GROUP-SPECIFIC BACTERIAL DIVERSITY IN THE GUT OF FUNGUS-CULTIVATING TERMITES (*MACROTERMES, ODONTOTERMES* AND *MICROTERMES* SPECIES) FROM KENYA

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Abstract
The mutualistic association between termites and their gut symbionts has continued to attract the curiosity of researchers over time. The aim of this study was to characterize group-specific bacterial community structure and diversity in the gut of three fungus cultivating termites *Macrotermes michaelseni, Odontotermes* and *Microtermes* species using PCR-denaturing gradient gel electrophoresis (PCR-DGGE) analysis. Group-specific primers targeting members of the phyla *Bacteroidetes, Firmicutes* and *Planctomycetes* were used for PCR amplification of total DNA mixture extracted from termite guts. The PCR products were used as templates in a second PCR with nested bacteria DGGE-PCR primer pairs whose products were separated by DGGE. Representative DNA bands were excised from the gels, re-amplified, purified and sequenced. The sequences were blast analyzed and together with other reference sequences retrieved from the public GenBank were used to infer phylogenetic trees. All sets of sequences were deposited in the public GenBank. Results from the DGGE band patterns revealed a sharp contrast between the bacterial communities of *M. michaelseni, Odontotermes* and *Microtermes* species. This underlines the difference in group-specific bacterial diversity in the three termites. Phylogenetic analysis of the 16S rRNA gene sequences indicated that they were affiliated with the three phyla: *Bacteroidetes, Firmicutes* and *Planctomycetes.* Sequences (40%) affiliated with the phylum *Planctomycetes* were isolated and clustered with 'Termite *planctomycete* cluster', indicating that they are termite gut specific members. Sequences (89%) isolated were often affiliated with sequences obtained from other termites' guts, demonstrating that a majority of the gut bacteria are autochthonous having mutualistic relationship with their hosts. Notably, the isolated sequences had less than 96% sequence similarity with the closest cultivated strains, indicating that the majority of termite gut bacterial lineages are still uncultured. The results will help better understand the bacterial symbionts-termites mutualistic associations.

Key words: Bacterial diversity, termites, PCR-DGGE

1.0 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. Termites rely on microbes in their guts for degradation of recalcitrant components of plant biomass and this has a major influence on soil structure and carbon mineralization (Brune and Friedrich, 2000; 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). Nonetheless, the majority of the microbial species are difficult or impossible to cultivate, thus limiting our knowledge on their role in the termite gut (Breznak, 2000). Therefore, alternative culture-independent methods can be used to increase our knowledge on the symbiont-host relationships.

Different approaches including 16S rRNA gene profiling have helped determining the microbial diversity in termites without cultivation (Hongoh *et al.,* 2003, 2005, 2006; Shinzato *et al.,* 2005, 2007; Fisher *et al.,* 2007; Long *et al.,* 2010; Mackenzie *et al.,* 2010; Mathew *et al.,* 2012; Köhler *et al.,* 2012). These culture-independent studies reported high bacterial diversity in the guts of termites including some termite-specific bacterial lineages (Hongoh *et al.,* 2003; Shinzato *et al.,* 2005). Recently, metagenomics studies have greatly expanded our knowledge of gut

symbiosis by providing key information on bacterial genes required for reductive acetogenesis, fermentation, lignocellulosic digestion, 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). However, there is little information on the evolutionary relationships between symbionts and host termites and the specificity of the bacterial lineages to their hosts. This has partly been attributed by the high abundance, cryptic and unpredictable foraging patterns (Ahmed *et al.,* 2011) coupled with the enormous diversity of their gut symbionts (Makonde *et al*., 2013a).

The fungus cultivating termites comprise many of the economically important termite species (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.,* 2010; Mathew *et al.,* 2012; Zhu *et al.,* 2012, Makonde *et al.,* 2013a), comparative analysis of group specific bacterial lineages of the genera *Odontotermes, Macrotermes* and *Microtermes* has not been reported. This is important not only for detecting host specific bacterial lineages, but also for generating data that can be used to infer host-symbionts evolutionary relationships. This study, therefore, attempted to characterize group-specific bacterial community structure and diversity in the gut of three fungus cultivating termites *Macrotermes michaelseni, Odontotermes* and *Microtermes* species using PCR-denaturing gradient gel electrophoresis (PCR-DGGE) analysis. The results will contribute knowledge of the specificity and mutualistic relationship between gut bacterial symbionts and termites.

2.0 Material and Methods

2.1 Collection and Identification of Termites

Samples were collected in March, 2011 from Juja in Kiambu County, Kenya (latitude 1° 5′ 54.68′′ N, longitude 37° 1′ 1.10′′ W). Termite mounds (Designated as mound B colonized by *Macrotermes* and *Microtermes* species at the bottom and upper parts, respectively and mound C, approximately 2 km far apart was colonized by *Odontotermes* sp.) were excavated to a depth of 0.5 – 1.0 m. 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 behavior 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.,* 2013b) and comparing it to the sequences of previously identified specimens.

2.2 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 *Macrotermes mchaelseni* [JQ247993] 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 and used for total DNA extraction as described elsewhere (Makonde *et al.,* 2013b) using the UltraClean® Mega soil DNA isolation kit (MO BIO Laboratories, Inc.) according to the manufacturer's protocol.

2.3 Group-specific PCR-DGGE Analysis

The primers used were those described in other studies (Muyzer *et al.,* 1993, 1998; Mühling *et al.,* 2008). Group-specific primers (Table 1) were used to PCR amplify total DNA from guts of *Macrotermes michaelseni, Microtermes* and *Odontotermes* species. 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 Mühling *et al*., 2008 and the PCR product size was checked on 1% agarose gel stained with ethidium bromide.

PCR products were used as templates in a second PCR with nested bacteria DGGE-PCR primer pairs. PCR conditions were as described by Mühling *et al.,* (2008) but with distinct annealing temperatures (AT) as indicated in Table 1. PCR products were separated by DGGE in an 8% (w/v) polyacrylamide gel with urea and formamide as denaturants using an Ingeny phorU system (Ingeny International BV, Goes, The Netherlands). Linear denaturing gradients between 40% and 60% (Mühling *et al.,* 2008) or 35% to 80% were used. Electrophoresis was performed in 1x TAE buffer at 60° C at an initial voltage of 200 V for 5 min, followed by a constant voltage of 100 V for 16 hours.

Polyacrylamide gels were stained with SYBRGold (MoBiTec, Göttingen, Germany) for 1 hour. DNA bands of interest were excised from the gels with a sterile scalpel, and the DNA was eluted overnight at 4^{o} C in 25 µl of 10 mM Tris-HCl buffer (pH 8.0).

2.4 PCR re-amplification and Sequencing

Eluates (3 µl) were used for re-amplification as described previously using corresponding primers without a GC clamp. PCR products were directly purified and sequenced at Helmholtz Centre for Infection Research (HZI), Braunschweig, Germany. Trace files were manually edited and assembled using Invitrogen vector NTI 11.5 software. Bands that resulted in poor sequences were cloned into pGEM-T® Easy vector system II (Promega) according to the manufacturer's recommendations 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). Representative clones were selected and then sequenced as described above. All sets of sequences were deposited in GenBank under the accession numbers JX421956 to JX421963 (DGGE *Bacteroidetes* sequences), JX421964 to JX421986 (DGGE *Firmicutes* sequences) and JX421987 to JX422007 (DGGE *Planctomycetes* sequences).

2.5 Phylogenetic Analysis

The sequences were quality checked for chimeric structures using the Mallard program (Ashelford *et al.,* 2006). A search for similar sequences using BLASTN (Altschul *et al.,* 1990) was performed, and sequence alignment was performed using the CLUSTAL Omega program (http://www.clustal.org) against the nearest neighbours. A neighbor-joining tree of the aligned sequences was constructed (Saitou and Nei, 1987) using MEGA V5.10 (Tamura, 2011). Evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura, 2004). Bootstrapping (Felsenstein, 1985) was conducted with 1000 replicates to obtain statistical support values. All sites, including gaps in the sequence alignment, were excluded pairwise in the phylogenetic analysis. The taxonomic assignment was confirmed at an 90% confidence level using the naïve Bayesian rRNA classifier on the RDP website (Cole *et al.,* 2005).

3.0 Results

3.1 Nested PCR-DGGE Analysis

Comparative analysis of the DGGE profiles from the gut contents of three termites (*Macrotermes michaelseni, Odontotermes* and *Microtermes* spp.) displayed visual differences in the DGGE band patterns (Figure 1). *Bacteroidetes* DGGE profiles had poor resolution and only three bands for *Odontotermes* sp. could be resolved. Likewise five bands for *Microtermes* sp. were analyzed (Figure 1A). Members from the phylum *Bacteroidetes* could not be resolved by DGGE analysis in *M. michaelseni* thus no clear bands were observed. The *Firmicutes* DGGE profile for *Microtermes* sp. displayed nine bands but only seven bands could be analyzed (Fig. 1B). Likewise, the DGGE profile for *Odontotermes* sp. had seven bands with different intensities, which were analyzed while nine bands for *M. michaelseni* were analyzed. Notably, band numbers 9, 10, 15, 16 and 17 were shared (Fig. 1B). The *Planctomycetes* DGGE profiles showed seven bands for *Odontotermes* sp., but displayed eight bands for *Microtermes* sp. and six bands for *M. michaelseni.* However, band numbers 24, 25, 28, 29 and 36 were shared by either two or all termites (Figure 1C, Table 2).

3.2 Phylogenetic Affiliation of Sequences

The 16S rDNA sequence analysis of the bands excised from DGGE gels revealed that the bands were affiliated with the phyla *Bacteroidetes, Firmicutes* and *Planctomycetes.* Phylogenetic analysis of the sequences grouped the respective bacterial phyla together with corresponding sequences previously isolated from termite guts (Figure 2A, B & C). Most of the newly isolated *Bacteroidetes* sequences were closely affiliated (97-99% sequence similarity) with other sequences obtained from gut of termites. The sequence DGGEBB8 [JX421763] was phylogenetically closely related to bacterial clones isolated from cockroach and *Pachnoda ephipiata* [FJ374177] (with 96-97% sequence identity) while sequences DGGEBB5-6 [JX421959-61] had less than 96% sequence similarity with clone sequences from sediments cockroach and human feces (Figure 1A; Table 2). This scenario was also observed for

the newly isolated *Firmicutes* and *Planctomycetes* sequences where majority of them were related to other sequences, which were often obtained from other termites (Table 2).

In the Phylogenetic tree of the *Firmicutes* (Figure 2B), two main clusters were formed. One cluster had three isolated sequences (DGGEFB9, DGGEF10 and DGGEF22) that were affiliated with *Lactococcus lactis* and other clones isolated from vegetable, termite gut and mouse (sequence identity of <97% with a bootstrap value of 99%). The other seven isolated sequences (DGGEFB12, DGGEFB14, DGGEFB15, DGGEFB16, DGGEFB17, DGGEFB21 and DGGEFB23) formed the second cluster (with a bootstrap value of 100%) and were often affiliated (sequence identity of 96-98%) with clone sequences from *Nasutiternes takasagoensis* and *Cubitermes orthognathus.* For the phylogenetic tree of the phylum *Planctomycetes*, only a single main cluster was formed with all newly isolated sequences affiliated (95-99% sequence similarity) with *Planctomycete* clones from different termite guts (*Microcerotermes, Cubitermes* spp., *Coptotermes formosanus* and *Reticulitermes* spp.) as indicated in Figure 2C and Table 2. Some of the sequences (highlighted in green) were shared between the termites. This indicates the presence of termite gut specific bacterial lineages previously observed in the phylum *Firmicutes.* The blast comparison and phylogenetic analysis also indicted that the co-migrating bands were 100% identical (Figure 2A, B & C; Table 2).

4.0 Discussion

The phylogenetic analysis of the group-specific bacterial 16S rRNA genes from the three termites revealed bacterial communities that are still uncultured. The affiliation of the isolated DGGE sequences with others previously obtained from termite guts underscores the existence of termite specific bacterial lineages (Hongoh *et al*., 2006; Shinzato *et al.,* 2007; Warnecke *et al.,* 2007; Makonde *et al.,* 2013a). The co-migration of some bands indicated that some of the bacterial lineages are shared in these termites. This scenario, however, can also emanate from either random acquisition of microorganisms from the environment (Curtis *et al.,* 2004) or variation in the hosts' diets (Tanaka *et al.,* 2006). Members from the phylum *Bacteroidetes* could not be resolved by DGGE in *M. michaelseni.* This could be due to poor separation of the bands as a result of the denaturation gradient (35-80%) used. The presence of unique DGGE bands in each termite may demonstrate the presence of termite genera specific bacterial lineages.

Previously, members of the phylum *Bacteroidetes* were detected as the dominant phylogenetic groups using 16S rRNA clone based methods (Hongoh *et al.,* 2006; Shinzato *et al.,* 2007; Mackenzie *et al.,* 2010; Zhu *et al.,* 2012, Makonde *et al.,* 2013a). However, the low resolution in the DGGE analysis limited the detection of majority members, which demonstrates the pitfall of the DGGE method in *Bacteroidetes* profiling. Notably, members of *Bacteroidetes* have been shown to play important roles in the termites; for instance, members of the genera *Prevotella* (*Prevotella ruminicola* 23 and *Prevotella intermedia* 17) can ferment both xylan and cellulose through carbohydrate-active enzymes such as xylanase, carboxymethylcellulase and endoglucanase (http:/www.cazy.org). Kodama *et al.* (2012) reported a *Bacteroidetes* isolate, *Dysgonomonas oryzarvi* from a microbial fuel cell, which implicates it in cellulose degradation. Genome studies have revealed *Bacteroides* involvement in biodegradation of polysaccharides and fermentation of sugars (Xu *et al.,* 2003; Sonnenburg *et al.,* 2010) by contributing glycosyl hydrolases for their hosts' digestion (Liu *et al.,* 2011). Some members of *Bacteroidetes* also benefit their host by excluding potential pathogens from colonizing the gut (Wexler, 2007).

The DGGE profile for the phylum *Firmicutes* indicated bands with different intensities, demonstrating difference in relative abundances of the respective bacterial members. Moreover, it shows the DGGE method can better resolve members of the *Firmicutes* (Fig. 2B). The unique DGGE bands (DGGEBB12-14 [421970-72], DGGEBB21-23 [421984-86]) detected in each termite gut emphasize specific bacterial-hosts associations while the presence of co-migration bands (DGGEBB9 [421964-66], DGGEBB10 [421967-68], DGGEBB15 [421973-75], DGGEBB16 [421976-78], DGGEBB17 [421979-80]) underlines the termite gut specific bacterial lineages previously reported (Hongoh *et al*., 2006; Shinzato *et al.,* 2007; Warnecke *et al.,* 2007; Makonde *et al.,* 2013a). Most of the newly isolated sequences were closely related to other clone sequences from termites. The termite gut specific bacteria may have important role for the survival of the termites. Moreover cultivated members of *Firmicutes* such as *Acetivibrio*

cellulosolvens, Acetivibrio multivorans, Acetivibrio ethanolgignens and *Acetivibrio cellulolyticus* (Tanaka *et al.,* 1991; Xu *et al.,* 2003) have been reported to ferment cellulosic substrates.
Notably, using the group-specific PCR-DGGE fingerprinting method, more 16S rRNA gene sequences affiliated with the phylum *Planctomycetes* were captured compared to previous clone-based analysis (Mackenzie *et al.,* 2010; Zhu *et al.,* 2012, Makonde *et al.,* 2013a) which comprehensively described the dominant phyla. All of the *Plactomycete* sequences clustered with those from other termites and belong to a large clade, the 'Termite *Planctomycete* cluster' (Köhler *et al.,* 2008), indicating that they are termite gut specific members. Again, this demonstrates that the low abundance of *Planctomycetes* previously detected in the gut of other fungus-cultivating termites (Shinzato *et al.,* 2005; Hongoh *et al.,* 2006; Mackenzie *et al.,* 2010; Long *et al.,* 2010; Zhu *et al.,* 2012) could be as a result of PCR bias (von Wintzingerode *et al.,* 1997). This, however, needs to be substantiated with more samples using general bacterial primers and group-specific primers.

5.0 Conclusion

The DGGE analysis confirmed the presence of common gut bacterial species in the investigated termites (*M. michaelseni, Odontotermes* and *Microtermes* spp.) that could be important for the termites' life styles. The presence of unique DGGE banding patterns for each termite may indicate termite genus-specific bacterial lineages. Though the method used in this study cannot help infer physiological roles for the uncultured bacteria in the termites, the findings provide more knowledge on specific bacterial community structure in the guts of fungus-cultivating termites and contribute to understanding gut bacterial diversity and their associations with termites.

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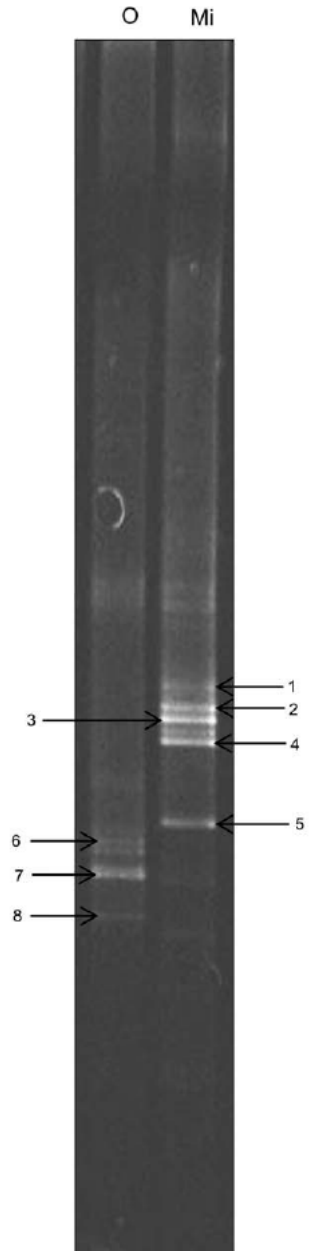
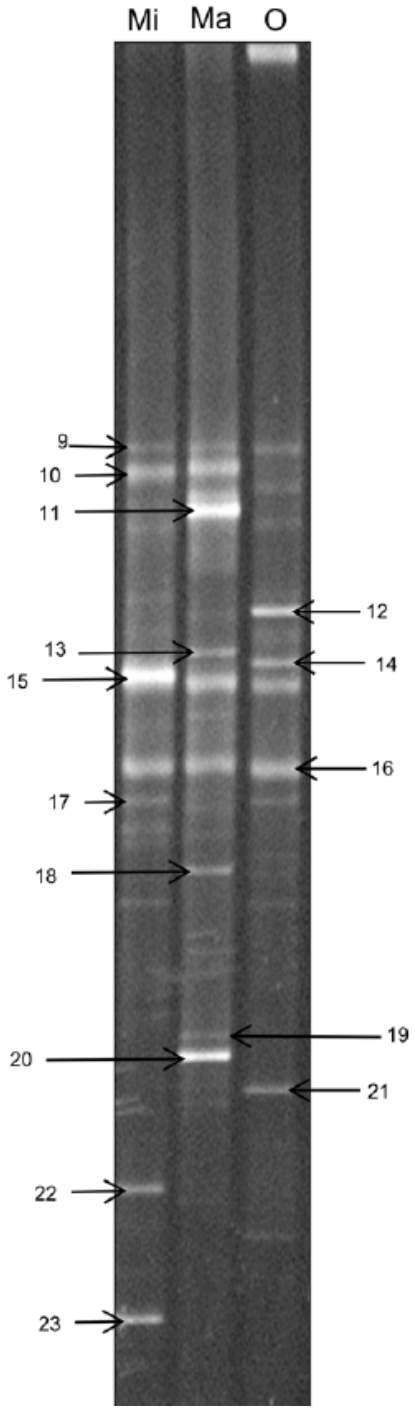
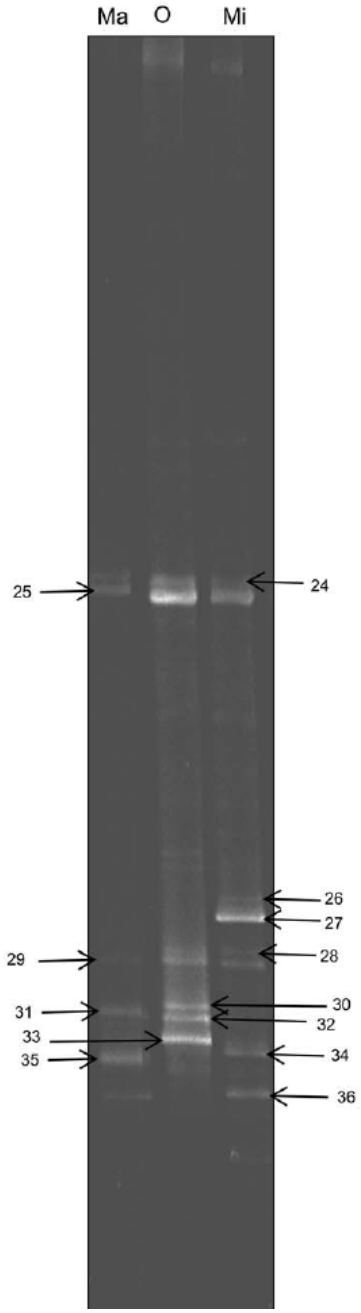
Figure 1

A. *Bacteroidetes*





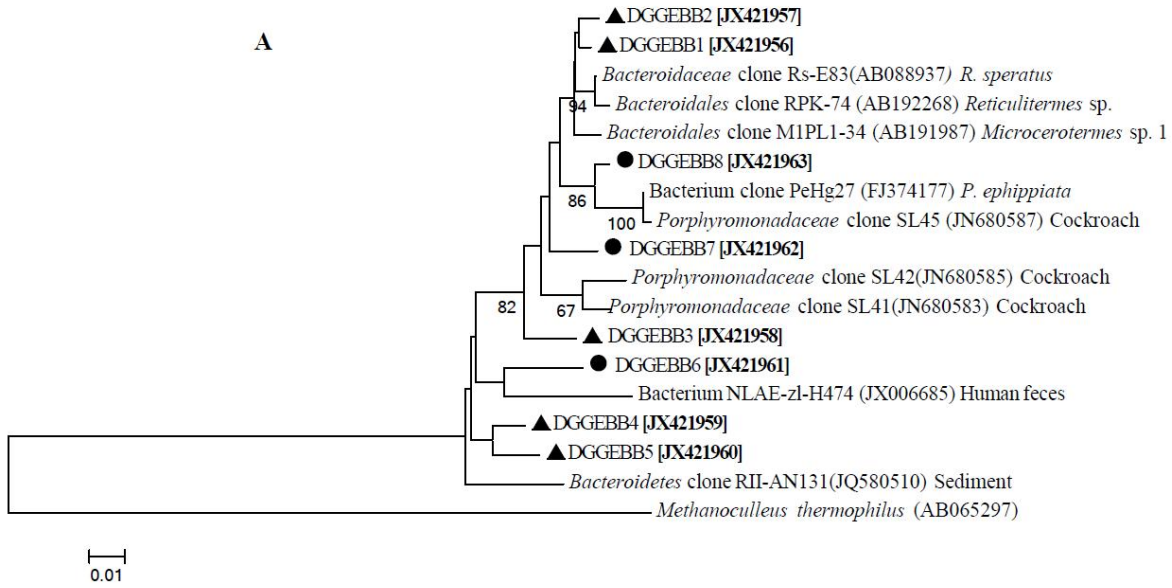
B. Firmicutes

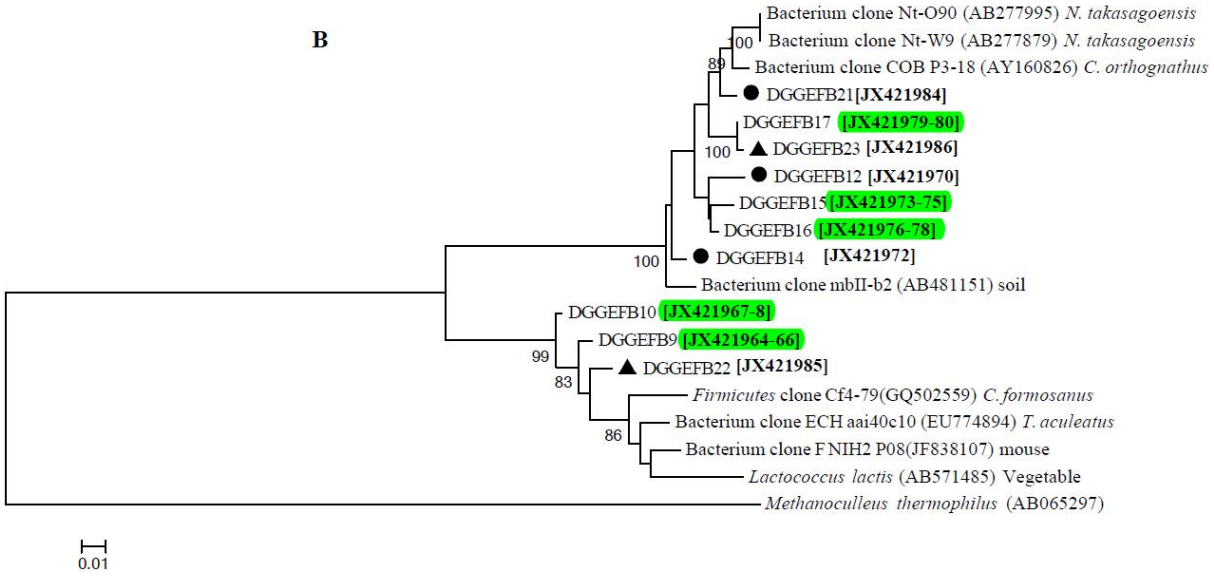


C. *Plantomycetes*

Figure 1: Nested PCR-DGGE band patterns for the phylum A. Bacteroidetes,B. Firmicutes and C. Planctomycetes for termites. Lanes: 'O' denotes Odontotermes sp., 'Ma' denotes M. michaelseni and 'Mi' denotes Microtermes sp. Numbers and arrows indicate DNA bands that were excised and sequenced. Co-migrating bands are indicated by one number

Figure 2


A
DGGEBB2 [JX421957]
DGGEBB1 [JX421956]
Bacteroidaceae clone Rs-E83(AB088937) R. speratus
Bacteroidales clone RPK-74 (AB192268) Reticulitermes sp.
Bacteroidales clone M1PL1-34 (AB191987) Microcerotermes sp. 1
DGGEBB8 [JX421963]
Bacterium clone PeHg27 (FJ374177) P. ephippiata
Porphyromonadaceae clone SL45 (JN680587) Cockroach
DGGEBB7 [JX421962]
Porphyromonadaceae clone SL42(JN680585) Cockroach
Porphyromonadaceae clone SL41(JN680583) Cockroach
DGGEBB3 [JX421958]
DGGEBB6 [JX421961]
Bacterium NLAE-zl-H474 (JX006685) Human feces
DGGEBB4 [JX421959]
DGGEBB5 [JX421960]
Bacteroidetes clone RII-AN131(JQ580510) Sediment
Methanoculleus thermophilus (AB065297)
94
86
100
82
67
0.01


B
Bacterium clone Nt-O90 (AB277995) N. takasagoensis
Bacterium clone Nt-W9 (AB277879) N. takasagoensis
Bacterium clone COB P3-18 (AY160826) C. orthognathus
DGGEFB21[JX421984]
DGGEFB17 [JX421979-80]
DGGEFB23 [JX421986]
DGGEFB12 [JX421970]
DGGEFB15[JX421973-75]
DGGEFB16 [JX421976-78]
DGGEFB14 [JX421972]
Bacterium clone mbII-b2 (AB481151) soil
DGGEFB10 [JX421967-8]
DGGEFB9 [JX421964-66]
DGGEFB22 [JX421985]
Firmicutes clone Cf4-79(GQ502559) C.formosanus
Bacterium clone ECH aai40c10 (EU774894) T. aculeatus
Bacterium clone F NIH2 P08(JF838107) mouse
Lactococcus lactis (AB571485) Vegetable
Methanoculleus thermophilus (AB065297)
100
89
100
100
99
83
86
0.01

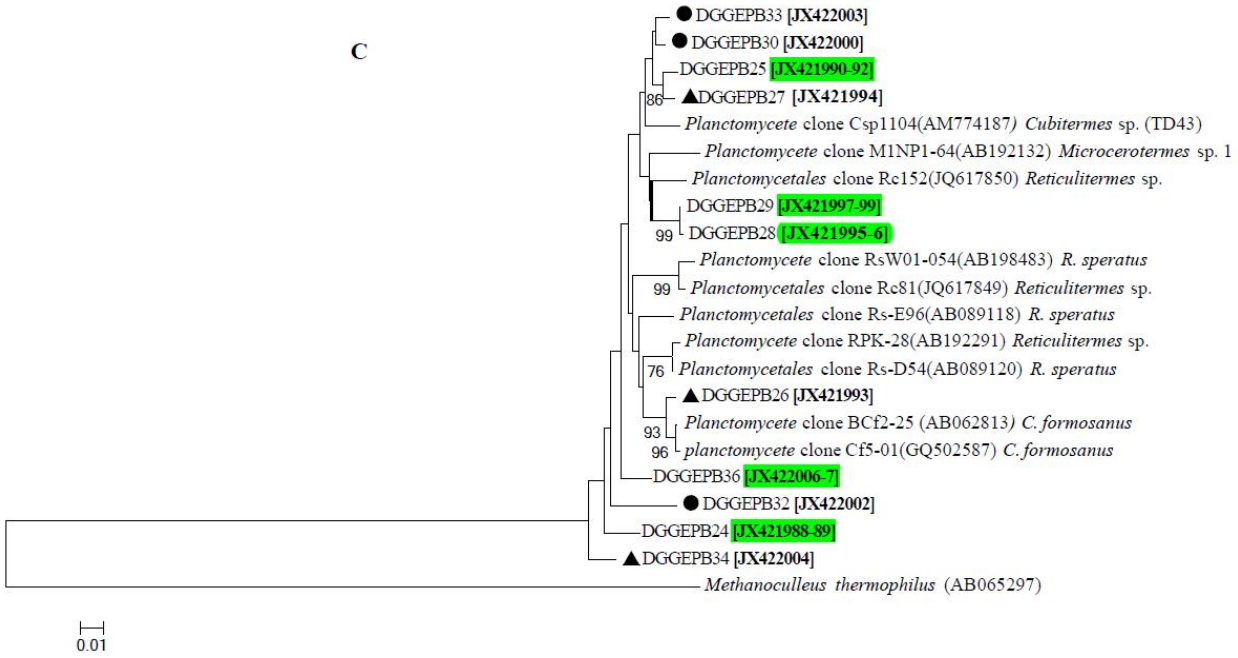


Figure 2: Evolutionary relationships between partial 16S rRNA gene sequences and selected taxa in the phyla A. Bacteroidetes, B. Firmicutes and C. Planctomycetes. The DGGE sequences highlighted in green were shared by either two or the three termites. Methanoculleus thermophiles (accession number, AB065297) was used to root the trees

Table 1: *Primers used for group-specific bacterial amplification. Primer sequences are described below*

Target Group	Primers used for group-specific PCR$_a$	AT PCR (^{0} C)	Primers used for re-PCR for DGGE	AT nested PCR (^{0} C)	Denaturing gradient used for DGGE (%)
Firmicutes	Firm350f/814r	58	518f-GC/785r	57	40-60
Planctomycetes	Plancto352f/920r	68	518f-GC/907r	60	40-60
Bacteroidetes	CFB555f/968r	61	CFB555f-GC/907r	58	35-80

Key:

Firm350f: 5'-GGC AGC AGT RGG GAA TCT TC-3' (Mühling *et al.,* 2008),

Firm814r: 5'-ACA CYT AGY ACT CAT CGT TT-3' (Mühling *et al.,* 2008),

Plancto352f: 5'-GGC TGC AGT CGA GRA TCT-3' (Mühling *et al.,* 2008),

Plancto920r: 5'-TGT GTG AGC CCC CGT CAA-3' (Mühling *et al.,* 2008),

CFB555f: 5'-CCG GAW TYA TTG GGT TTA AAG GG-3' (Mühling *et al.,* 2008),

CBF968r: 5'-GGT AAG GTT CCT CGC GTA-3' (Mühling *et al.,* 2008),

907r: 5'-CCG TCA ATT CMT TTG AGT TT-3' (Muyzer *et al.,* 1993),

518f: 5'-CCA GCA GCC GCG GTA AT-3' (Muyzer *et al.,* 1993),

785r: 5'-CTA CCA GGT ATC TAA TCC-3' (Lee *et al.,* 1993).

GC clamp: CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG G (Muyzer *et al.,* 1998),

'AT' denotes annealing temperature.

Table 2: List of DGGE bands for termite guts 16S rRNA Gene sequences and their closest affiliations

		Accession number					
Phylum	**Band No:[a]**	**O**	**Ma**	**MI**	**Taxonomical Affiliation/ isolation source**	**Accession no.**	**% ID**
Bacteroidetes	1	-	-	JX421956	Clone Rs-E83 (*R. speratus*)	AB088937	99
	2	-	-	JX421957	Clone M1PL1-34 (*Microcerotermes* sp. 1)	AB191987	98
	3	-	-	JX421958	Clone RPK-74 (*Reticulitermes* sp.)	AB192268	96
	4	-	-	JX421959	Clone RII-AN131 (Sediment)	JQ580510	95
	5	-	-	JX421960	Clone SL42 (Cockroach)	JN680585	94
	6	JX421961	-	-	Bacterium NLAE-zl-H474 (Human feces)	JX006685	93
	7	JX421962	-	-	Clone SL41 (Cockroach)	JN680583	96
	8	JX421963	-	-	Bacterium clone PeHg27 (*P. ephippiata*)	FJ374177	97
Firmicutes	**9**	JX421964	JX421966	JX421965	Firmicutes clone Cf4-79 (*C. formosanus*)	GQ502559	95
	10	-	JX421968	JX421967	Bacterium clone F_NIH2_P08 (Mouse)	JF838107	94
	12	JX421970	-	-	*Lactococcus lactis* (Vegetable)	AB571485	96

	13	-	JX421971	-	Bacterium clone COB P3-18 (*C. orthognathus)*	AY160826	96
	14	JX421972	-	-	Bacterium clone mbII-b2 (soil)	AB481151	98
	15	JX421975	JX421973	JX421974	Bacterium clone COB P3-18 (*C. orthognathus)*	AY160826	97
	16	JX421977	JX421976	JX421978	Bacterium clone COB P3-18 (*C. orthognathus)*	AY160826	96
	17	JX421979	-	JX421980	Bacterium clone COB P3-18 (*C. orthognathus)*	AY160826	98
	20	-	JX421983	-	Bacterium clone ECH_aai40c10 (*T. aculeatus*)	EU774894	96
	21	JX421984	-	-	Bacterium clone COB P3-18 (*C. orthognathus)*	AY160826	96
	22	-	-	JX421985	*Lactococcus lactis* (Vegetable)	AB571485	96
	23	-	-	JX421986	Bacterium clone COB P3-18 (*C. orthognathus)*	AY160826	97
Planctomycetes	**24**	JX421987	-	JX421988	Planctomycete clone M1NP1-4 (*Microcerotermes* sp. 1)	AB192132	96
	25	JX421991	JX421992	JX421990	Planctomycete clone BCf2-25 (*C. formosanus*)	AB062813	97
	26	-	-	JX421993	Planctomycete clone Cf5-01 (*C. formosanus*)	GQ502587	99
	27	-	-	JX421994	Planctomycete clone BCf2-25 (*C. formosanus*)	AB062813	97
	28	JX421996	-	JX421995	Planctomycetales clone Rc152	JQ617850	97

					(*Reticulitermes* sp.)		
	29	JX421998	JX421999	JX421997	Planctomycetales clone Rc152 (*Reticulitermes* sp.)	JQ617850	97
	30	JX422000	-	-	Planctomycete clone Csp1104 (*Cubitermes* sp.)	AM774187	97
	32	JX422002	-	-	Planctomycetales clone Rs-E96 (*R. speratus*)	AB089118	95
	33	JX422003	-	-	Planctomycete clone BCf2-25 (*C. formosanus*)	AB062813	97
	34	-	-	JX422004	Planctomycete clone RsW01-054 (*R. speratus*)	AB198483	95
	36	-	JX422006	JX422007	Planctomycetales clone Rs-E96 (*R. speratus*)	AB089118	97

Key: 'DGGEBB' denotes denaturing gradient gel electrophoresis Bacteroidetes band, 'DGGEFB' denotes denaturing gradient gel electrophoresis Firmicutes band, and 'DGGEPB' denotes denaturing gradient gel electrophoresis Planctomycetes band. Number in bold indicates bands that were shared. '-'denotes absence of band, 'O' denotes Odontotermes sp., 'Ma' denote Macrotermes michaelseni and 'Mi' denotes Microtermes sp.. [a]Number in bold indicates bands that were shared. '-'denotes absence of band sequence.