

Diversity and Function of Bacteria Related to the Newly Isolated Organism JT5, a Possible Lignocellulose-Degrading Species from the Gut of an Evolutionarily Higher Termite

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Abstract

Recently, a novel bacterium (strain JT5) related to the genus *Dysgonomonas* was isolated from an evolutionarily-higher termite, *Gnathamitermes perplexus*. This bacterium was determined to be an abundant member of the gut community and to possess genes for enzymes involved in lignocellulose degradation. We hypothesized that numerous bacteria related to JT5 would reside in *G. perplexus* and in a variety of other termite species. To test the hypothesis, we created inventories of SSU rRNA genes from *G. perplexus* gut community DNA. We targeted subsets of SSU rRNA genes closely related to JT5, as well as those more broadly associated with the *Dysgonomonas* genus, in the gut bacterial population of *G. perplexus* and other termite species. Investigations revealed a lower than expected diversity of bacteria predicted to share species-level relationships with JT5 in *G. perplexus*, but a higher than expected diversity at genus level. We also found that many of termite samples we analyzed contained bacteria predicted to be related to JT5. These results indicate that JT5 represents one of potentially several bacterial species within the genus *Dysgonomonas* that inhabit the guts of termite species.

Introduction

Termites are one of the most successful groups of insects on the planet and are one of only two insects known capable of subsisting solely on lignocellulosic plant biomass. The remarkable digestive capability of termites is attributed to highly complex microbial communities inhabiting their guts. In evolutionarily-lower termites, this role has been primarily attributed to flagellate protozoa. Such protozoa do not inhabit the guts of evolutionarily-higher termites, thus it has been proposed that the role of lignocellulose degradation has been taken over by bacteria¹.

Remarkably little is known about termite gut bacteria because the vast majority of species cannot currently be isolated and grown in pure culture³. Recently, however, a bacterium has been isolated from the gut of the evolutionarily-higher termite *Gnathamitermes perplexus*. This isolate (JT5) is numerically abundant in the termite gut, possesses many genes for enzymes that are predicted to break down lignocellulose and it grows well in pure culture.

Here we investigated the ecological diversity of bacteria related to JT5 in termite guts, as this organism might represent a good model for understanding bacterially-mediated lignocellulose degradation occurring in termites. This in turn, can be applied in the development of biofuels out of woody crops, agricultural waste, and grasses².



Figure 1. *Gnathamitermes perplexus*, a phylogenetically higher termite and bacterium JT5. *G. perplexus* (panel A) was collected at Joshua Tree National Park in southern California. Bacterial strain JT5 (panel B) was isolated from the gut of *G. perplexus* using anaerobic growth medium amended with lignocellulosic material as a carbon source.

Methods

Three gut segments were analyzed by PCR for the presence or absence of bacteria related to JT5 to identify which portion of the gut JT5 inhabits. Community DNA was amplified using primers that target bacteria in general as well as primers that target the genus *Dysgonomonas* and primers that target species similar to JT5. Similarly, whole-gut community DNA from 8 different termite species was assessed to determine if members of the genus *Dysgonomonas* or species similar to JT5 inhabits other termite species.

Community diversity was assessed by performing restriction fragment-length polymorphism RFLP analysis using the general workflow described below.

1. PCR amplify whole gut community DNA using each of the above primer sets
2. Clone each PCR product separately into plasmids and transform *E. coli* cells
3. Pick 96 colonies from each round of transformation to produce 3 clone inventories
4. Restriction digest and visualize RFLP patterns of clones in each inventory
5. Grow *E. coli* cells that contained plasmids with unique RFLP patterns overnight in nutrient broth to generate plasmid DNA for sequencing

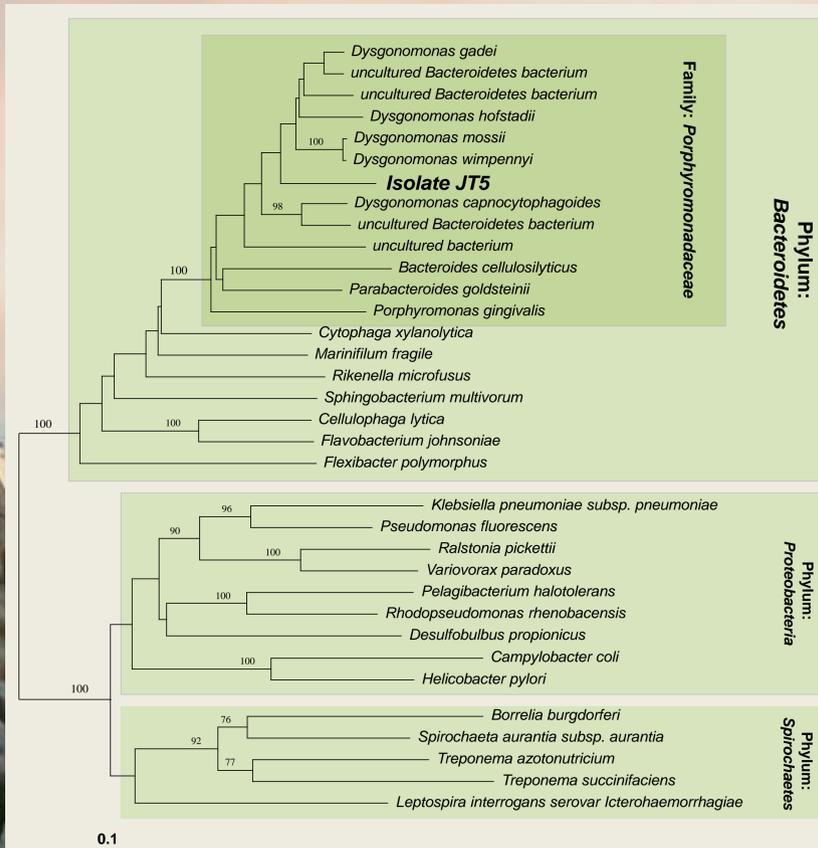


Figure 2. Isolate JT5 is a member of phylum *Bacteroidetes* and groups with genus *Dysgonomonas* within the family *Porphyromonadaceae*. The DNA distance tree was constructed in ARB using the Kimura algorithm and was based on bacterial SSU rRNA sequences with 1,204 aligned nucleotides for each sequence. Bootstrap values (based on 100 replicates) are given when >75%. Scale bar represents 10% difference in nucleotide sequences. Bacteria from the phyla *Proteobacteria* and *Spirochaetes* are used as outgroups.



Figure 3. *G. perplexus* segments used for community analysis. Guts were removed from insects and dissected into three sections with a 22 gauge needle. Gut segments (approx. 30 of each) were pooled and the microbial community DNA extracted and used as template for PCR. **Anterior sample** includes the crop (C), midgut (M), mixed segment (MS), and proctodeal segment 1 (P1). **P2 sample** includes only proctodeal segment 2 (P2), an unusually distended portion of the gut not typically seen in termites. **Posterior sample** includes proctodeal segment 3 (P3) (analogous to the hind gut paunch in phylogenetically lower termites) the colon (P4) and rectum (P5).

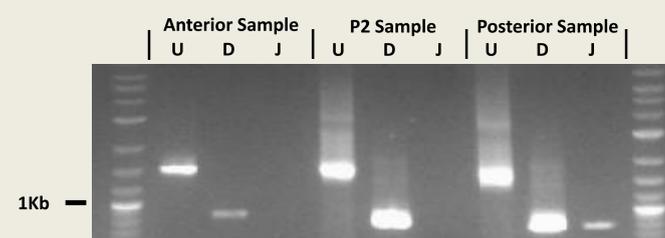


Figure 4. Isolate JT5 inhabits the anterior portion of the *G. perplexus* gut. PCR amplifications of different gut sections show a JT5 signal only in the posterior section using JT5-specific primers (J) while a strong signal was observed for the posterior section, the P2 section and, to a lesser extent, the anterior gut section using *Dysgonomonas*-specific primers. As expected, all sections produced a strong signal when universal bacterial primers (U) were used.

Table 1

Termite Species	Ecosystem	Food	Soil Contact	Universal Bacterial	Dysgonomonas	JT5-Specific
Higher Termites						
<i>Gnathamitermes perplexus</i>	Warm temperate desert	Dry grass, soil	High	+++	++	+
<i>Rhynchoterme</i> sp. Cost004	Wet rainforest transition	Leaf litter	High	+++	++	-
<i>Amitermes</i> sp. Cost010	Wet rainforest transition	Roots, Soil	High	+++	+	-
<i>Nasutitermes</i> sp. Cost003	Wet rainforest transition	Wood	Low	+++	+	-
<i>Microceroterme</i> sp. Cost008	Lowland moist forest	Palm	Low	+++	+	-
Lower Termites						
<i>Insisitermes minor</i>	Low-altitude residential	Wood	High	+++	+	-
<i>Reticulitermes hesperus</i>	High-altitude pine forrest Wood	High	High	+++	+	-
<i>Zootermopsis nevadensis</i>	High-altitude pine forrest Wood	Low	Low	+++	-	-

Relative signal intensity given by (+) symbols. Where the signal is indicated as (-), no PCR amplification was observed.

Results and Discussion

PCR amplification of a sample from the anterior section, the P2 segment, and the posterior section of the *G. perplexus* gut showed the presence of *Dysgonomonas* species in all 3 segments. The *Dysgonomonas* signal was weakest in the anterior section, indicating the bacteria belonging to this genus reside primarily in the hindgut of this termite. A PCR signal indicating the presence of JT5 was seen only in the anterior gut segment, which makes it likely that JT5 is located in the P3 gut compartment where lignocellulose degradation is thought to occur. These findings support the idea that JT5 is an important part of the microbial community facilitating lignocellulose breakdown in *G. perplexus*.

Bacteria predicted to belong to the genus *Dysgonomonas* were present in all 5 higher termites studied, as well as in two lower termites, *Reticulitermes hesperus* and *Insisitermes minor*. The JT5 species was found only in the higher termite *G. perplexus*, indicating that this species may be specific to the termite *G. perplexus*.

Libraries of a *G. perplexus* whole gut sample using general bacterial primers, primers for the *Dysgonomonas* genus, and primers specific for JT5 yielded 70, 17, and 2 unique RFLP types, respectively. These results indicate that species closely related to JT5 while abundant, were not very diverse. In contrast, a diversity of bacteria predicted to belong to the genus *Dysgonomonas* was observed in this sample, suggesting a major role for these bacteria in the digestive capabilities of termites.

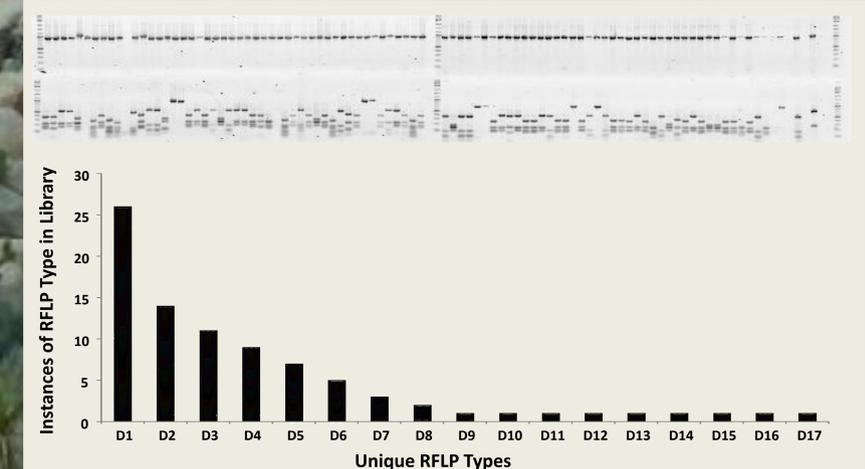


Figure 5. RFLP analysis of *Dysgonomonas* library. PCR products and restriction digested PCR products are shown in the upper panel. The lower panel shows the analysis of the library, indicating 17 different RFLP types. The most abundant of which (D1) comprised over 25% of the inventory.

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All termite specimens were collected under permit by state, federal, or international governing bodies and were handled according to accepted methods for animal care and use.

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