranean soil/grass feeders were found to be dominated by a novel cluster of *Firmicute*-like FTHFS types. Also presented herein is the development of microfluidic digital PCR for molecular characterization of individual bacteria from environmental samples. We used this technique to retrieve FTHFS and 16S rRNA gene sequences from single bacterial cells, thereby discovering the 16S rRNA sequences of uncultured acetogens in the termite gut. This technique should provide a valuable tool for molecular analyses of termite gut acetogens, and can potentially be adapted for the characterization of uncultured bacteria that carry any metabolic gene of interest.

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