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Comparison of the gut microbiota from soldier and worker castes of the termite *Reticulitermes grassei*

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Summary. The bacterial microbiota from the whole gut of soldier and worker castes of the termite *Reticulitermes grassei* was isolated and studied. In addition, the 16S rDNA bacterial genes from gut DNA were PCR-amplified using *Bacteria*-selective primers, and the 16S rDNA amplicons subsequently cloned into *Escherichia coli*. Sequences of the cloned inserts were then used to determine closest relatives by comparison with published sequences and with sequences from our previous work. The clones were found to be affiliated with the phyla Spirochaetes, Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Synergistetes, Verrucomicrobia, and candidate phyla Termite Group 1 (TG1) and Termite Group 2 (TG2). No significant differences were observed with respect to the relative bacterial abundances between soldier and worker phylotypes. The phylotypes obtained in this study were compared with reported sequences from other termites, especially those of phylotypes related to Spirochaetes, *Wolbachia* (an Alphaproteobacteria), Actinobacteria, and TG1. Many of the clone phylotypes detected in soldiers grouped with those of workers. Moreover, clones CRgS91 (soldiers) and CRgW68 (workers), both affiliated with 'Endomicrobia', were the same phylotype. Soldiers and workers also seemed to have similar relative protist abundances. Heterotrophic, poly-β-hydroxyalkanoate-accumulating bacteria were isolated from the gut of soldiers and shown to be affiliated with Actinobacteria and Gammaproteobacteria. We noted that *Wolbachia* was detected in soldiers but not in workers. Overall, the maintenance by soldiers and workers of comparable axial and radial redox gradients in the gut is consistent with the similarities in the prokaryotes and protists comprising their microbiota. [**Int Microbiol** 2011; 14(2):83-93]

Keywords: *Reticulitermes grassei* · termite castes · termite gut microbiota · Spirochaetes · Actinobacteria · 'Endomicrobia' · *Wolbachia* · PHA-accumulating bacteria

Introduction

Termites (Dictyoptera, Isoptera) share with wood-feeding cockroaches (family Cryptocercidae; Blattaria, Dictyoptera) the unusual ability to degrade lignocellulosic plant material [4]. In the termites, the family Termitidae comprises 80% of all termite species, while the six remaining wood-eating families, often misleadingly called "lower termites", the Mastotermitidae, Kalotermitidae, Hodotermitidae, Termopsidae, Rhinotermitidae and Serritermitidae, comprise the remaining 20%. These latter six small families, which probably are still in the initial stages of evolution for several significant traits, are very important to decipher the origin of termite evolution. From this point of view, *Reticulitermes grassei*, which is the subject of the present study, is of special interest in terms of combination of ancestral and derived characters within the family Rhinotermitidae [13,25,28].

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Termites are unique among social insects because they undergo incomplete metamorphosis and display a diversified caste polyphenism. Worker, soldier, reproductive, and undifferentiated immature forms cooperate in an integrated manner in termite society. Developmental pathways are complex and vary between different termite families. In Rhinotermitidae, larvae develop into nymphs or workers. Nymphs can then develop either into (i) alates, with wings and eyes (imagoes, first form), that disperse and become primary colony founders, or (ii) brachypterous neotenic reproductives (second form), with rudimentary wings and eyes, which do not disperse but supplement or replace the reproductives within the colony. Workers can (i) become apterous neotenic reproductives (third form), with neither wings nor eyes, (ii) remain workers, or (iii) become pre-soldiers that eventually molt into soldiers [10,38,39].

The gut of wood-eating ("lower") termites contains a diverse population of prokaryotes and flagellated protists that degrade lignin, cellulose, and hemicelluloses to fermentable carbohydrates. These microbial populations are indispensable to their termite hosts. By contrast, Termitidae ("higher" termites) typically lack motile protists but maintain a complex prokaryotic community. Protists found in the digestive tract of termites and *Cryptocercus* cockroaches belong to the orders Trichomonadida, Cristamonadida, Hypermastigida, and Oxymonadida [9]. A single gut contains approximately 10^3 to 10^5 protist cells, accounting for at least 90% of the volume of the hindgut [5,19]. Each wood-feeding termite species harbors specific protists and hosts between 1 and 20 morphologically distinguishable species. A feature of the gut microbiota of wood-feeding termites is the symbiosis of bacteria and protists. The symbiotic bacteria of protists comprise diverse taxonomic groups: Spirochaetes, Bacteriodetes, candidate phylum Termite Group 1 (TG1), Deltaproteobacteria, Mycoplasma, and Synergistetes [19]. The taxonomic composition of gut bacteria has been examined in several termite species by 16S rRNA clone analysis. At present, the identified phylotypes have been classified into 15 phyla belonging to *Bacteria* and only one to *Archaea*. Euryarchaeota comprise methanogens and constitute 0–10% of the phylotypes detected [16,19]. These studies are based on gut samples from termites belonging to the worker caste.

Because of their different activities and roles in the societies, individuals of different castes could be thought at first glance to harbor different gut microbiota. However, we expected exactly the contrary since gut microbiota are suppressed by every moult and are then re-transmitted during later food exchange between workers and soldiers. Whatever the complex ontogenetic trajectory of every individual, and its current role in termite society, soldiers and workers of *R. grassei* may be thus expected to harbor similar microbiota.

In this work, we compared the whole-gut microbiota of soldier and worker castes from the wood-feeding termite *Reticulitermes grassei* to determine whether, in the same physiological state, the compositions of their microbiota are also the same. A previous article by Hongoh et al. [17] compared the gut bacterial microbiota among castes of the Termitidae termite *Macrotermes gilvus*. Using terminal restriction fragment length polymorphism (T-RFLP), they showed that molecular community profiles are more closely related to differences in age than in caste. In this context, the present study is the first description and inventory of the gut microbiota of soldier and worker castes from *R. grassei*, a European wood-eating Rhinotermitidae termite.

Materials and methods

Termites. Individuals of *Reticulitermes grassei* (Rhinotermitidae) were obtained from the laboratory of Dr. Miquel Gaju (University of Cordoba, Spain). Infested wood samples were kept in boxes at room temperature. Soldier and worker castes were used in this study.

Isolation of bacterial DNA. The whole gut of the insects was removed and then homogenized using a FastPrep System (MP Biomedicals Europe) with 0.1-mm glass beads. Bulk DNA was extracted using a PureLink genomic DNA purification kit (Invitrogen, San Diego, CA, USA). Ten individuals of the soldier caste and ten of the worker caste were analyzed.

PCR and cloning. PCR was performed with the Bacteria-specific primer pair 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1391R (5′-GACGGGCGGTGWGTRCA-3′) [4]. PCR amplification was carried out in a 50-µl reaction mix containing 1 µl DNA template, 20 pmol of each primer, 40 nmol dNTPs, 1.5 mM $MgCl_2$ and 1 U Taq platinum polymerase (Invitrogen). Samples were pre-heated at 94°C for 9 min followed by 30 cycles of denaturation at 94°C for 45 s, annealing at 51°C for 30 s, and elongation at 72°C for 1.5 min, and finally an elongation step at 72°C for 15 min. The PCR amplification products were examined by electrophoresis in a 1% agarose gel. DNA was stained with ethidium bromide and visualized under short-wavelength UV light. Purification and cloning of the PCR products were done, respectively, using a Pure Link kit (Invitrogen) and the TOPO TA cloning kit (Invitrogen) according to the manufacturers' recommendations.

Sequencing and phylogenetic analysis. Purified PCR products were sequenced and analyzed as described in Berlanga et al. [4]. The primer used for 16S rRNA sequencing of *Bacteria* was 515F (5′-GTGCCAGCMG CCGCCGCGGTAA-3′). PCR mixtures consisted of 2 µl PCR product, 1 µl primer at 5 µM, 1 µl BigDye, and 3 µl BigDye solution in a final volume of 20 µl. Cycle sequencing was done using an ABI 9700, with one cycle at 96°C for 1 min, 25 cycles of denaturation at 96°C for 10 s, annealing at 55°C for 5 s, and extension at 60°C for 4 min. Sequencing reactions were run on an ABI 3100 DNA sequencer. Partial 16S rRNA sequences (ca. 850 bp) were compared to known sequences in GenBank using the advanced gapped BLAST (basic local alignment search tool) algorithm. Phylogenetic analyses

were carried out with MEGA version 2.1. The dendrogram was constructed using the neighbor-joining algorithm and the p-distance estimation method. One thousand bootstrap trees were generated, and bootstrap confidence levels were determined using the MEGA 2.1 program. Chimeric sequences were identified according to the chimera check program Mallard v1.09.

Nucleotide sequence accession numbers. Partial 16S rRNA gene sequences of clones representing novel phylotypes defined in this study and published sequences are available for electronic retrieval from the EMBL, GenBank nucleotide sequence databases (JN703741–JN703777).

Isolation of poly-β-hydroxyalkanoates(PHA)-accumulating heterotrophic bacteria from the gut of *R. grassei* soldiers. The whole gut was removed from three termites and homogenized in 1 ml of Ringer's ¼. A dilution series was made in Ringer's ¼ to obtain 30–300 colony-forming units (CFU) per plate. Aliquots of 0.1 ml were plated onto trypticase soy agar (TSA) (Scharlau, Barcelona, Spain). The plates were incubated for 24–48 h at 30ºC. Colonies that developed on agar were differentiated by color, elevation, form, and edge appearance. Isolates of axenic colonies were randomly picked and cultured in solid mineral salt medium (MSM) containing (in g/l): agar 15, $Na_2HPO_4 \cdot 7H_2O$ 6.7, NaCl 10, KH_2PO_4 1.5, NH_4Cl 0.1, $MgSO_4 \cdot 7H_2O$ 0.2, $CaCl_2$ 0.01, ferrous ammonium citrate 0.06, and 1 ml trace elements. MSM was supplemented with 5 g glucose/l and 0.5 mg Nile red (Sigma, St. Louis, MO, USA)/ml (final concentration), dissolved in dimethylsulfoxide [2]. Petri dishes were incubated for 4–5 days at 30ºC. Nile red produces orange fluorescence when it binds to polymer granules in the cells [34]. We selected ten strains that exhibited fluorescence and thus potentially accumulate PHA. Strains were grown in trypticase soy broth (TSB) (Sharlau) overnight at 30°C. DNA was extracted using the PureLink genomic DNA purification kit (Invitrogen). The strains were identified by partial sequencing of their 16S RNA, carried out using the BigDye V3.1 solution (Applied Biosystems). Partial 16S rRNA sequences were compared to known sequences in GenBank.

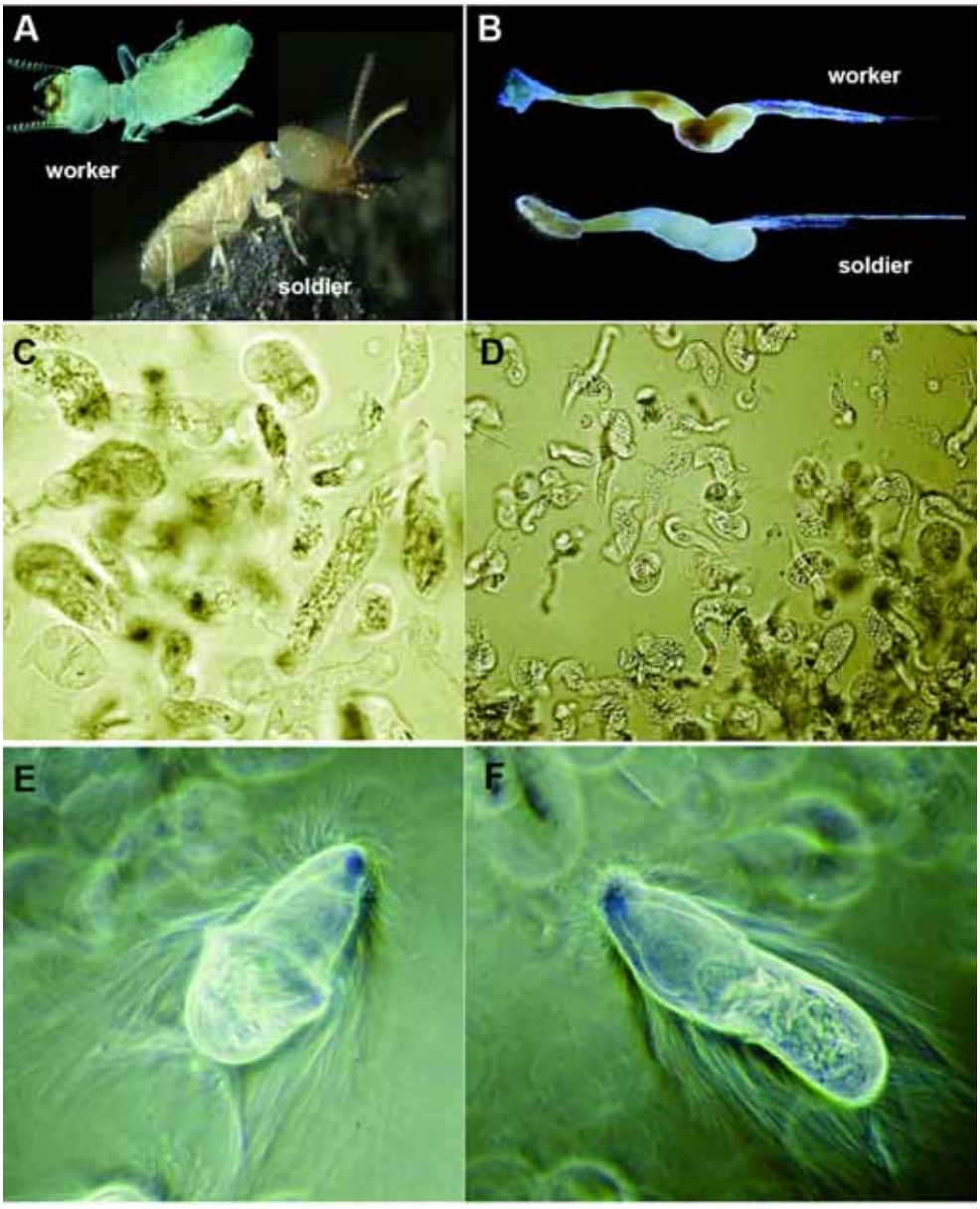

Fig. 1. (**A**) External appearance of the termite *Reticulitermes grassei*, soldier and worker castes. (**B**) Comparison of the intestinal tract of soldier and worker castes. (**C**) Microbiota from the whole-gut of a worker caste, and (**D**) that of a soldier caste. (**E**) A protist from a worker gut, and (**F**) a protist from a soldier hindgut. (Photographs by R. Duro.)

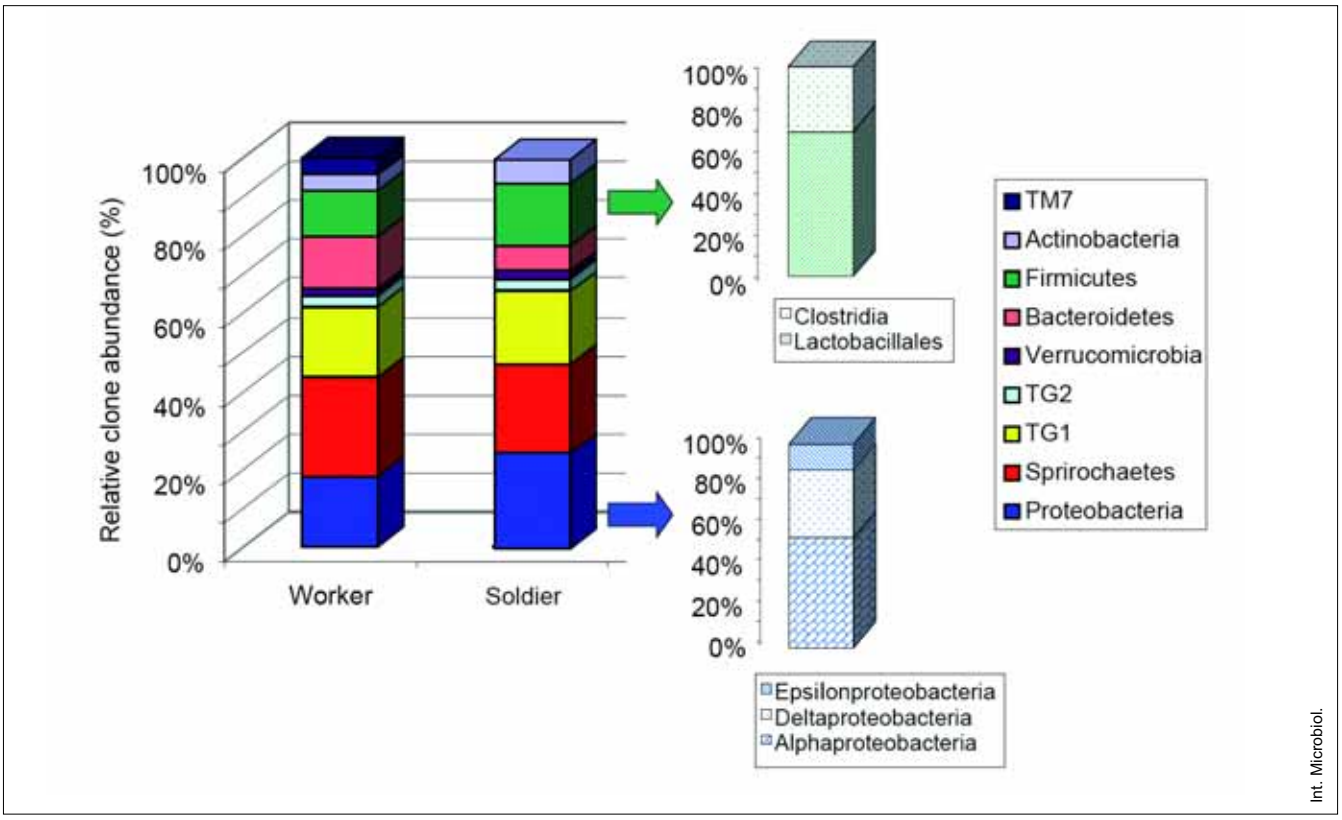

Fig. 2. Relative clone abundance (%) of bacterial phylotypes from the whole gut of soldier and worker castes of *R. grassei*.

Results

Protists of the guts of soldiers and workers from *R. grassei*. Whole guts of *R. grassei* soldiers and workers were approximately 4 mm long and 0.5–1 mm in diameter at their widest point, which was the paunch region. The intestinal tract of soldiers was slightly different in appearance from that of workers (Fig. 1). Contrast-phase microscopy of the paunch contents of the soldier gut revealed an abundance of the bacteria and protists typically associated with wood-eating termites (Fig. 1C,D). Based on a comparison of several photographs of the intestinal tracts of soldiers and workers, similar relative protist morphotypes abundances were determined, in accordance with previous studies in *Reticulitermes flavipes* [26].

Bacterial microbiota of the guts of soldiers and workers from *R. grassei*. Analysis of the 16S rRNA clone library provided a relative census of the bacterial microbiota of the whole gut of soldiers from *R. grassei*. The clones were affiliated with the phyla Spirochaetes, Proteobacteria, Firmicutes, Bacteroidetes, Actinobacteria, Synergistetes, Verrucomicrobia, candidate phylum TG1, and candidate phylum Termite Group 2 (TG2). Among the clones analyzed, no significant differences in the relative abundances of phyla were observed between soldiers and workers. The exception was Alphaproteobacteria (Fig. 2), as some of the alphaproteobacterial clones belonging to the genus *Wolbachia* were detected in clones from soldiers but not from those of workers. The predominant Firmicutes were lactobacilli (class Bacillales, subclass Lactobacillales).

The phylotypes obtained were compared with reported sequences from other termites, especially sequences related to Spirochaetes, TG1, *Wolbachia* and Actinobacteria. Except for Actinobacteria, these phyla form one or more monophyletic clusters within each bacterial phylum that coevolved with its termite host [3,16,30,35,42].

Spirochaetes. From the whole-gut communities of soldiers (CRgS phylotypes) and workers (CRgW phylotypes), 125 and 55 clones (16S rDNA cloning libraries), respectively, were analyzed. Of these, 28 and 14, respectively, were attributable to Spirochaetes. Several phylotypes were detected

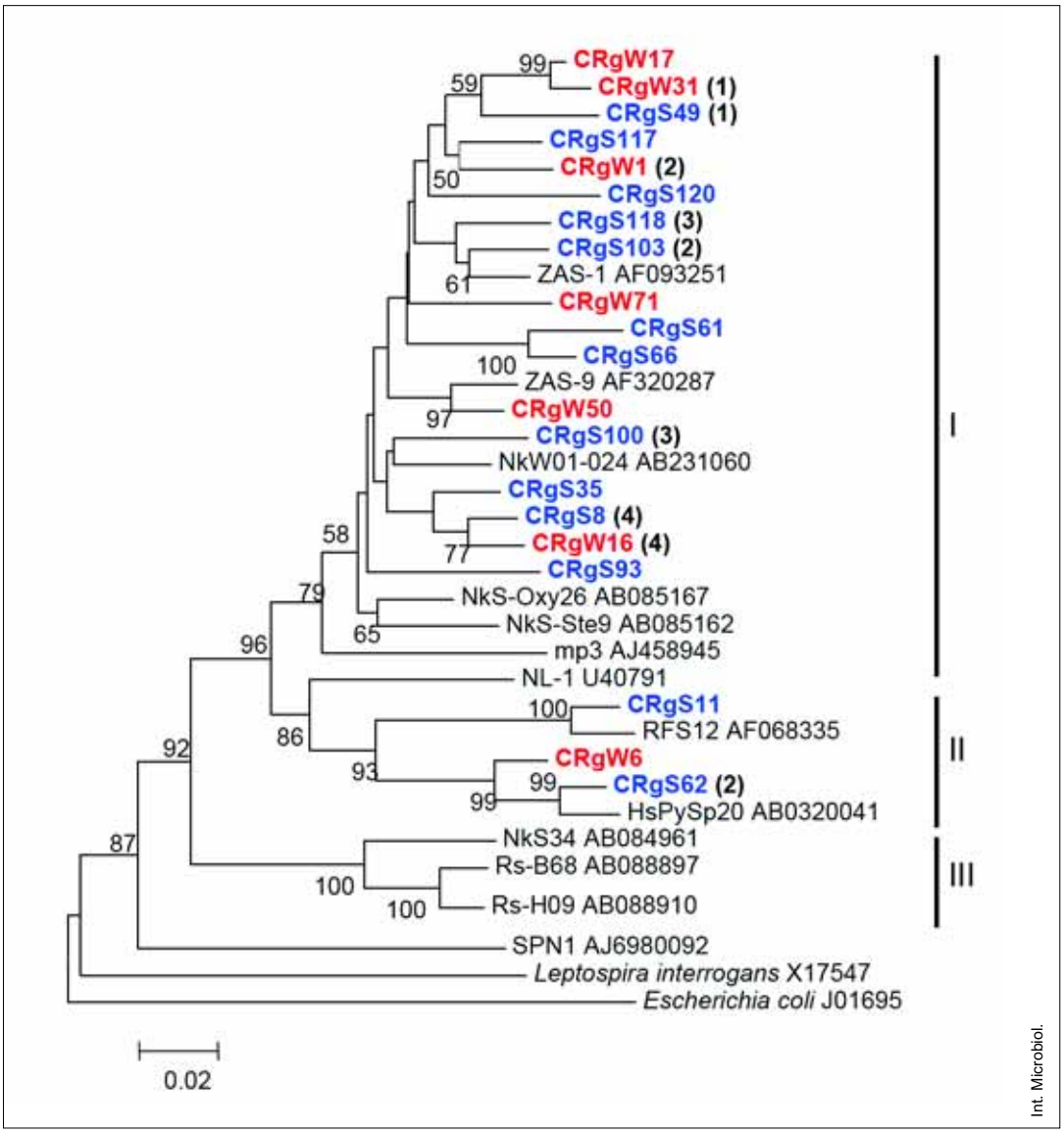

Fig. 3. Phylogenetic tree of the 16S rDNA partial sequences of spirochetes. *Treponema* clusters I, II, and III are indicated on the right side of the tree. In blue, phylotypes CRgS from the *R. grassei* soldier caste; in red, CRgW, clones from the worker caste. In parentheses, the number of phylotypes found repeatedly (clones sharing >97% sequence identity were grouped into the same phylotype). One thousand bootstrap trees were generated, and bootstrap confidence levels, as percentages (only values >50%), are shown at tree nodes. Bar = 0.02 difference in a nucleotide sequence.

repeatedly (Fig. 3, number in parentheses after the phylotype). Almost all phylotypes grouped with *Treponema* cluster I; five phylotypes were assigned to *Treponema* cluster II and one phylotype was affiliated with *Treponema* cluster III (affiliated with the genus *Spirochaeta*) [2]. There were no differences in the grouping of spirochetal phylotypes from soldiers and workers. Cluster I comprises both ectosymbionts attached to protists and free-swimming gut spirochetes [2,29]. Based on the affiliation with reported sequences, phylotypes CRgW50, CRgS103 and CRgS118 were considered to be free-swimming spirochetes because they grouped with the culturable spirochetes *Treponema primitia* ZAS-1 and *T. azotonutricium* ZAS-9 (from *Zootermopsis angusticollis*) [12]. Members of cluster II are ectosymbiotic spirochetes of oxymonad protists and were originally described in *Reticulitermes speratus* and *Hodotermopsis sjoestedti*) [30].

Termite group 1. Microorganisms of TG1 are regularly encountered in the guts of termites and wood-feeding cockroaches, but they are also present in many other habitats [14].

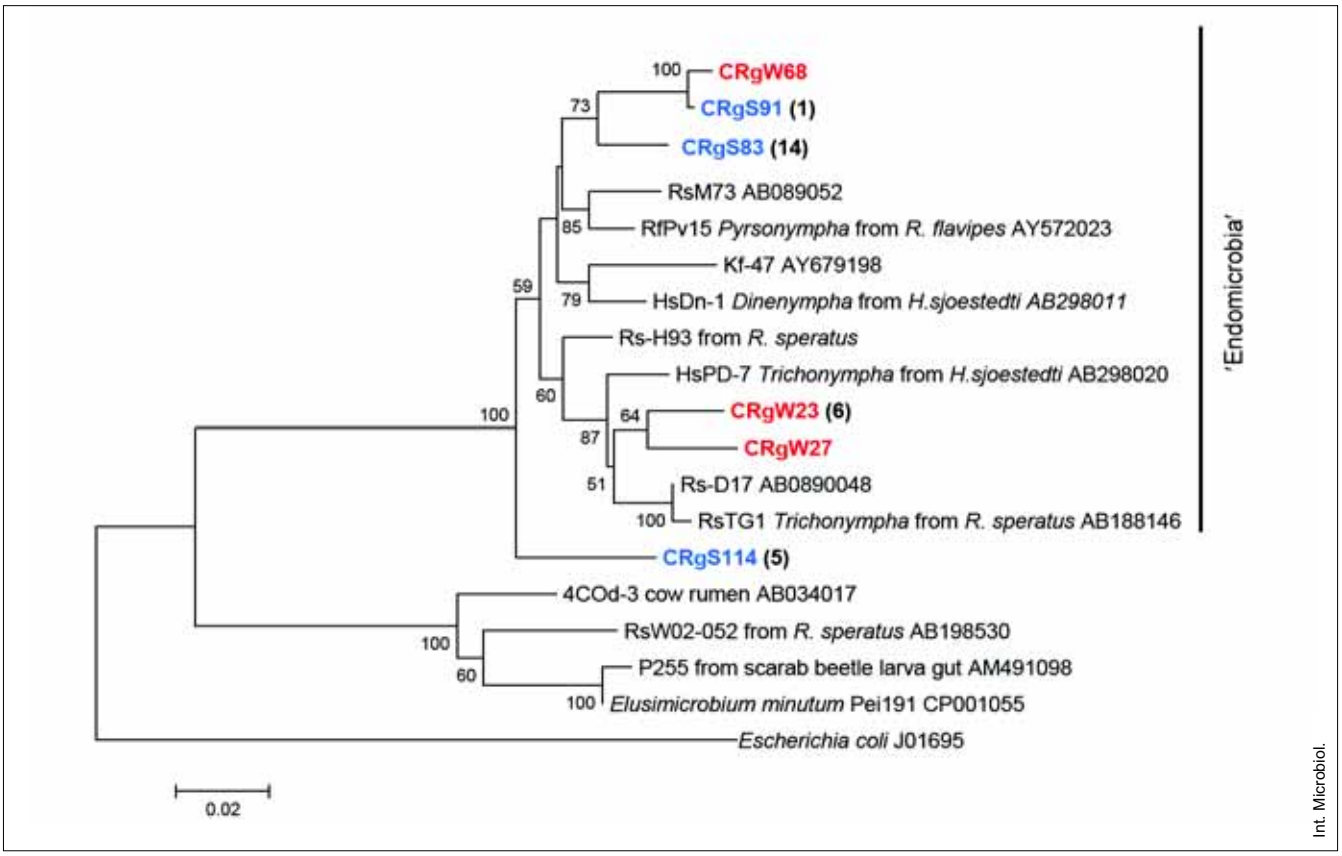

Fig. 4. Phylogenetic tree of the 16S rDNA partial sequences of 'Endomicrobia,' a group belonging to the candidate phylum Termite Group I (TG1). In blue, phylotype CRgS from *R. grassei* soldier termites; in red, phylotype CRgW from worker termites. In parentheses, the number of phylotypes found repeatedly. One thousand bootstrap trees were generated, and bootstrap confidence levels, as percentages (only values >50%), are shown at tree nodes. Bar = 0.02 difference in a nucleotide sequence.

The name 'Endomicrobia' has been proposed for TG1 bacteria that are intracellular symbionts of flagellate protists found exclusively in the guts of termites and wood-feeding cockroaches [21]. Phylotypes CRgS83 (high relative abundance with 12 repeated phylotypes), CRgS91, and CRgW68 grouped with endosymbionts from the protist *Pyrsonympha*. CRgS91 (soldier caste) and CRgW68 (worker caste) were the same phylotype (99% homology). These results again corroborated the absence of significant differences between the gut microbiota of soldiers and workers. Phylotypes CRgW23 and CRgW27 were affiliated with endosymbionts from the protist *Trichonympha*. Phylotype CRgS114 did not cluster with either 'Endomicrobia' or 'Elusimicrobia,' a new group representative of the TG1 phylum and consisting of sequences derived from invertebrate guts and bovine rumen [15] (Fig. 4).

Alphaproteobacteria. The genus *Wolbachia* (class Alphaproteobacteria) encompasses obligate intracellular bacteria that are cytoplasmically transmitted in arthropods and filarial nematodes [27]. Based on the 16S rRNA gene phylogenies of *Wolbachia,* there are eight major clades (A–H). Clades A and B include most of the parasitic *Wolbachia* found in arthropods, clades C and D the majority of the mutualistic W*olbachia* present in filarial nematodes, and clades E–H *Wolbachia* from various arthropods. For clades E–H, the host effects are currently unknown; clade F is notable in that its members infect arthropods—especially termites—, but also nematodes [27].

Phylotypes obtained in this work from soldiers of *R. grassei* were affiliated with clade F (Fig. 5). No clones affiliated with *Wolbachia* were detected in the clone library obtained from workers.

Actinobacteria. Members of actinobacteria are present in the intestinal tract of termites although these bacteria have not strictly co-speciated with their termite hosts. They do not form one or more monophyletic clusters, unlike spirochetes

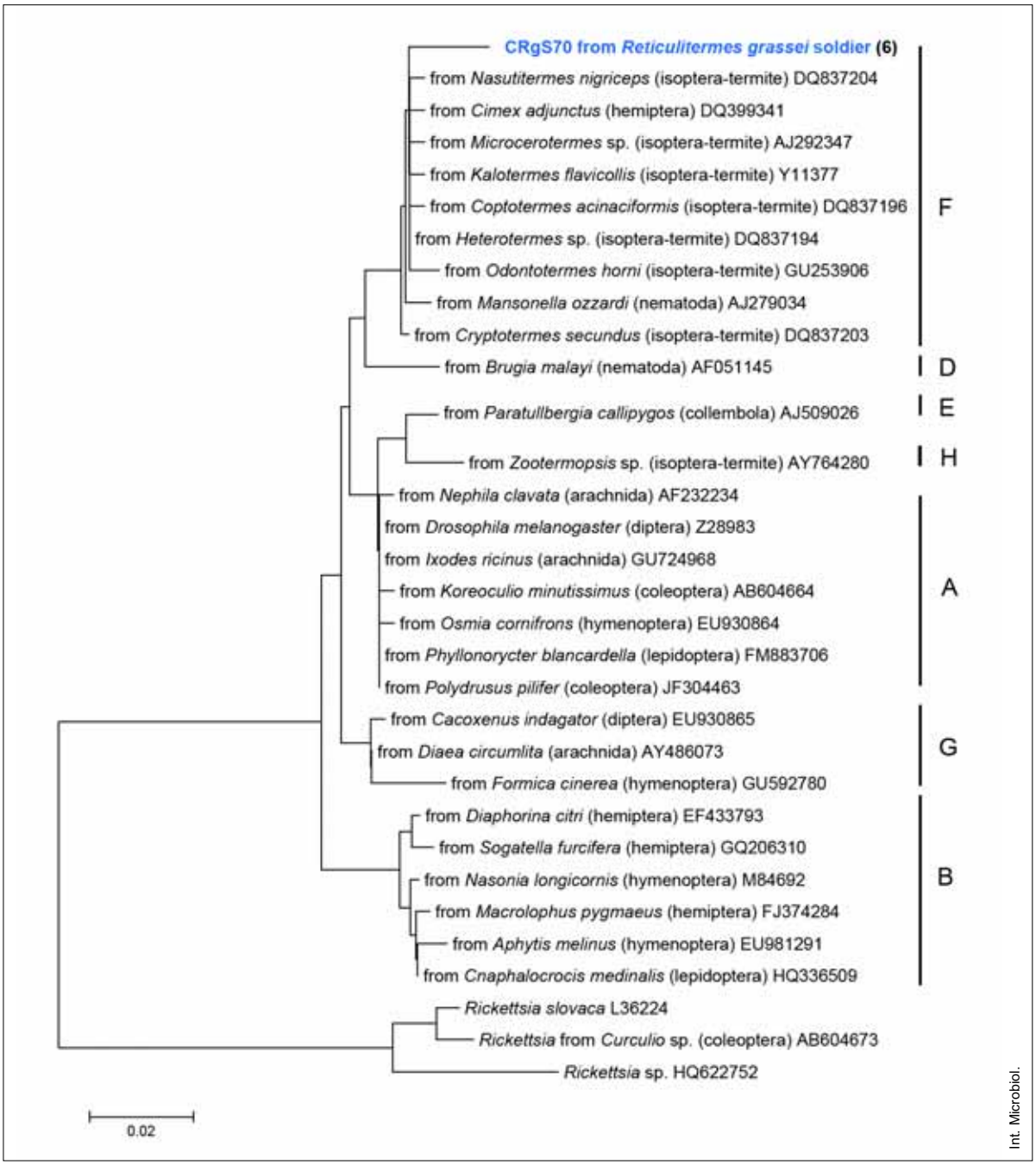

Fig. 5. Phylogenetic tree of the 16S rRNA partial sequences of *Wolbachia*. *Wolbachia* clades are indicated on the right side of the tree. In blue, phylotype CRgS70, isolated repeatedly (6 clones) from the *R. grassei* soldier caste. This clone was compared with other *Wolbachia* sequences detected in several host species. One thousand bootstrap trees were generated, and bootstrap confidence levels, as percentages (only values >50%), are shown at tree nodes. Bar = 0.02 difference in a nucleotide sequence.

or 'Endomicrobia.' In this study, both the clone-library phylotypes and strains isolated from the whole gut of *R. grassei* soldiers grouped with the genera *Microbacterium* (CRgS47, RgS-A1, RgS-A7, RgS-A12) and *Rhodococcus* (CRgS72, RgS-A2, RgS-A9, RgS-A8) (Fig. 6).

Isolation of PHA-accumulating heterotrophic bacteria from the gut of *R. grassei* soldiers. Ten strains that potentially accumulate PHA were selected for further study (see Materials and methods). Strains RgS-A1, RgSA7, and RgS-A12, belonging to the genera *Microbac-*

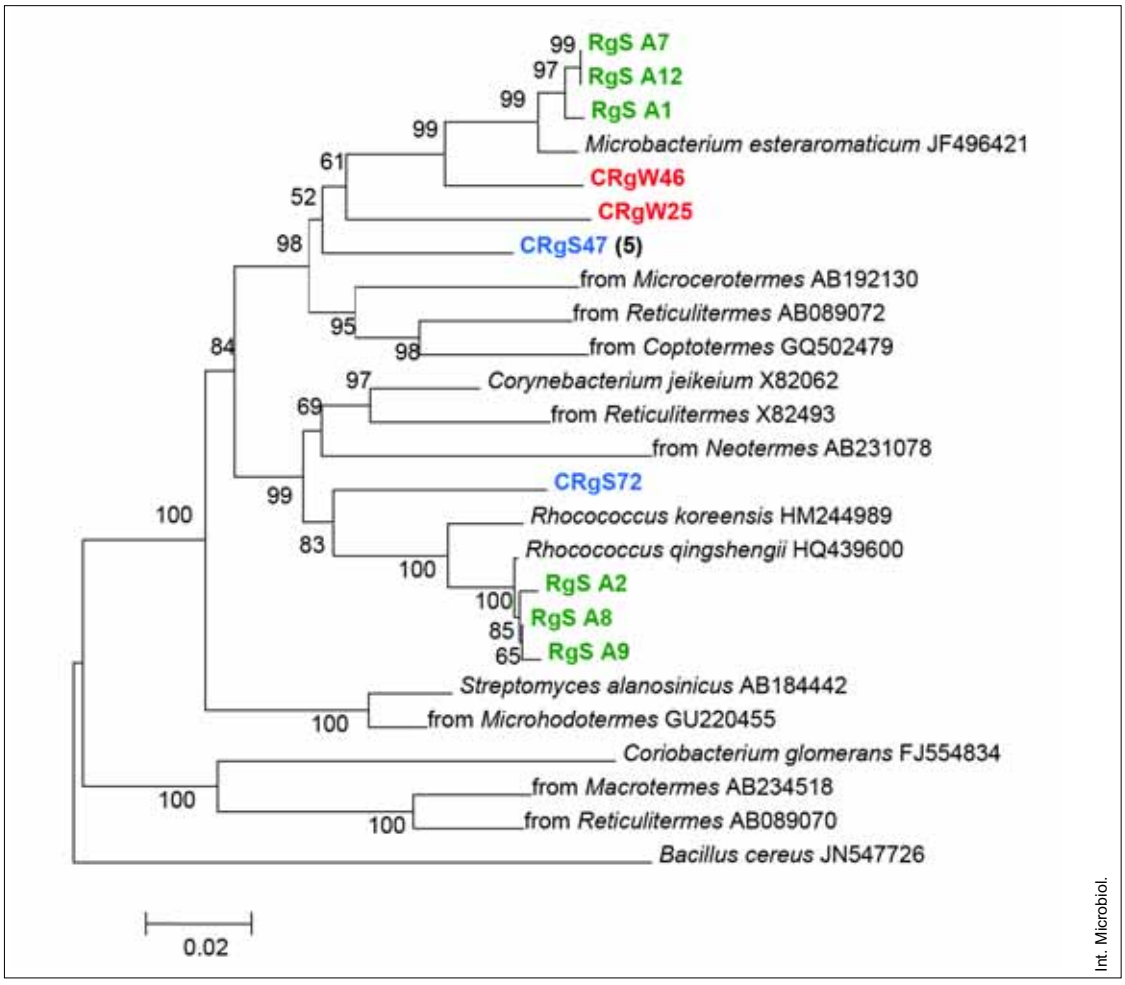

Fig. 6. Phylogenetic tree of the 16S rDNA partial sequences of Actinobacteria. In blue, phylotypes CRgS isolated from the *R. grassei* soldier caste; in red, CRgW from the worker caste; in green, RgS-A from isolates in axenic culture. In parentheses, the number of phylotypes found repeatedly. One thousand bootstrap trees were generated, and bootstrap confidence levels, as percentages (only values >50%), are shown at tree nodes. Bar = 0.02 difference in a nucleotide sequence.

terium (actinobacteria), showed 99.827% homology with *Microbacterium profundi* and 99% homology with each other. Three other isolates, RgS-A2, RgS-A8, and RgS9 (99.0% homology with each other), were shown to be affiliated with *Rhodococcus qingshengii* (99.524%, Actinobacteria). Four isolates (RgS-A3, RgS-A5, RgS-A6 and RgS-A11) grouped with *Enterobacter mori* (99.519% homology), a Gammaproteobacteria. Note that this group was not detected molecularly, i.e., by clone-library sequencing.

Discussion

Termites are eusocial insects that display distinct castes and life stages, including reproductive, soldier, and worker forms. Each caste plays a significant role within the colony. Reproductives maintain colony population; soldiers protect the colony from invaders; workers, the most numerous life stage in a colony, build and maintain the galleries, mind the larvae, and feed other colony members. Workers transfer food stomodeally (regurgitated) and/or proctodeally (hindgut contents). Microorganisms are vertically transmitted from workers to other individuals of the colony via proctodeal trophallaxis.

Since, in insects, the proctodeal part of the intestine, i.e., the hindgut, is shed during ecdysis, the gut microbiota of newly molted termite workers and soldiers depends on the contributions of fellow workers to restore the protists lost during molting. Soldiers have a specialized morphology allowing colony defense, and their feeding activity is

dependent on other colony members. While young soldiers, before they develop large mandibles, can chew wood, adult soldiers, owing to their large mandibles, cannot, although they are able to digest proctodeal wood particles that have passed through the gut of workers and are thus partially digested. Consequently, in wood-eating termites, the gut of soldiers, like that of workers, harbors protists and bacteria throughout the entire termite life cycle. In *R. grassei*, the relative protist abundances are similar in soldiers and workers, as shown in previous studies in *R. flavipes* [26].

Proctodeal trophallaxis explains the observed similarities in the microbiota of soldier and worker castes. Analysis of the 16S rRNA clone library showed affiliations with the phyla Firmicutes, Bacteroidetes. Proteobacteria, Actinobacteria, Spirochaetes, Synergistetes, Verrucomicrobia, and the candidate phyla TG1 and TG2 (Fig. 2). The phylotypes obtained in this study were compared with reported sequences from other termites, especially those related to Spirochaetes, TG1, *Wolbachia*, and Actinobacteria (Figs. 3–6). Spirochetes are specific symbionts that have coevolved with termites, are stably harbored, and are closely related with a particular termite species but also within the same termite family [2]. In the termite gut, spirochetes can be observed both as free-swimming bacteria in the lumen and as protists-ectosymbionts [29].

Termites also support characteristic communities of gut protists. Furthermore, many protist species are simultaneously associated with different bacterial ectosymbionts, and common spirochetal phylotypes are often shared among different protist species [1,29]. Indeed, ectosymbiotic spirochetes associated with a single protist include at least three phylotypes (species) of spirochetes [20]. This relationship between protists and spirochetes is completely different from the symbiosis of 'Endomicrobia.' Although part of the TG1 phylum, 'Endomicrobia' endosymbionts are host-specific intracellular symbionts of termites and *Cryptocercus* gut flagellates, and 'Endomicrobia' sequences from each flagellate host represent distinct phylotypes. These observations support the hypothesis that the diversity of 'Endomicrobia' reflects the diversity of their flagellate hosts [21,36]. In this study, 'Endomicrobia' from the protists *Pyrsonympha* and *Trichonympha* were detected (Fig. 4). The complete genome sequence of the 'Endomicrobia' endosymbionts suggests a capacity to provide amino acids and cofactors to the host flagellate [18].

The genus *Wolbachia* (Alphaproteobacteria) includes obligate intracellular bacteria that are cytoplasmically transmitted in arthropods and filarial nematodes. *Wolbachia* comprises both mutualistic and parasitic lineages grouped in eight major clades [27]. Basically, two phylogenetically diverse types of *Wolbachia* infect Isoptera (termites), clade F and clade H. In this study, all phylotypes detected, only in the soldier caste of *R. grassei,* were affiliated with clade F (Fig. 5). *Wolbachia* clade F infects termites species that are phylogenetically "recent," while clade H is found in the phylogenetically "old" genera [27]. *Wolbachia* are associated with four distinct reproductive phenotypes in a wide range of Arthropoda: feminization, parthenogenesis, male killing, and cytoplasmic incompatibility. However, nothing is known about possible phenotypes linked to *Wolbachia* in Isoptera [42].

Actinobacteria are frequently studied because of their diverse metabolic capabilities. One of the environments that has been explored for the isolation of Actinobacteria is the termite gut [24,41]. In this study, clones detected and isolated from Actinobacteria were grouped into *Microbacterium* and *Rhodococcus* genera. Traditionally, Actinobacteria is a group that is always present in the termite gut and in the environment of the colony (environmental colonization). Nevertheless, recent studies have shown that there is no significant overlap between the gut microbiota of soil-feeding termites and the surrounding soils used as natural food [11,24]. Functional metagenomic analysis of bacteria from the Termitidae termites *Nasutitermes ephratae* and *N. corniger* identified two groups of bacteria, Fibrobacteres and Spirochaetes (major groups involved in the degradation of lignocellulose) [40]; however, other microorganisms with cellulase activities, such as Actinobacteria, may also play a role in the degradation of these products [23].

The accumulation of intracellular storage polymers such as poly-β-hydroxyalkanoates (PHA) is a bacterial strategy that increases survival in a changing environment [22,33]. PHA serves as an endogenous source of carbon and energy during starvation. PHA accumulation indicates an environment suitable for bacteria, one that contains high concentrations of organic carbon sources [37]. Among the strains present in the gut of *R. grassei* soldiers, ten that potentially accumulate PHA were selected: Strains RgS-A1, RgSA7 and Rgs-A12 belonged to the genera *Microbacterium* (actinobacteria); RgS-A2, RgS-A8 and RgS9 were affiliated with *Rhodococcus qingshengii* (Actinobacteria); and RgS-A3, RgS-A5, RgS-A6, and RgS-A11 grouped with the Gammaproteobacteria *Enterobacter*. Actinobacteria strains might provide lignocellulosic degradation products as a renewable carbon source for PHA production. However, further studies are needed to metabolically characterize these isolates with respect to their function in the termite intestinal tract and to explore their potential industrial applications [23].

The guts of wood-feeding termites typically contain hundreds of microbial species [31] that degrade lignocellulose into usable catabolites and are able to provide nitrogen to the host, whose food is typically low in nitrogen. Elucidation of the roles played by bacteria, flagellates, and the animal host is a first but important step in understanding the complex interactions in this tripartite symbiosis. The insect gut is surrounded by aerobic tissues aerated by the insect's tracheal system. Oxygen penetrates the peripheral hindgut to a depth of up to 150–200 mm below the epithelium. Oxygen removal by the respiratory activity of the gut microbiota creates a microoxic periphery around an anoxic center [6,7]. A stable, active physiological state supports high microbial diversity, probably to maintain a sharp O_2–H_2 gradient in the gut. Hydrogen is the central free intermediate in termite-gut lignocellulose digestion. In wood-eating termites, H_2 formation is mainly attributed to the dense populations of cellulolytic flagellates and to bacteria such as spirochetes. The main compounds produced by spirochetes are acetate (acetogenesis), H_2, and CO_2. Termite-gut treponemes (*Treponema primitia* ZAS-2 and *Treponema azotonutricium* ZAS-9) isolated from the termite *Zootermopsis angusticollis* and grown together in vitro as a consortium have been shown to express several genes that are beneficial to the host. Although the functional significance of most of these changes in gene expression are not yet understood, they are no doubt representative of the broad, comprehensive, and mutualistic interactions between closely related, co-resident gut symbionts [32].

The present work indicates that, in *R. grassei*, the microbiota of both the soldier and the worker castes are similar. The fact that adult soldiers, although unable to eat whole wood, partially digest degraded wood particles that have passed through the gut of workers (proctodeal trophallaxis) could explain such similarity. During molting, the hindgut is shed and the newly molted soldiers acquire bacteria and protists from workers. Although initial innoculation depends on workers, the maintenance of a similar microbiota by soldiers suggests that soldiers' gut should have similar physiological conditions (redox potential, O_2–H_2 gradients, etc.), and that they do digest partially degraded lignocellulosic material provided by the workers.

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