Formyltetrahydrofolate synthetase gene diversity in the guts of higher termites with different diets and lifestyles

Elizabeth A Ottesen and Jared R Leadbetter

Supplemental Materials

Supplemental Figure 1. Mitochondrial cytochrome oxidase II phylogeny of termites and roaches.

Supplemental Figure 2. Phylogenetic analysis of termite and roach FTHFS sequences.

Supplemental Figure 3. UniFrac Analysis of FTHFS library compositions.

Supplemental Figure 4. Phylogenetic analysis of FTHFS sequences used for UniFrac analysis.

Supplemental Table 1. Operational taxonomic unit grouping of FTHFS sequences identified in this study

Supplemental Table 2. Sequences used in FTHFS phylogenetic analysis

Supplemental Table 3. Sequences used in COII phylogenetic analysis

Supplemental References.
Supplemental Figure 1. Mitochondrial cytochrome oxidase II phylogeny of termites and roaches. Species from which gut FTHFS diversity has been examined marked in bold, species examined in this study underlined. Tree calculated using Phylip PHYML and 393 unambiguous, aligned DNA bases. Open circles indicate nodes also supported by either Fitch distance or Phylip parsimony methods. Closed circles indicate nodes supported by all three algorithms. Scale bar represents 0.1 base pair changes per alignment position.
Supplemental Figure 2. Non-acetogenic Firmicutes. Tree constructed using 346 unambiguous, aligned amino acids and the PhyML maximum likelihood algorithm. Open circles indicate nodes also supported by either Fitch distance or Phylip parsimony methods. Closed circles indicate nodes supported by all three algorithms. Scale bar indicates 0.1 changes per alignment position. The 13 acetogenic isolates in Figure 1 (main text) were used as an outgroup.
**Supplemental Figure 3.** UniFrac Analysis of FTHFS library compositions. Analyses conducted utilizing the phylogenetic tree shown in Supplemental Figure 4. As the UniFrac Jacknife re-sampling algorithm does not correct for sequence de-replication (6), sequences representing multiple clones were copied and added back into the tree before analysis. Environments were clustered using the abundance-weighted, normalized metric for the Lovell cluster (A) or full tree (B). Nodes supported by >90% of Jacknife re-sampling tests are marked. Principal component analyses were conducted using the abundance-weighted, normalized metric for the Lovell cluster (A) or the full tree (B). Abbreviations: P, *P. americana*; Ca, *C. punctulatus* adult; Cn, *C. punctulatus* nymph; Z, *Z. nevadensis*; R, *R. santonensis*; C, *C. secundus*; I, *Incisitermes* sp. Pas1; N, *Nasutitermes* sp. Cost003; M, *Microcerotermes* sp. Cost008; Rh, *Rhynchorotermes* sp. Cost004; G, *Gnathamitermes* sp. JT5; Ac, *Amitermes* sp. Cost010; Aj, *Amitermes* sp. JT2.
Supplemental Figure 4. Phylogenetic analysis of FTHFS sequences used for UniFrac analysis. Tree constructed using 301 unambiguous, aligned amino acids and the PhyML maximum likelihood algorithm. Scale bar indicates 0.1 changes per alignment position. As the PCR primers used in these studies specifically target acetogen-like FTHFS types, UniFrac analyses were conducted for both the Lovell cluster only (node A) and for the Lovell cluster plus the Clone E, *C. acidiurici* and *M. thermoacetica* groups. Clones affiliated with non-target groups such as *Bacteroidetes*, sulfate-reducing *Proteobacteria*, and most non-acetogenic *Firmicutes* were judged to represent non-specific amplification events and were excluded from the analysis. The number of RFLP types represented by each sequence is listed; for *C. secundus* and *R. santonensis*, abundances were published at the phylotype only, so the total number of hits was distributed amongst the representative clones for each group.
SUPPLEMENTAL TABLE 1. Operational Taxonomic Grouping of FTHFS sequences identified in this study

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1 Group representative (sequence used in published phylogenetic trees)
2 Defined as percent of full-length, non-chimeric clones
3 Sequenced RFLP type clones associated with each group
### SUPPLEMENTAL TABLE 2. Sequences used in FTHFS phylogenetic analysis

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