Antonie van Leeuwenhoek (2013) 104:869–883
DOI 10.1007/s10482-013-0001-7

ORIGINAL PAPER

16S-rRNA-based analysis of bacterial diversity in the gut of fungus-cultivating termites (*Microtermes* and *Odontotermes* species)

Huxley M. Makonde · Hamadi I. Boga · Zipporah Osiemo · Romano Mwirichia · Lucy M. Mackenzie · Markus Göker · Hans-Peter Klenk

Received: 6 February 2013 / Accepted: 8 August 2013 / Published online: 14 August 2013

Abstract The interaction between termites and their gut symbionts has continued to attract the curiosity of researchers over time. The aim of this study was to characterize and compare the bacterial diversity and community structure in the guts of three termites (*Odontotermes somaliensis, Odontotermes* sp. and *Microtermes* sp.) using 16S rRNA gene sequencing of clone libraries. Clone libraries were screened by restriction fragment length polymorphism and representative clones from *O. somaliensis* (100 out of 330 clones), *Odontotermes* sp. (100 out of 359 clones) and *Microtermes* sp. (96 out 336 clones) were sequenced. Phylogenetic analysis indicated seven bacterial phyla were represented: *Bacteroidetes*, *Spirochaetes*, *Firmicutes*, *Proteobacteria*, *Synergistetes*, *Planctomycetes* and *Actinobacteria*. Sequences representing the phylum *Bacteroidetes* (>60 %) were the most abundant group in *Odontotermes* while those of *Spirochaetes* (29 %) and *Firmicutes* (23 %) were the abundant groups in *Microtermes.* The gut bacterial community structure within the two *Odontotermes* species investigated here was almost identical at the phylum level, but the *Microtermes* sp. had a unique bacterial community structure. Bacterial diversity was higher in *Odontotermes* than in *Microtermes.* The affiliation and clustering of the sequences, often with those from other termites' guts, indicate a majority of the gut bacteria are autochthonous having mutualistic relationships with their hosts. The findings underscore the presence of termite-specific bacterial lineages, the majority of which are still uncultured.

Keywords Termites-symbiont mutualism · 16S rRNA gene · Macrotermitinae · Biodiversity

Electronic supplementary material The online version of this article (doi:10.1007/s10482-013-0001-7) contains supplementary material, which is available to authorized users.

H. M. Makonde (✉) · M. Göker · H.-P. Klenk
Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstraße 7b, 38124 Brunswick, Germany
e-mail: huxcly@yahoo.com; huxleymakonde@gmail.com

H. M. Makonde · H. I. Boga · Z. Osiemo · R. Mwirichia
Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000, Nairobi, Kenya

H. M. Makonde
Technical University of Mombasa, P.O. Box 90420-80100, Tom Mboya Street, Mombasa, Kenya

L. M. Mackenzie
Institute of Infection and Global Health, University of Liverpool, Liverpool, Merseyside, UK

Introduction

Termites play diverse roles in semi-arid and humid ecosystems and have an impact on the turnover of organic matter in tropical and subtropical regions. The

microbiota associated with termites are crucial for the degradation of recalcitrant components of plant biomass and this has a major influence on soil structure and carbon mineralization (Brune and Friedrich 2000; Ohkuma et al. 2003). Termites rely on microbes in their guts that are exchanged between colony members and transferred to the next generation through trophallaxis. These microbes assist termites in breaking down lignocellulose (Brune and Ohkuma 2011). The isolation and cultivation of several bacterial strains from termite guts has partially enabled their classification as decomposers of lignocellulose, uric acid and/or other aromatic compounds; as nitrogen-fixers; and/or as H_2/CO_2-acetogens (Breznak 2000). However, the majority of the microbial species are difficult or not currently possible to cultivate, thus limiting our understanding of their role in the gut ecosystem (Breznak 2000).

Molecular approaches based on 16S rRNA gene analyses have been helpful for assessing the microbial diversity in termites without cultivation (Schmitt-Wagner et al. 2003; Hongoh et al. 2003, 2006; Shinzato et al. 2005, 2007; Fisher et al. 2007; Long et al. 2010; Mackenzie et al. 2007; Mathew et al. 2012; Schauer et al. 2012; Köhler et al. 2012). These studies not only revealed high bacterial diversity in the guts, but also termite-specific bacterial lineages (Hongoh et al. 2003; Shinzato et al. 2005). Moreover, metagenomic studies yielded key information on bacterial genes required for fermentation, lignocellulosic digestion, reductive acetogenesis, and nitrogen fixation within the host-symbiont association (Warnecke et al. 2007; Tartar et al. 2009; Mattéotti et al. 2011; Liu et al. 2011; Köhler et al. 2012). Although such studies have greatly expanded our knowledge of gut symbiosis, the evolutionary relationships between symbionts and host termites is still inadequately addressed owing to their enormous diversity (Shinzato et al. 2007).

The *Macrotermitinae* originate from Africa and comprise many of the economically important termite species (Ahmed et al. 2011). Their high abundance and cryptic and unpredictable foraging patterns, coupled with the diversity of their gut symbionts, challenge the development of termite control strategies (Ahmed et al. 2011). Although comprehensive studies have been conducted on these termites (Hongoh et al. 2006; Shinzato et al. 2007; Long et al. 2010; Mackenzie et al. 2007; Mathew et al. 2012; Zhu et al. 2012), information on the gut microbial diversity of the members of the genus *Odontotermes* is still inadequate. Furthermore, such information is lacking entirely for the members of the genus *Microtermes* on account of its rarity and lack of adequate termite experts in Africa (Ahmed et al. 2011). Therefore, in this study, we characterize the gut bacterial diversity and compare its community structure and specificity in three termites representing these genera. The findings will extend the clone-based inventories of the termite gut microbiota and contribute to understanding of the specificity and mutualistic relationship of gut symbionts with their hosts.

Materials and methods

Collection and identification of termites

Samples were collected in March, 2011 from Thika district, Kenya (latitude 1°5′54.68″N, longitude 37°1′1.10″W). Termite mounds [C and C1, approximately 0.5 km far apart, were colonized by *Odontotermes* sp. (JQ247986) and *O. somaliensis* (JQ247985), respectively; mound B, located approximately 2 km from mound C, was colonized by *Microtermes* sp. (JQ247991)] were excavated to a depth of 0.5–1.0 m as described elsewhere (Makonde et al. 2013). Termites ($n = 200$ workers and 50 soldiers) were sampled into sterile plastic boxes. Worker-caste termites were used in the experiments due to their foraging behaviour during establishment and renewal of the fungus gardens. The identity of the termites was confirmed by sequencing the mitochondrial cytochrome oxidase II gene in DNA extracted from the heads of soldiers (Austin et al. 2004; Makonde et al. 2013) and comparing it to the sequences of previously identified specimens (Inward et al. 2007).

DNA extraction and PCR amplification

The exterior surfaces of the termites were washed with 70 % ethanol and then rinsed with sterile distilled water. The guts were aseptically removed with forceps (Schmitt-Wagner et al. 2003). A total of 26 guts (approximately 144 mg) from *O. somaliensis* and *Odontotermes* sp. (JQ247986) and 74 guts (approximately 143 mg) from *Microtermes* sp. (JQ247990) were put separately into three sterile micro tubes

containing 0.2 ml of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0). They were then homogenized using a sterile glass rod. The corresponding homogenates were then transferred into sterile tubes and used for total DNA extraction using the UltraClean®. Mega soil DNA isolation kit (MO BIO Laboratories, Inc.) according to the manufacturer's protocol.

Clone libraries, construction and sequencing

Purified DNA was quantified photometrically (NanoDrop; Thermo Fisher Scientific, Schwerte, Germany) and used as a template for amplification of 16S rRNA genes using the universal bacterial primers (27F 5′-TAG AGT TTG ATC CTG GCT CAG-3′ forward primer and 1392R 5′-GAC GGG CGG TGT GTA CA-3′ reverse primer) according to their position in relation to the *Escherichia coli* gene sequence (Lane 1991). For each PCR, 1 µl (25 ng/µl) of the template was mixed with TaKaRa Ex Taq™ HS (5 units/µl) according to the manufacturer's protocol. The PCR conditions were as described by Mackenzie et al. 2007 except the final extension was at 72 °C for 10 min. PCR product size was checked on 1 % agarose gels stained with ethidium bromide. The amplicons were gel purified using the Macherey-Nagel NucleoSpin extract II kit (740609.50) and eluted in 30 µl of TE Buffer (5 mM, pH 8.0). The quality of the purified PCR product was determined through electrophoresis on a 1 % agarose gel.

Purified PCR products were ligated into pGEM-T® Easy vector system II (Promega) and transfected through heat shock into *E. coli* JM109 high efficiency competent cells (Promega). Selection of transformants and extraction of plasmid DNA followed described protocols (Ausubel 1995). Further screening was done via restriction fragment length polymorphism (RFLP) to select representative clones for sequencing. Restriction digests of cloned PCR products were performed using the restriction enzyme *Hae*III (New England Biolabs). Representative clones from the three termites were selected for sequencing at Helmholtz Centre for Infection Research (HZI), Braunschweig, Germany. Trace files were manually edited and assembled using Invitrogen vector NTI 11.5 software. The obtained sets of sequences were deposited in GenBank under the accession numbers JQ710341 to JQ710433 (for the *O. somaliensis* sequences), JX421772–JX421863 (for the *Odontotermes* sp. sequences), JX421864–JX421955 (for the *Microtermes* sp. sequences).

Phylogenetic analysis

We checked all sequences for chimeric structures using the Mallard program (Ashelford et al. 2006). A search for similar sequences using BLASTN (Altschul et al. 1990) against the National Center for Biotechnology Information (NCBI) database was performed, and sequence alignment between the query sequences and the identified nearest neighbours was performed using the CLUSTAL Omega program (http://www.clustal.org). A neighbour-joining tree of the aligned sequences was constructed (Saitou and Nei 1987) using MEGA V5.10 (Tamura et al. 2011). Evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al. 2004). To obtain support values for the branches, bootstrapping (Felsenstein 1985) was conducted with 1,000 replicates. All sites, including gaps in the sequence alignment, were excluded pairwise in the phylogenetic analysis. Using the resultant neighbour-joining tree, we assigned each clone to a taxonomic group. We defined ribotypes as those sequences sharing at least 98 % sequence identity with each other (Hongoh et al. 2003). The taxonomic assignment was confirmed at an 80 % confidence level using the naïve Bayesian rRNA classifier on the RDP website (Cole et al. 2005).

Comparison of bacterial communities in different termites

To compare the bacterial community structures in different termite species based on the relative abundances of different bacterial phyla in the guts, 16S rRNA gene sequences obtained from clone libraries of different termites were downloaded from the NCBI Genbank database. Details of the information on the downloaded sequences can be found in the Online Supplementary Table S1. The retrieved set of sequences downloaded, together with our sequences, were separately subjected to the RDP naïve Bayesian rRNA Classifier Version 2.5 program on the RDP website and then compared against the Genbank 16S rRNA gene sequence database (www.ncbi.nlm.nih.gov/BLAST/). The relative abundances (%) of the bacterial groups (Supplementary Table S1) were then used for correlation analysis using principal

components analysis (PCA) as implemented in XLSTAT version 2013.2.

Results

Affiliation of 16S rRNA gene sequences from termite guts

A total of 689 clones from *O. somaliensis* and *Odontotermes* sp., and 336 clones from *Microtermes* sp. were analysed by RFLP (Supplementary Figure S1). 296 unique RFLP patterns from *O. somaliensis* (100 clones), *Odontotermes* sp. (100 clones) and *Microtermes* sp. (96 clones) were sequenced. Of the sequenced clones, 277 were non-chimeric and were included in the subsequent analysis. The clones (prefixed as OTG in *O. somaliensis,* OGH in *Odontotermes* sp. and MIGH in *Microtermes* sp.) were affiliated with 151 different phylotypes. Phylogenetic analyses showed the clones corresponded to diverse bacterial species affiliated with seven bacterial phyla (*Bacteroidetes, Spirochaetes, Firmicutes, Proteobacteria, Synergistetes, Planctomycetes* and *Actinobacteria*) (Figs. 1, 2, 3, 4). The percentage numbers indicate the relative abundances of the bacterial groups from the total number of clones.

Gut bacterial community structure in *Odontotermes* spp.

The gut bacterial community structure of the two *Odontotermes* species was almost identical at the phylum level, as shown in Fig. 1. The phylum *Bacteroidetes* accounted for 68 and 64 % of the total clones in *O. somaliensis* and *Odontotermes* sp., respectively (Fig. 1). The majority of the phylotypes (accounting for 34 % clones in *O. somaliensis* and 28 % clones in *Odototermes* sp.) belonged to the genus *Alistipes* and often clustered with clones previously obtained from guts of termites, humans, cockroach and mouse faeces (Fig. 2). Several phylotypes, among them OGH174 (4.2 %) and OTG026 (8.8 %), formed a cluster with clones from *Odontotermes formosanus* and *Macrotermes gilvus* and were distantly related to those of bacteria in the genus *Alistipes* (<94 % sequence identity to *Alistipes finegoldii* [AB554230] and *Alistipes* sp. JC136 [JF824799]) (Fig. 2). Phylotypes OGH158 and OTG019 clustered with clones from *M. gilvus* and mouse faeces. These were distantly affiliated with bacteria in the genus *Alistipes* (90–95 % sequence identity to *Alistipes* sp. JC136 [JF824799], *Alistipes shahii* [AB554233] and *Alistipes putredinis* [AB554232]), indicating that they are part of the gut intestinal tract microbiota. Other phylotypes including OGH182 and OTG004 were often closely affiliated with clones from *M. gilvus* and *Macrotermes michaelseni* but were only distantly related to *Alistipes* sp. JC136 and *A. finegoldii* (<94 % sequence similarity) (Fig. 2). Phylotype OTG053 (5.5 %) and several others including phylotypes OGH183 and OTG003 belonged to the order *Bacteroidales* and had between 89 and 98 % sequence similarities with other clones from different environments (termite gut, anaerobic reactor, industrial sediments, sludge and soil) (Fig. 2). Phylotypes OGH177, OGH41 and several others were assigned to the order *Bacteroidales* and formed a

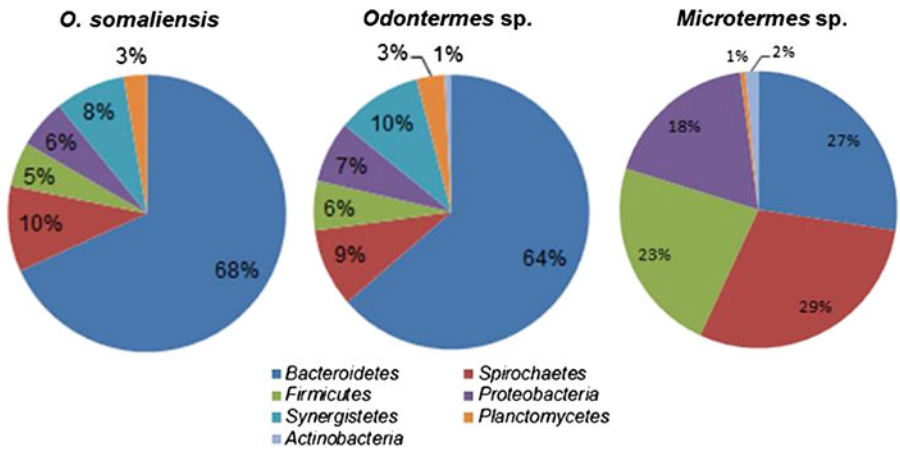

Fig. 1 Percentage of the representative phyla in the gut of *Odontotermes* and *Microtermes* species

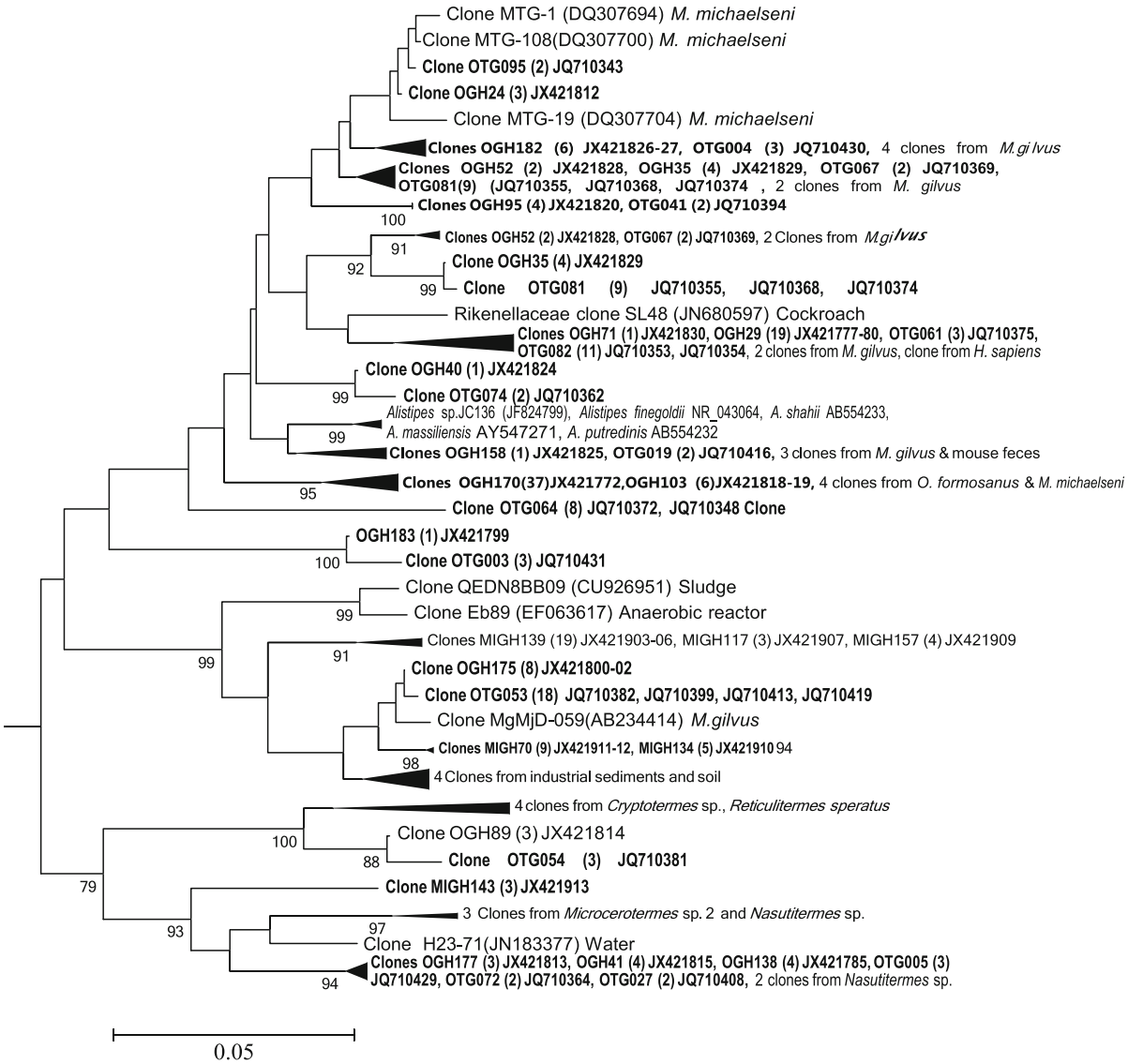

Fig. 2 Evolutionary relationships between partial 16S rRNA gene clone sequences and selected taxa in the phylum Bacteroidetes. *OTG* denotes clone sequences from *O. somaliensis*. *OGH* denotes clone sequences from *Odontotermes* sp., *MIGH* denotes clone sequences from *Microtermes* species. *Methanoculleus thermophiles* (AB065297) was used to root the tree

cluster with clones from *Nasutitermes* species (Fig. 2). Eight phylotypes (OTG024, OTG044, OTG066, OTG073, OGH45, OGH119, OGH144 and OGH176) were affiliated with the genus *Bacteroides* (Fig. 3). Phylotypes OGH176 and OTG066 formed a subcluster with some *Bacteroides* species isolated from human stools and mouse guts. Phylotypes OGH45 and OTG073 clustered together with clone ImMB5 from *Incisitermes minor* (Fig. 3). Phylotypes OGH58 and OTG069 were distantly related to bacteria in the genus *Prevotella* (89–90 % sequence identity with *Prevotella nanceiensis* and *Prevotella* sp. 310-5) as the nearest cultivated neighbours (Fig. 3). Two phylotypes, OTG012 and OGH165, formed a large cluster with several clones from *O. formosanus, Reticulitermes speratus* and *Nasutitermes* sp. and were distantly related to *Bacteroides* species (90 % sequence identity). Phylotypes OGH20 and OTG096

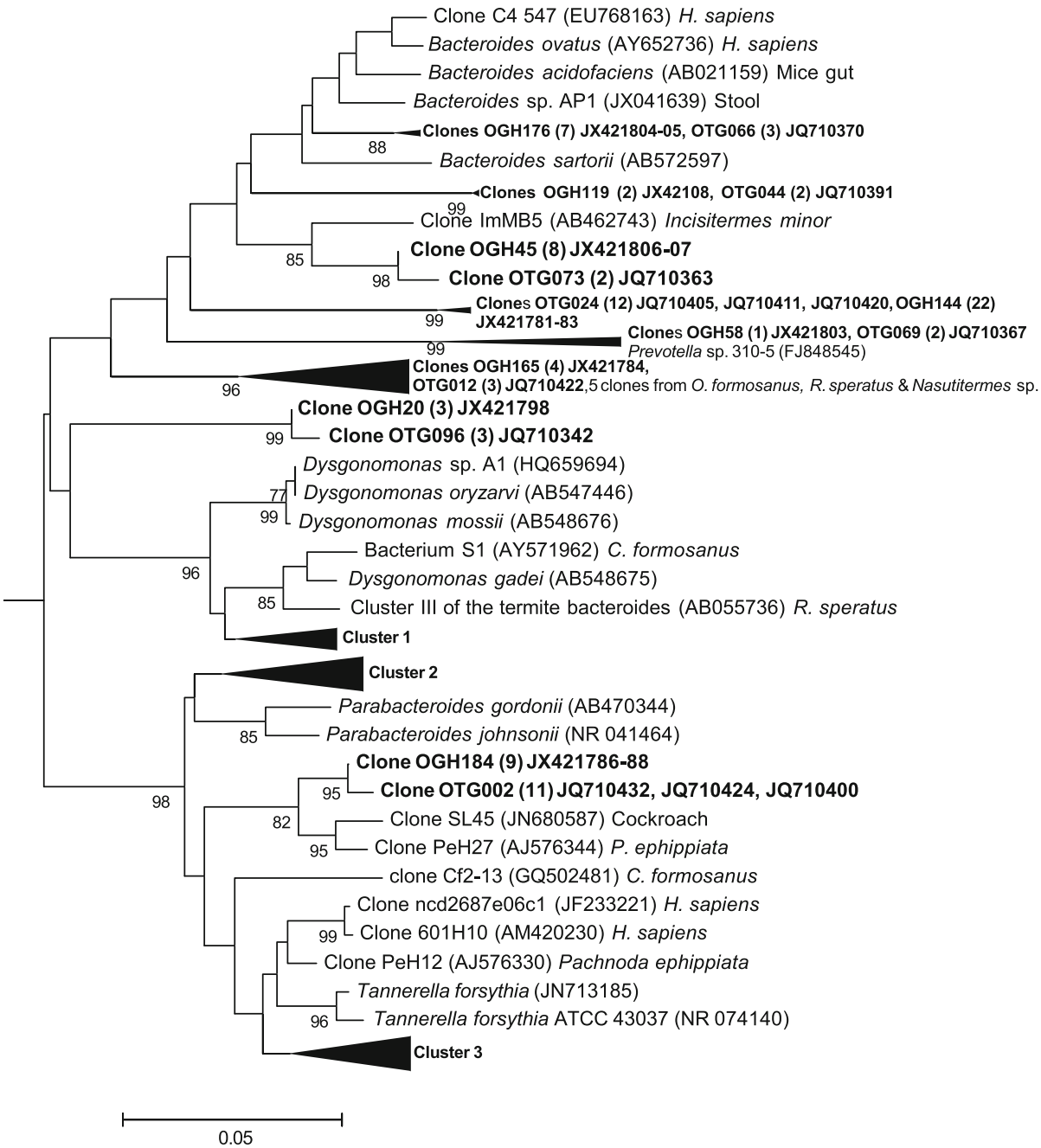

Fig. 3 Continuation of Fig. 2 showing evolutionary relationships between partial 16S rRNA gene clone sequences and selected taxa in the phylum Bacteroidetes. *OTG* denotes clone sequences from *O. somaliensis*. *OGH* denotes clone sequences from *Odontotermes* sp., *MIGH* denotes clone sequences from *Microtermes* species

formed a separate cluster and were distantly related to *Dysgonomonas mossii* (AB548676) and *Dysgonomonas oryzarvi* (AB547446) (90 % sequence similarity). Cluster one (Fig. 3) consisted of four phylotypes OTG085 (two clones [JQ710351]), OGH23 (three clones [JX421796]), OGH60 (three clones [JX421797]) and OTG075 (two clones [JQ710361]) together with clone Cf2-02 (GQ502485) from *Coptotermes formosanus.* These were related to bacteria in the genus *Dysgonomonas* (92–94 % sequence identity

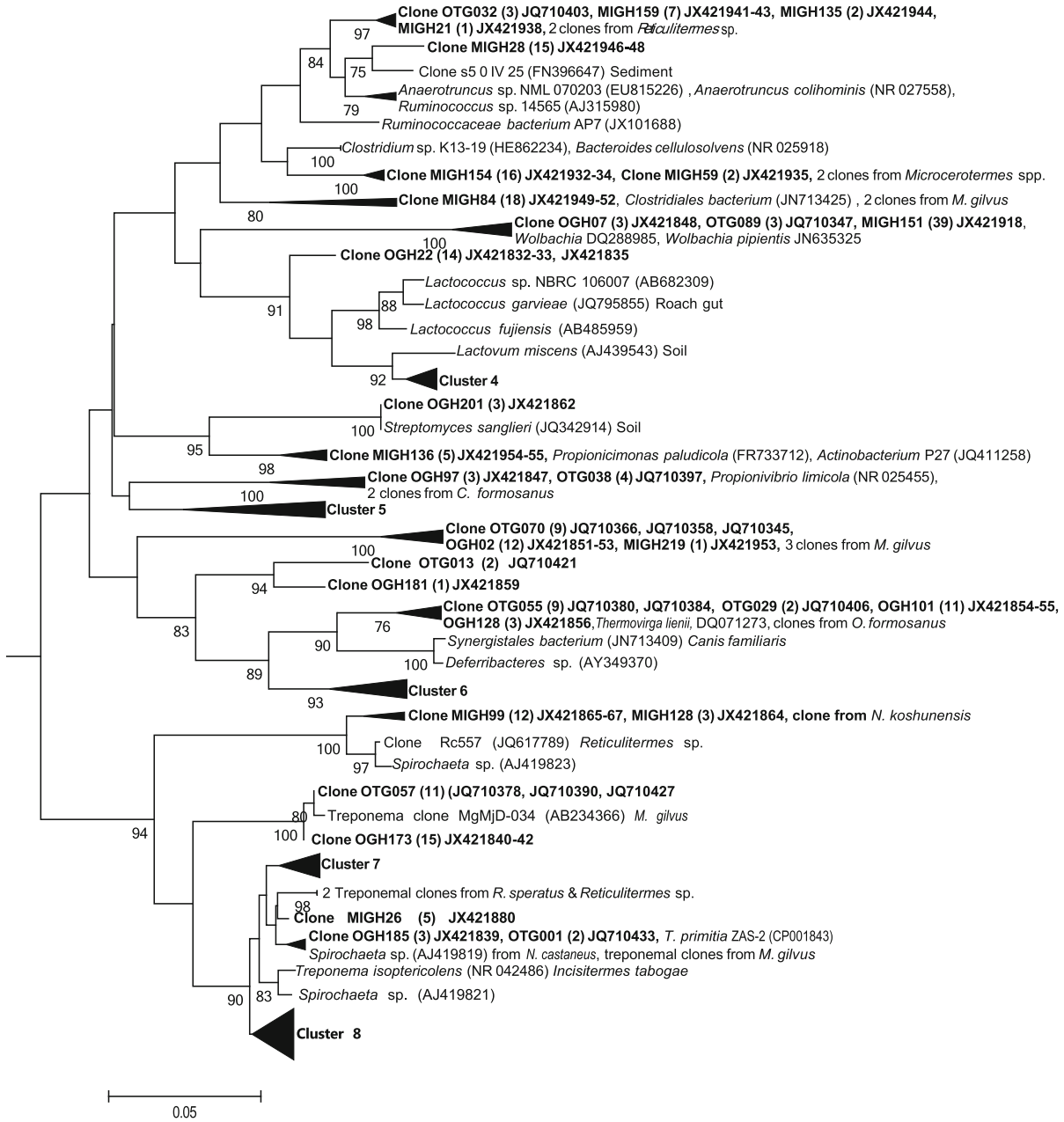

Fig. 4 Evolutionary relationships between partial 16S rRNA gene clone sequences and selected taxa in the phyla *Spirochaetes, Firmicutes, Synergistetes, Planctomycetes, Proteobacteria and Actinobacteria*. *OTG* denotes clone sequences from *O. somaliensis*. *OGH* denotes clone sequences from *Odontotermes* sp., *MIGH* denotes clone sequences from *Microtermes* species. *Methanoculleus thermophiles* (AB065297) was used to root the tree

with *Dysgonomonas hofstadii* [FN356023] and *Dysgonomonas gadei* [AB548675]). Members of the genus *Dysgonomonas* have been reported to inhabit intestinal guts, blood, wounds, urine, peritoneal fluid, stools and gallbladder (Hansen et al. 2005). Several other phylotypes i.e. OTG043 (12 clones [JQ710392]), OTG047 (three clones [JQ710388]), OTG060 (three clones [JQ710376]), OTG084 (three clones [JQ710352]), OGH27 (seven clones [JX421793-94]), OGH76 (four clones [JX421791-92]), OGH99 (eight

clones [JX421789-90]) and OGH155 (nine clones [JX421809-11]) formed cluster two together with other clones often obtained from *O. formosanus* and *M. gilvus* (Fig. 3). These clones belonged to the genus *Parabacteroides* and were distantly affiliated with *Parabacteroides gordonii* (AB4703344) and *Parabacteroides johnsonii* (NR_041464) (89–91 % sequence identity) (Fig. 3). Phylotypes OGH184 and OTG002 were assigned to the genus *Tennerella* (93 % sequence identity with *Tannerella forsythia*) (JN713185) and *Tannerella forsythia* ATCC 43037 (NR_074140) and formed a subcluster with clones from insects (cockroach and *Pachnoda ephippiata*).

The phylum *Synergistetes* accounted for ten phylotypes. Phylotypes OGH147 (seven clones [JX421858]), OGH135 (two clones [JX421944]), OTG011 (eight clones [JQ710423, JQ710404]) and OTG020 (six clones [JQ710432, JQ710385]) were assigned to the family *Synergistaceae* and formed cluster six (Fig. 4) together with *Candidatus Tammella caduceiae* (AB299516) and some clones often obtained from *M. gilvus.* Phylotypes OTG055, OGH101, OGH128 and OTG029 belonged to the genus *Thermovirga* (<80 % sequence identity with *Thermovirga lienii* [DQ071273] and were distantly related to members in the genera *Deferribacter* and *Flexistipes* (93–94 % sequence identities with *Flexistipes*-like sp. [AY005447], *Deferribacteres* sp. [AY349370] and *Synergistales bacterium* [JN713409]) and clustered with some clones from *O. formosanus* (Shinzato et al. 2007). Two phylotypes OGH181 and OTG013 belonged to the family *Synergistaceae* and were distantly affiliated with clones obtained from soil (90–93 % sequence identity). A total of eight phylotypes were related to the phylum *Spirochaetes* (Fig. 4). Phylotypes OTG057 and OGH173 clustered with treponemal clones from *M. gilvus.* Four phylotypes OGH15 (three clones [JX421837]), OGH39 (12 clones [JX421838, JX421843, JX421844]), OTG063 (17 clones [JQ710373, JQ710360, JQ710357]) and OTG087 (three clones [JQ710349]) formed cluster seven (Fig. 4) together with *Spirochaeta* sp. (AJ419819), *Treponema azotonutricium* ZAS-9 (CP001841), *Treponema* sp. ZAS-1 (AF09325) (92–94 % sequence identity) and other treponemal clones often from *Nasutitermes* species and *M. gilvus.* Two phylotypes OGH185 and OTG001 formed a cluster with other treponemal clones obtained from *M. gilvus* and were related to *Spirochaeta* sp. (AJ419819) from *Neotermes castaneus* and *Treponema primitia* ZAS-2 (CP001843) (93–94 % sequence identity) (Fig. 4). Eight phylotypes were distributed in three subdivisions of the *Proteobacteria*. Two phylotypes, OTG038 and OGH97, were assigned to the genus *Propionivibrio* (*ß-Proteobacteria*) and clustered with *Propionivibrio limicola* (NR_025455) (<94 % sequence identity) and clone Cf6-10 (GQ502594) from *C. formosanus* (Fig. 4). Phylotypes OGH07 and OTG089 belonged to the subdivision *α-Proteobacteria*, with 98–99 % sequence affiliation to *Wolbachia* species from guts of termites and insects. Four phylotypes, OGH145 (13 clones [JX421846, JX421849]), OGH148 (seven clones [JX421845, JX421850]), OTG017 (nine clones [JQ710418, JQ421412]), and OTG092 (three clones [JQ710346]) formed cluster five (Fig. 4) together with clones from *M. michaelseni.* These were assigned to the subdivision *δ-Proteobacteria.*

Phylotype OTG071 (14 clones [JQ710350, JQ710365, JQ710371]) was the most abundant in the phylum *Firmicutes* and formed cluster four (Fig. 4) together with clones from *O. formosanus* and *C. formosanus.* Phylotype OTG071 was related to bacteria in the genus *Lactovum* (94 % sequence identity with *Lactovum miscens* [AJ439543]). Phylotype OGH22 partly clustered with *Lactococcus* species while phylotype OTG032 was assigned to the family *Ruminococcaceae* and was distantly affiliated with members in the genus *Ruminococcus* (92 % sequence similarity with *Ruminococcus* sp. 14565 [AJ315980] and *Ruminococcaceae bacterium* AP7 [JX101688]). The genus *Ruminococcus* includes *Ruminococcus flavefaciens*, an anaerobic cellulolytic bacterium found in the rumen and in the hindgut of monogastric domestic and wild mammals (Bayer et al. 2008). These and other cellulolytic bacteria play an important role in the digestion of hemicellulose and cellulose plant cell walls (Bayer et al. 2008). Phylotype OGH102 was assigned to the order *Clostridiales* and was 92 % related to *Ruminococcus* sp. 14565. *Planctomycetes* accounted for a single phylotype in each termite (OTG070 and OGH02) and formed a subcluster with other clones from *M. gilvus* (Fig. 4). The phylum *Actinobacteria* was represented by a single phylotype OGH201 from *Odontotermes* sp. that was affiliated with *Streptomyces sanglieri* (JQ342914) isolated from soil.

Gut bacterial community structure in *Microtermes* sp.

The gut bacterial community structure of *Microtermes* sp. showed differences from that of *Odontotermes* species (Fig. 1). Phylotypes (27 % of the clones) belonging to the phylum *Bacteroidetes* were assigned to different genera. Phylotypes MIGH04 (five clones [JX421895]), MIGH32 (two clones [JX421896]), MIGH45 (one clone [JX421894]), MIGH50 (four clones [JX421893]), MIGH86 (five clones [JX421892]), MIGH88 (four clones [JX421897]), MIGH142 (five clones [JX421891]) and MIGH150 (three clones [JX421898]) were assigned to the genus *Tannerella* and formed large cluster three (Fig. 3) with clones often from guts of termites (*R. speratus, Hodotermopsis sjoestedti, Cubitermes orthognathus*) and insects. Phylotype MIGH143 formed a cluster with clones from other termites and was affiliated with *Bacteroidetes* bacterial clone from water (95 % sequence similarity). Only a single phylotype MIGH10 (5.1 %) was related to bacteria in the genus *Alistipes* (95 % sequence identity with *A. finegoldii* [AB554230]) and several other clones from *M. gilvus, Odontotermes* species and mouse gut (Fig. 2). Five phylotypes (MIGH70, MIGH134, MIGH139, MIGH117 and MIGH157) belonged to the order *Bacteroidales* and were distantly related (94–96 % sequence identity) to other clones obtained from different environments (termite gut, anaerobic reactor, industrial sediments, sludge and soil) (Fig. 2). Notably, seventeen phylotypes (29 % of the clones) were affiliated with the phylum *Spirochaetes* and formed a large cluster with treponemal clones and some *Spirochaeta* species from several termite guts, particularly wood feeding termites (Fig. 4). Phylotype MIGH131 (6.8 %), which was the most abundant in this group, formed part of cluster seven (Fig. 4) together with other clones from *Odontotermes* species, *M. gilvus* and *Nasutitermes* species. Phylotypes MIGH99 and MIGH128 were affiliated with bacteria in the genus *Spirochaeta* (95–96 % sequence similarity with *Spirochaeta* sp. AJ419823 from *Zootermopsis angusticollis*) and formed a subcluster with clones from *Neotermes koshunensis* and *Reticulitermes* species (Fig. 4). Several other phylotypes i.e. MIGH115 (16 clones [JX421877-79]), MIGH47 (three clones [JX421884]), MIGH89 (three clones [JX421885]), MIGH39 (two clones [JX421874]), MIGH52 (four clones [JX421875]), MIGH36 (three clones [JX421876]), MIGH69 (two clones [JX421881]), MIGH121 (four clones [JX421882]), MIGH138 (three clones [JX421883]), MIGH141 (four clones [JX421886]), MIGH60 (one clone [JX421887]), MIGH73 (nine clones [JX421888-89]), MIGH53 (one clone [JX421890]) formed a large monophyletic group (cluster eight) together with *Spirochaeta* species (AJ419817, AB015812) and other clones from termites (*Nasutitermes* sp., *C. orthognathus* and *Termes comis*) (Fig. 4).

The phylum *Firmicutes* accounted for 23 % of the total clones. Phylotypes MIGH123 (four clones [JX421936]), MIGH80 (eight clones [JX421937]), MIGH133 (three clones [JX421940]) and MIGH100 (one clone [JX421939]) formed part of cluster four (Fig. 4) together with other clones from *Odontotermes* species. These were affiliated with the genus *Lactovum* and clustered with *L. miscens.* Phylotypes MIGH59 and MIGH154 belonged to the family *Ruminococcaceae* and formed a subcluster with clones from *Microcerotermes* species (Fig. 4). These two phylotypes (MIGH59 and MIGH154) were affiliated with *Clostridium* sp. K13-19 (HE862234) and *Bacteroides cellulosolvens* (NR_025918) (94–95 % sequence identity) (Fig. 4). *Clostridium* sp. K13-19 and *Bacteroides cellulosolvens* are anaerobic cellulose-degrading bacteria that may have a role in degradation of plant biomass. For instance, *Bacteroides cellulosolvens* is known to bind tightly and degrade crystalline forms of cellulose (Giuliano and Khan 1984). Three phylotypes (MIGH159, MIGH135 and MIGH21) had <93 % sequence similarity with *Ruminococcus* sp. 14565 (AJ315980) and clustered with clones from *Odontotermes* and *Reticulitermes* species (Fig. 4). Phylotype MIGH28 belonged to the genus *Anaerotruncus* and was distantly related to *Anaerotruncus* sp. NML 070203 [EU815226] (94 % sequence identity). Phylotype MIGH84 (5.4 %) belonged to the order *Clostridiales* and was the most abundant in this phylum, forming a cluster with *Clostridiales bacterium* (JN713425) and two clones from *M. gilvus* (Fig. 4). One phylotype MIGH51 belonged to *δ-Proteobacteria* and clustered with clones from other termites. Phylotype MIGH151 (11.6 %), the most abundant in the clone library belonged to the subdivision *α-Proteobacteria* and was closely affiliated with *Wolbachia* species (>97 % sequence identity) (Fig. 4). The phyla *Planctomycetes*

and *Actinobacteria* were the least represented, with a single phylotype from each phylum (MIGH219 and MIGH136, respectively). Phylotype MIGH136 formed a cluster with *Actinobacterium* P27 (JQ411258) and *Propionicimonas paludicola* (FR733712) (Fig. 4). Phylotype MIGH219 was closely affiliated with clones from *M. gilvus* and those from *Odontotermes* species.

The PCA (Fig. 5), indicated that the relative abundances of *Alistipes* and *Treponema* are the major effect determining the overall variance of the genus compositions in the samples, followed by the relative abundance of *genus Hespellia*. Differences regarding the other genera detected in the termite gut are negligible. *Hespellia* abundance increases in the direction of the soil feeder, *Cubitermes* sp., while abundance of *Alistipes* increases towards *M. gilvus*. *Treponema,* however, increases towards the wood feeder, *R. flavipes.* The relative abundance of the bacterial genera in the guts of fungus-cultivating termites, which are represented by more than one sample, showed considerable divergence but no genus-specific pattern.

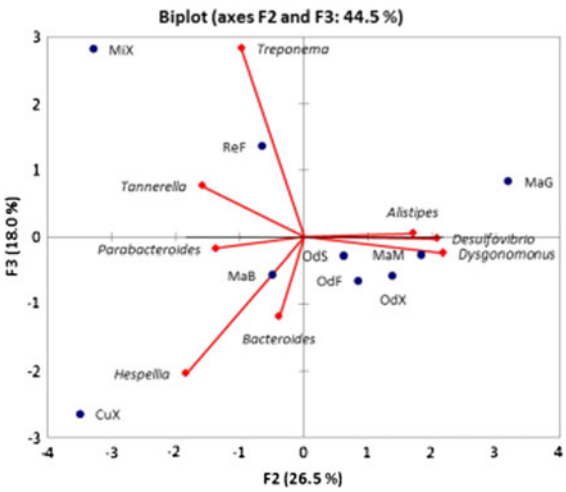

Fig. 5 Principal-component analysis of bacterial communities based on the relative abundances of the bacterial genera. The vectors indicate the direction and impact of each detected bacterial genus on the overall variance. Those with a negligible influence were not annotated. Abbreviations in figure: CuX *Cubitermes* sp., OdF *O. formosanus*, OdS *O. somaliensis*, OdX *Odontotermes* sp., ReF *R. flavipes*, MiX *Microtermes* sp.

Discussion

The phylogenetic analysis of the 16S rRNA gene sequences from the three termites revealed diverse gut bacterial communities that are still uncultured. The affiliation of the cloned sequences with others previously obtained from termite guts underlines the existence of termite-specific bacterial lineages (Hongoh et al. 2003, 2005, 2006; Schmitt-Wagner et al. 2003; Shinzato et al. 2007; Warnecke et al. 2007) (Figs. 2, 3, 4). Notably, the results indicated a higher gut bacterial diversity in *Odontotermes* than in *Microtermes*. The bacterial community structure within the two here examined *Odontotermes* species was almost identical at the phylum level (Fig. 1). The phylum *Synergistetes* was exclusively detected in *Odontotermes* species (Fig. 4). Previously, members of *Synergistetes* were reported in guts of *M. gilvus* (Hongoh et al. 2006), *M. michaelseni* (Mackenzie et al. 2007) and *O. formosanus* (Shinzato et al. 2007), wood-feeding termites (Hongoh et al. 2005; Köhler et al. 2012) and in cockroaches (Schauer et al. 2012). However, they were not detected in the guts of soil-feeding termites (Schmitt-Wagner et al. 2003) and *Macrotermes barneyi* (Zhu et al. 2012). Members of the phylum *Synergistetes* mainly inhabit anaerobic environments, including gastrointestinal tracts, human clinical specimens, soil, oil wells and wastewater treatment plants (Vartoukian et al. 2007). Their presence at pathology related sites suggests they may be opportunistic pathogens; however, other species of *Synergistetes* are significant contributors in the degradation of sludge for production of biogas in anaerobic digesters (Riviere et al. 2009). Though their precise role in termites remains unclear, some *Synergistetes* species (e.g. *Thermovirga lienii*) have been implicated in amino-acid-degradation (Dahle and Birkeland 2006), which is an important process in the gut of the termites.

The PCA indicated that the relative similarity in the bacterial communities across the termites is mainly impacted by the genera *Alistipes, Treponema, Tannerella, Dysgonomonus* and *Hespellia* (Fig. 5). Their relative abundances considerably varied across the different termite samples (Fig. 5). Members of *Treponema* seem to be more predominant in wood feeders such as *R. flavipes* and in the *Microtermes* sp. (see Supplementary Table S1) while *Hespellia* appear more dominant in the soil-feeders such as *Cubitermes*

species. However, it cannot be generalized at this point because these two guilds (wood feeders and soil-feeders) were represented by a single sample only, respectively. Rather, the considerable divergence in the relative abundances of these genera within the fungus-cultivating termites, which were represented by many samples, indicates that other factors besides feeding habits influence the abundances of the phyla within the gut bacterial communities (Sanyika et al. 2012).

The phylum *Bacteroidetes* showed differences in group abundance; for instance, the genera *Parabacteroides, Bacteroides* and *Dysgonomonas* were detected in *Odontotermes* but undetected in *Microtermes* (Supplementary Table S1). The genus *Alistipes* was represented by more phylotypes from *Odontotermes* (Fig. 2) than in *Microtermes*, which had more phylotypes affiliated with the genus *Tannerella* (Fig. 3) than with *Odontotermes*. The described species from the genus *Alistipes* (*A. finegoldii, A. onderdonkii, A. shahii, A. putredinis* and *A. indistinctus*) (Könönen et al. 2010; Nagai et al. 2010) have also been isolated from the intestines of healthy humans but their precise role is not yet known. The high abundance of *Bacteroidetes* in the termite guts (Figs. 2, 3) is in agreement with findings in other fungus-cultivating termites (Hongoh et al. 2006; Mackenzie et al. 2007; Zhu et al. 2012), suggesting that they play significant roles in the termites; for examples, members of the genera *Tannerella,* and *Prevotella* (*Prevotella ruminicola* 23, *Prevotella intermedia* 17) can ferment both xylan and cellulose through carbohydrate-active enzymes such as xylanase, carboxymethylcellulase and endoglucanase (http://www.cazy.org). Recently, *D. oryzarvi* was isolated from a microbial fuel cell (Kodama et al. 2012), which implicates it in cellulose degradation. Moreover, genome analysis has shown *Bacteroides* involvement in breaking down polysaccharides and metabolizing their sugars (Xu et al. 2003; Sonnenburg et al. 2010) by contributing glycosyl hydrolases for their hosts' digestion (Liu et al. 2011). *Bacteroidetes* also benefit their host by excluding potential pathogens from colonizing the gut (Wexler 2007). However, some species such as *Bacteroidetes fragilis* have been implicated in diseases (Wexler 2007; Saulnier et al. 2011). Although the involvement of *Bacteroidetes* in degradation and fermentation of plant biomass partly implicates them in termite nutrition, it remains to be specifically determined.

Interestingly, *Spirochaetes* were more predominant in *Microtermes* than in *Odontotermes* (Fig. 4). This finding contrasts the literature (Shinzato et al. 2007; Zhu et al. 2012; Mathew et al. 2012), which shows infrequent detection of *Spirochaetes* in the guts of other fungus-cultivating termites. Notably, many members of *Spirochaetes* are host-associated and inhabit the oral cavity, intestinal tract and genital areas of humans or other mammals, as well as the gut contents of wood-feeding insects (Norris et al. 2010). They form an abundant group in the guts of most termites (Breznak 1984) (Fig. 4), especially wood-feeding termites (Hongoh et al. 2005; Köhler et al. 2012); this concurs with our results regarding *Microtermes* sp. (Fig. 4), where majority of the phylotypes were affiliated with treponemal clones from wood-feeding termites. The relative abundance of treponemal species in the gut substantiates their importance in termites. For instance, Ballor et al. (2012) revealed the presence of termite gut treponemes with multiple iron-hydrogenases and *Treponema azotonutricium* (an isolate from the lower termite *Z. angusticollis*) produces molecular hydrogen as a by-product of carbohydrate fermentation (Graber et al. 2004), which validates the suggestion (Köhler et al. 2012) that *Spirochaetes* are partly responsible for hydrogen production in termites. In addition, examination of *Treponema* strains ZAS-1, ZAS-2 and ZAS-9 revealed that they possess two homologues of *nifH* and each exhibited nitrogenase activity, demonstrating their involvement in nitrogen fixation (Lilburn et al. 2001).

It should be noted that members of the phyla *Firmicutes* and *Proteobacteria* are regularly encountered in termite guts (Fig. 4) and may play key functions. Two phylotypes (MIGH59 and MIGH154) detected in *Microtermes* sp. (Fig. 4) were affiliated with *Clostridium* sp. K13-19 (HE862234) and *Bacteroides cellulosolvens* (NR_025918) (94–95 % sequence identity). *Clostridium* sp. K13-19 and *Bacteroides cellulosolvens* are anaerobic cellulose-degrading bacteria that may have a role in degradation of plant biomass. For instance, *Bacteroides cellulosolvens* is known to bind and degrade crystalline forms of cellulose (Giuliano and Khan 1984). Some clones that were detected had affiliation with the genus *Lactovum* (Fig. 4) and clustered with *L. miscens* (isolated from acidic forest soil) that is reported to be involved in mixed fermentative metabolism (Matthies et al. 2004). Moreover, members of the

subdivision *δ-proteobacteria* such as *Desulfovibrio* spp. isolated from termite guts display high rates of hydrogen-dependent oxygen reduction (Kuhnigk et al. 1996). Members of the genus *Propionivibrio* (*β-Proteobacteria*) (represented by phylotypes OTG038 and OGH97) that were detected in the clone libraries were related to *P. limicola* (NR_025455) (Fig. 4). *P. limicola* has been shown to be fermentative and specialize in the degradation of hydroaromatic compounds (Brune et al. 2002). Members of *α-Proteobacteria* such as *Wolbachia* species that were also present in our clone libraries (Fig. 4) are associated with four distinct reproductive phenotypes in a wide range of *Arthropoda*: parthenogenesis, male killing, feminization and cytoplasmic incompatibility; nonetheless, little is known about possible phenotypes linked to *Wolbachia* in *Isoptera* (Werren et al. 2008).

There was low detection of *Planctomycetes* and *Actinobacteria* in all termites (Fig. 4). This trend has been reported in other termites (Shinzato et al. 2005; Fisher et al. 2007; Mackenzie et al. 2007; Long et al. 2010; Zhu et al. 2012). Nevertheless, *Actinobacteria* have diverse metabolic capabilities and some isolates from termite guts (Pasti and Belli 1985; Watanabe et al. 2003; Mackenzie et al. 2007) have cellulolytic activity (Pasti and Belli 1985; le Roes-Hill et al. 2011) and lignin-solubilizing activity (Pasti and Belli 1985; Pasti et al. 1990). In addition, *Actinobacteria* excrete antimicrobial peptides (Bulmer and Crozier 2004), which have been shown to inhibit the growth of some *Pseudoxylaria* and *Termitomyces* (Visser et al. 2012), hence preventing contamination in the farming of fungus gardens (Moriya et al. 2005).

Notably, the majority of the representative phylotypes found in our clone libraries are affiliated with sequences previously isolated from termites gut (Figs. 2, 3, 4). For instance, in *O. somaliensis* a total of forty-three phylotypes out 53 were affiliated with termite-related clones. Similarly, in *Odontotermes* sp. 27 phylotypes out of the 51 clustered with clones from fungus-cultivating termites. This trend has been reported elsewhere (Shinzato et al. 2007). However, in the *Microtermes* sp. several phylotypes were closely affiliated with clones previously obtained from non-fungus cultivating termites (Figs. 2, 3, 4). Previously, Hongoh et al. (2005) observed consistency in the bacterial phylogeny and the community structure within a genus of termites, which is in agreement with our findings regarding the two *Odontotermes* species (Fig. 1). Such observations suggest that majority of termite gut bacteria are specific symbionts that have coevolved with their hosts (Shinzato et al. 2005). The vertical mode of transmission of such gut microbes could be one of the major factors contributing to formation of the termite-specific bacterial lineages observed. The presence of clones affiliated with those originating from non-termite environments could emanate from either random acquisition of microorganisms from the environment (Curtis and Sloan 2004) or variation in the hosts' diets (Tanaka et al. 2006). However, the factors determining the community structure of termite guts are still unclear (Shinzato et al. 2005) and further research is needed to address the mechanisms establishing the microbial community structures in the different termite species. It should be noted that there could be an underestimation of the diversity since individual taxa present in smaller numbers are difficult to detect due to PCR bias (von Wintzingerode et al. 1997; Farris and Olson 2007) and the RFLP screening method used (Supplementary Figure 1).

Conclusion

The findings of this study reveal a high level of bacterial diversity in the guts of fungus-cultivating termites, the majority of which are still uncultivated. This fact, coupled with the great diversity of termite species (Ahmed et al. 2011), challenge our ability to resolve the physiology and metabolic functions of the bacteria in the gut ecosystem. Nonetheless, the affiliation of the clones with those from guts of other termites demonstrates that the majority of the gut bacteria are autochthonous and have mutualistic relationship with their hosts (Hongoh et al. 2006; Shinzato et al. 2007).

Therefore, combined efforts using both culture and culture-independent methods are needed to comprehensively characterize the microbial species' richness and their specific roles in the termite gut. Although the approaches used in this study cannot help infer physiological roles for the uncultured bacteria in the termites, the results provide key insights into bacterial community structure in the guts of fungus-cultivating termites and contribute to understanding gut bacterial diversity and their mutualism with termites.

Acknowledgments This work was supported by a Ph.D. scholarship from the Deutscher Akademischer Austauschdienst (DAAD) and a Grant (NCST/5/003/PG/224) from the Kenya National Council for Science and Technology (NCST). Kenya Wildlife Services (KWS) and National Environmental Management Act (NEMA) provided permits for sample collection in Kenya. We kindly thank J. Simon, G. Gresenz, M. Scharfe and S. Schmidt for excellent technical support.

References

Ahmed BM, Nkunika POY, Sileshi WG, French JRJ, Nyeko P, Jain S (2011) Potential impact of climate change on termite distribution in Africa. Br J Environ Clim Chang 1:172–189

Altschul SF, Gish W, Miller W et al (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Ashelford KE, Chuzhanova NA, Fry JC, Jones AJ, Weightman AJ (2006) New screening software shows that most recent large 16S rRNA gene clone libraries contain chimeras. Appl Environ Microbiol 72:5734–5741

Austin JW, Szalanski AL, Cabrera BJ (2004) Phylogenetic analysis of the subterranean termite family *Rhinotermitidae* (*Isoptera*) by using the mitochondrial cytochrome oxidase II gene. Ann Entomol Soc Am 97:548–555

Ausubel FM (1995) Current protocols in molecular biology. Wiley, New York

Ballor NR, Paulsen I, Leadbetter JR (2012) Genomic analysis reveals multiple [FeFe] hydrogenases and hydrogen sensors encoded by treponemes from the H_2-rich termite gut. Microb Ecol 63:282–294

Bayer EA, Lamed R, White BA, Flint HJ (2008) From cellulosomes to cellulosomics. Chem Rec 8:364–377

Breznak JA (1984) Hindgut spirochaetes of termites and *Cryptocercus puntulatus*. In: Krieg NR, Holt JG (eds) Bergey's manual of systematic bacteriology, vol 1. Williams & Wilkins, Baltimore, pp 67–70

Breznak JA (2000) Ecology of prokaryotic microbes in the guts of wood- and litter-feeding termites. In: Abe T, Bignell DE, Higashi M (eds) Termites: evolution, sociality, symbiosis, ecology. Kluwer, Dordrecht, pp 209–231

Brune A, Friedrich M (2000) Microecology of the termite gut: structure and function on a microscale. Curr Opin Microbiol 3:263–269

Brune A, Ohkuma M (2011) Role of the termite gut microbiota in symbiotic digestion. In: Bignell DE, Roisin Y, Lo N (eds) Biology of termites: a modern synthesis. Springer, Dordrecht, pp 439–475

Brune A, Ludwig W, Schink B (2002) *Propionivibrio limicola* sp. nov., a fermentative bacterium specialized in the degradation of hydroaromatic compounds, reclassification of *Propionibacter pelophilus* as *Propionivibrio pelophilus* comb. nov. and amended description of the genus *Propionivibrio*. Int J Syst Evol Microbiol 52:441–444

Bulmer MS, Crozier RH (2004) Duplication and diversifying selection among termite antifungal peptides. Mol Biol Evol 21:2256–2264

Cole JR, Chai B, Farris RJ et al (2005) The Ribosomal Database Project (RDP-II): sequences and tools for high-throughput rRNA analysis. Nucleic Acids Res 33:D294–D296

Curtis TP, Sloan WT (2004) Prokaryotic diversity and its limits: microbial community structure in nature and implications for microbial ecology. Curr Opin Microbiol 7:221–226

Dahle H, Birkeland NK (2006) *Thermovirga lienii* gen. nov., sp. nov., a novel moderately thermophilic, anaerobic, amino-acid-degrading bacterium isolated from a North Sea oil well. Int J Syst Evol Microbiol 56:1539–1545

Farris MH, Olson JB (2007) Detection of actinobacteria cultivated from environmental samples reveals bias in universal primers. Lett Appl Microbiol 45:376–381

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Fisher M, Miller D, Brewster C, Husseneder C, Dickerman A (2007) Diversity of gut bacteria of *Reticulitermes flavipes* as examined by 16S rRNA gene sequencing and amplified rDNA restriction analysis. Curr Microbiol 55:254–259

Giuliano C, Khan AW (1984) Cellulase and sugar formation by Bacteroides cellulosolvens, a newly isolated cellulolytic anaerobe. Appl Environ Microbiol 48:446–448

Graber JR, Leadbetter JR, Breznak JA (2004) Description of *Treponema azotonutricium* sp. nov. and *Treponema primitia* sp. nov., the first spirochetes isolated from termite guts. Appl Environ Microbiol 70:1315–1320

Hansen PS, Jensen TG, Gahrn-Hansen B (2005) *Dysgonomonas capnocytophagoides* bacteraemia in a neutropenic patient treated for acute myeloid leukaemia. APMIS 113:229–231

Hongoh Y, Ohkuma M, Kudo T (2003) Molecular analysis of bacterial microbiota in the gut of the termite *Reticulitermes speratus* (Isoptera; Rhinotermitidae). FEMS Microbiol Ecol 44:231–242

Hongoh Y, Deevong P, Inoue T, Moriya S, Trakulnaleamsai S, Ohkuma M, Vongkaluang C, Noparatnaraporn N, Kudo T (2005) Intra- and interspecific comparisons of bacterial diversity and community structure support coevolution of gut microbiota and termite host. Appl Environ Microbiol 71:6590–6599

Hongoh Y, Ekpornprasit L, Inoue T, Moriya S, Trakulnaleamsai S, Ohkuma M, Noparatnaraporn N, Kudo T (2006) Intracolony variation of bacterial gut microbiota among castes and ages in the fungus-growing termite *Macrotermes gilvus*. Mol Ecol 15:505–516

Inward DJG, Vogler AP, Eggleton P (2007) A comprehensive phylogenetic analysis of termites (*Isoptera*) illuminates key aspects of their evolutionary biology. Mol Phylogenet Evol 44:953–967

Kodama Y, Shimoyama T, Watanabe K (2012) *Dysgonomonas oryzarvi* sp. nov., isolated from a microbial fuel cell. Int J Syst Evol Microbiol 62(12):3055–3059. doi:10.1099/ijs.0.039040-0

Köhler T, Dietrich C, Scheffrahn RH, Brune A (2012) High-resolution analysis of gut environment and bacterial microbiota reveals functional compartmentation in the gut of wood-feeding higher termites (*Nasutitermes* spp.). Appl Environ Microbiol 78:4691–4701

Könönen E, Song Y, Rautio M, Finegold SM (2010) Genus II. *Alistipes*. In: Krieg NR, Staley JT, Brown DR, Hedlund BP, Paster BJ, Ward NL, Ludwig W, Whitman W (eds)

Bergey's manual of systematic bacteriology 4, 2nd edn. Springer, New York, pp 84–88

Kuhnigk T, Branke J, Krekeler D, Cypionka H, König H (1996) A feasible role of sulfate-reducing bacteria in the termite gut system. Appl Microbiol 19:139–149

Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics. Wiley, New York, pp 115–175

Le Roes-Hill M, Rohland J, Burton S (2011) Actinobacteria isolated from termite guts as a source of novel oxidative enzymes. Antonie Van Leeuwenhoek 100:589–605

Lilburn TG, Kim KS, Ostrom NE, Byzek KR, Leadbetter JR, Breznak JA (2001) Nitrogen fixation by symbiotic and free-living spirochetes. Science 292:2495–2498

Liu N, Yan X, Zhang M, Xie L, Wang Q, Huang Y, Zhou X, Wang S, Zhou Z (2011) Microbiome of fungus-growing termites: a new reservoir for lignocellulase genes. Appl Environ Microbiol 77:48–56

Long Y-H, Xie L, Liu N, Yan X, Li M-H, Fan M-Z, Wang Q (2010) Comparison of gut-associated and nest-associated microbial communities of a fungus-growing termite (*Odontotermes yunnanensis*). J Insect Sci 17:265–276

Mackenzie LM, Muigai AT, Osir EO, Lwande W, Keller M, Toledo G, Boga HI (2007) Bacterial diversity in intestinal tract of fungus-cultivating termite *Macrotermes michaelseni* (Sjöestedt). Afr J Biotechnol 6:658–667

Makonde HM, Boga HI, Osiemo Z, Mwirichia R, Stielow B, Göker M, Klenk H-P (2013) Diversity of *Termitomyces* associated with fungus-farming termites assessed by cultural and culture-independent methods. PLoS ONE 8:e56464. doi:10.1371/journal.pone.0056464

Mathew GM, Ju Y-M, Lai C-Y, Mathew DC, Huang CC (2012) Microbial community analysis in the termite gut and fungus comb of *Odontotermes formosanus*: the implication of *Bacillus* as mutualists. FEMS Microbiol Ecol 79:504–517

Mattéotti C, Thonart P, Francis F, Haubruge E, Destain J, Brasseur C, Bauwens J, De Pauw E, Portetelle D, Vandenbol M (2011) New glucosidase activities identified by functional screening of a genomic DNA library from the gut microbiota of the termite *Reticulitermes santonensis*. Microbiol Res 166:629–642

Matthies C, Gößner A, Acker G, Schramm A, Drake HL (2004) *Lactovum miscens* gen. nov., sp. nov., an aerotolerant, psychrotolerant, mixed-fermentative anaerobe from acidic forest soil. Res Microbiol 155:847–854

Moriya S, Inoue T, Ohkuma M, Yaovapa T, Johjima T, Suwanarit P, Sangwani U, Vongkaluang C, Noparatnaraporn N, Kudo T (2005) Fungal community analysis of fungus gardens in termite nests. Microbes Environ 20:243–252

Nagai F, Morotomi M, Watanabe Y, Sakon H, Tanaka R (2010) *Alistipes indistinctus* sp. nov. and *Odoribacter laneus* sp. nov., common members of the human intestinal microbiota isolated from faeces. Int J Syst Evol Microbiol 60: 1296–1302

Norris SJ, Paster BJ, Smibert RM (2010) Genus IV. *Treponema*. In: Krieg NR, Staley JT, Brown DR, Hedlund BP, Paster BJ, Ward NL, Ludwig W, Whitman W (eds) Bergey's manual of systematic bacteriology 4, 2nd edn. Springer, New York, pp 529–550

Ohkuma M, Shimizu H, Thongaram T, Kosono S, Moriya K, Trakulnaleamsai S, Noparatnaraporn N, Kudo T (2003) An alkaliphilic and xylanolytic *Paenibacillus* species isolated from the gut of a soil-feeding termite. Microbes Environ 18:145–151

Pasti MR, Belli ML (1985) Cellulolytic activities of actinomycetes isolates from termites (*Termitinae*) gut. FEMS Microbiol Lett 26:107–112

Pasti MB, Pometto AL III, Nuti MP, Crowford DL (1990) Lignin-solubilizing ability of actinomycetes isolated from termite (*Termitidae*) gut. Appl Environ Microbiol 56:2213–2218

Riviere D, Desvignes V, Pelletier E, Chaussonnerie S, Guermazi S, Weissenbach J, Li T, Camacho P, Sghir A (2009) Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge. ISME J 3:700–714

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Sanyika TW, Rashamuse KJ, Hennessy F, Brady D (2012) Luminal hindgut bacterial diversities of the grass and sugarcane feeding termite *Trinervitermes trinervoides*. Afr J Microbiol Res 6:2639–2648

Saulnier DM, Riehle K, Mistretta TA, Diaz MA, Mandal D, Raza S, Weidler EM, Qin X, Coarfa C, Milosavljevic A et al (2011) Gastrointestinal microbiome signatures of pediatric patients with irritable bowel syndrome. Gastroenterology 141:1782–1791

Schauer C, Thompson CL, Brune A (2012) The bacterial community in the gut of the cockroach *Shelfordella lateralis* reflects the close evolutionary relatedness of cockroaches and termites. Appl Environ Microbiol 78:2758–2767

Schmitt-Wagner D, Friedrich MW, Wagner B, Brune A (2003) Phylogenetic diversity, abundance, and axial distribution of bacteria in the intestinal tracts of two soil-feeding termites (*Cubitermes* spp.). Appl Environ Microbiol 69:6007–6017

Shinzato N, Muramatsu M, Matsui T, Watanabe Y (2005) Molecular phylogenetic diversity of the bacterial community in the gut of the termite *Coptotermes formosanus*. Biosci Biotechnol Biochem 69:1145–1155

Shinzato N, Muramatsu M, Matsui T, Watanabe Y (2007) Phylogenetic analysis of the gut bacterial microflora of the fungus-growing termite *Odontotermes formosanus*. Biosci Biotech Biochem 71:906–915

Sonnenburg ED, Zheng H, Joglekar P, Higginbottom SK, Firbank SJ, Bolam DN, Sonnenburg JL (2010) Specificity of polysaccharide use in intestinal *Bacteroides* species determines diet-induced microbiota alterations. Cell 141:1241–1252

Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbour-joining method. Proc Natl Acad Sci USA 101:11030–11035

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–2739

Tanaka H, Aoyagi H, Shina S, Dodo Y, Yoshimura T, Nakamura R, Uchiyama H (2006) Influence of the diet components on the symbiotic microorganisms community in hindgut of *Coptotermes formosanus* Shiraki. Appl Environ Biotechnol 71:907–917

Tartar A, Wheeler MM, Zhou X, Coy MR, Boucias DG, Scharf ME (2009) Parallel metatranscriptome analyses of host and symbiont gene expression in the gut of the termite *Reticulitermes flavipes*. Biotechnol Biofuels 2:25

Vartoukian SR, Palmer RM, Wade WG (2007) The division "Synergistes". Anaerobe 13:99–106

Visser AA, Nobre T, Currie CR, Aanen DK, Poulsen M (2012) Exploring the potential for actinobacteria as defensive symbionts in fungus-growing termites. Microb Ecol 63:975–985

von Wintzingerode F, Göbel UB, Stackebrandt E (1997) Determination of microbial diversity in environmental samples: pitfalls of PCR-based rRNA analysis. FEMS Microbiol Rev 21:213–229

Warnecke F, Luginbuhl P, Ivanova N, Ghassemian M, Richardson TH, Stege JT, Cayouette M, McHardy AC, Djordjevic G, Aboushadi N, Sorek R, Tringe SG, Podar M, Martin HG, Kunin V, Dalevi D, Madejska J, Kirton E, Platt D, Szeto E, Salamov A, Barry K, Mikhailova N, Kyrpides NC, Matson EG, Ottesen EA, Zhang X, Hernández M, Murillo C, Acosta LG, Rigoutsos I, Tamayo G, Green BD, Chang C, Rubin EM, Eric J, Mathur EJ, Robertson DE, Hugenholtz P, Leadbetter JR (2007) Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. Nature 450:560–565

Watanabe Y, Shinzato N, Fukatsu T (2003) Isolation of actinomycetes from termites' guts. Biosci Biotechnol Biochem 67:1797–1801

Werren JH, Baldo L, Clark ME (2008) *Wolbachia*: master manipulators of invertebrate biology. Nat Rev Microbiol 6:740–751

Wexler HM (2007) Bacteroides: the good, the bad, and the nitty-gritty. Clin Microbiol Rev 20:593–621

Xu J, Bjursell MK, Himrod J, Deng S, Carmichael LK, Chiang HC, Hooper LV, Gordon JI (2003) A genomic view of the human *Bacteroides thetaiotaomicron* symbiosis. Science 299:2074–2076

Zhu YY, Li J, Liu H, Yang H, Xin S, Zhao F, Zhang X, Tian Y, Lu X (2012) Phylogenetic analysis of the gut bacterial microflora of the fungus-growing termite *Macrotermes barneyi*. Afr J Microbiol Res 6:2071–2078