

Genome analysis of unculturable, fermentative symbiotic bacteria in termite guts

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Research aims

Termites feed only on dead plant matter and contribute greatly to the global carbon cycle on Earth. Their ability to efficiently digest lignocellulose is mostly attributable to the symbiotic gut microbiota that is unique to termites and has attracted interest in both basic and applied scientific research. However, because most of the diverse gut microbes (protists, bacteria, and archaea) are as-yet uncultivable, the exact details of the symbiotic mechanisms remain unclear. This study aims to elucidate the contribution of an uncultivable, dominant bacterium, *Candidatus Symbiothrix dinenymphae* (order Bacteroidales) (Fig. 1), to the symbiotic lignocellulolytic system.

Methods

Bacteria in the gut of the termite *Reticulitermes speratus* were sorted to single cells on a fluorescently activated cell sorting (FACS) system, each of which was subjected to isothermal whole-genome amplification. The single-cell amplicons were screened by PCR amplification and Sanger sequencing to identify samples with *Ca. S. dinenymphae* cells. Each identified single-cell amplicon was subjected to paired-end and mate-pair nucleotide sequence analysis on an Illumina MiSeq platform. The generated sequence reads were assembled to contigs and scaffolds, which were

screened on the basis of sequence homology and GC content to remove contaminating fragments. In addition to the genome sequence analysis, transmission electron microscopy (TEM) was conducted for detailed observation of the cell structure and the attachment site (Fig. 1) (1).

Results

The total length of the draft genome of *Ca. S. dinenymphae* obtained was 3.5 Mb, and the estimated genome coverage and size were approximately 82% and 4.3 Mb, respectively, on the basis of the conserved single-copy gene repertoires. This bacterium is a permanently associated ectosymbiont of a protist cell (1), but its genome size has not been reduced, unlike that of the intracellular symbiont *Candidatus Azobacteroides pseudotrichonymphae* (order Bacteroidales) obligately associated with a termite-gut protist (2). The *Ca. S. dinenymphae* genome shares several functional characteristics with *Ca. A. pseudotrichonymphae*: it ferments monosaccharides to acetate via glycolysis and also undergoes fumarate respiration to generate ATPs, and it retains biosynthetic pathways for most amino acids. On the other hand, *Ca. S. dinenymphae* lacks the genes required for nitrogen fixation, a process that is the prominent feature of *Ca. A. pseudotrichonymphae*.

Strikingly, the *Ca. S. dinenymphae* genome contains many genes for various glycoside hydrolases (GHs) involved in the degradation of the plant cell wall. These have never been found in the genomes of intracellular symbionts

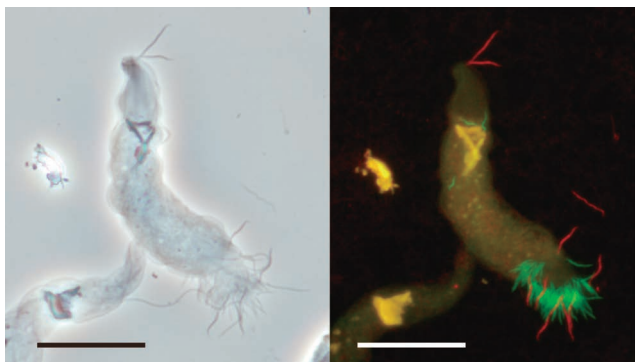


Fig. 1 The oxymonad protist *Dinenumpha porteri* type II in the gut of the termite *Reticulitermes speratus* and its ectosymbionts, *Candidatus Symbiothrix dinenymphae* (6-FAM-labeled, green) and spirochetes (Texas red-labeled, red), detected by FISH analysis using specific probes. Images are from phase-contrast (left) and epifluorescence (right) microscopy analyses. Bar: 20 μ m. See details in Ref. (1).

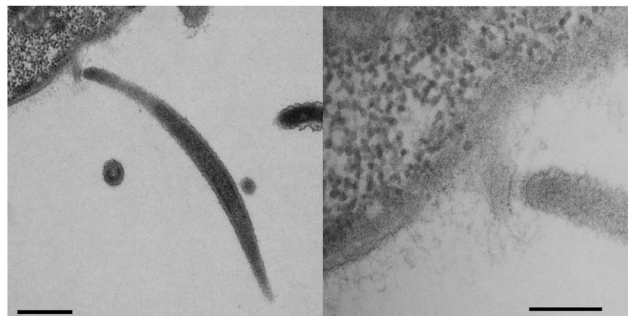


Fig. 2. Transmission electron micrographs of a *Candidatus Symbiothrix dinenymphae* cell attached to the cell surface of the protist *Dinenumpha* sp. A magnified image (right) shows a socket-like structure, probably derived from the host, and filamentous substances holding a tip of the ectosymbiont cell. Bars: 500 nm (left) and 200 nm (right). See details in Ref. (3).

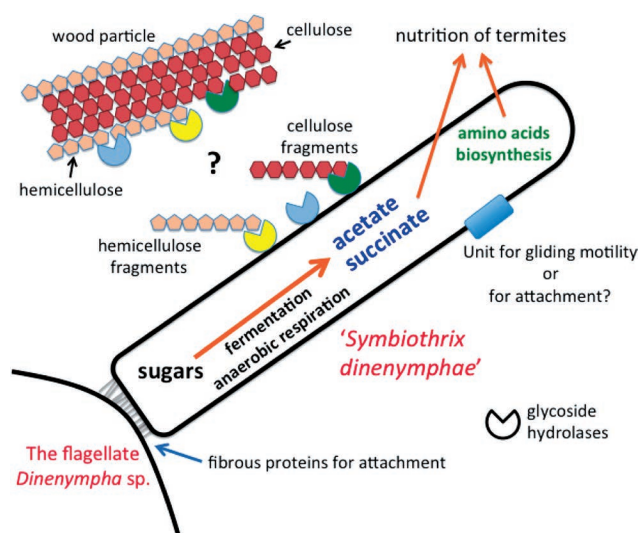


Fig. 3. Outline of the functions of the uncultivable bacterium *Candidatus Symbiothrix dinenymphae*, based on single-cell genome analysis.

such as *Ca. A. pseudotrichonymphae* (2). The GHs were classified into 16 families and included endo- β -1,4-endoglucanase and β -glucosidase, which are required for cellulose degradation. A gene for cellobiohydrolase was not found; this bacterium can degrade the amorphous site of cellulose but not its crystalline structure. In addition to the cellulases, diverse hemicellulases such as endo- β -1,4-xylanase, endo-1,5- α -l-arabinosidase, and α -mannosidase were identified. This bacterium secretes these hydrolytic enzymes from its cell while attached to the host protist cell surface; however, the localization of the enzymes, whether within the bacterial cell wall to capture soluble fragments

of lignocellulose or on wood particles to digest their surface, remains unidentified (Fig. 3).

Interestingly, this ectosymbiotic bacterium possesses a gene cluster responsible for gliding motility, which is occasionally observed in certain Bacteroidetes bacteria. This unexpected finding indicates that *Ca. S. dinenymphae* can detach from the host protist surface or possibly that the genes are just reminiscent of their ancestral free-living lifestyle.

Conclusions

Candidatus Symbiothrix dinenymphae, an uncultivable, dominant bacterium accounting for 2.5% of the total bacterial cells in the gut of *Reticulitermes speratus*, is an ectosymbiont of the cellulolytic protist *Dinenympha*. Single-cell genome analysis revealed that this bacterium also has the potential to digest lignocellulose and contribute to the nutrition of the termite host. On the other hand, the benefit to the host protist remains to be clarified.

References

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