Microecology of the termite gut: structure and function on a microscale
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Long considered simply as anoxic fermentors, termite guts are in fact axially and radially structured environments with physicochemically distinct microhabitats. Recent developments in termite gut microecology, which combined traditional and modern techniques, have focused on the spatial organization of important microbial populations and their in situ activities, and have significantly furthered our understanding of functional interactions within highly structured microenvironments.

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Introduction
The symbiotic association of termites with their diverse intestinal microbiota has many facets, touching on subjects in microbiology, biochemistry, protozoology, insect physiology and ecology, sociobiology, evolutionary biology, and even in atmospheric chemistry [1–3,4†]. For the microbial ecologist, termite guts represent excellent models of highly structured microenvironments, which allow case studies of, for example, microbial diversity and community structure, physiological adaptations to various environmental factors, metabolic interactions among microbial populations, and carbon and electron fluxes at the community level.

The intestinal tracts of termites comprise one or several dilated hindgut compartments, which harbor the bulk of the intestinal microbiota and were initially considered as ‘fermentation chambers’ analogous to the rumen of sheep and cattle (i.e. anoxic environments for an anaerobic, oxygen-sensitive microbiota). The most important metabolic activities traditionally attributed to the gut microbiota are, first, hydrolysis of cellulose and hemicelluloses, second, fermentation of the depolymerization products to short-chain fatty acids, which are then resorbed by the host, and third, intestinal nitrogen cycling and dinitrogen fixation [5,6*]. In the phylogenetically lower termites, a large fraction of hindgut volume (up to one-third of the body weight of a termite) is occupied by anaerobic flagellates, which phagocytize and degrade the wood particles comminuted by the termite [7†]. However, all phylogenetically higher termites, which represent the majority of all termite species, do not harbor flagellates within their gut. Instead, an acquisition of cellulases with the food (in case of the fungus-cultivating termites) or a host origin of the cellulolytic activities has been suggested [5].

Such simplistic concepts may appear rather appealing, but they have their obvious limitations. Driven by the methodological progress of the past decade, new aspects of termite gut microbiology have emerged, and new concepts are being developed. In this review, we summarize the most recent advances and outline future focus areas. Because of space limitation, the phylogeny of gut flagellates will not be treated. For more background information, the reader is referred to a number of previous reviews [5,6*,7†,8] and to a forthcoming monograph [4†].

Radial and axial compartmentation
The existence of anoxic conditions in termite guts is not trivial since the steep oxygen gradients at the oxic–anoxic interface drive a continuous influx of O2 into the gut [6*]. Microsensor measurements showed that O2 may penetrate 50–200 μm into the gut before it is removed by the respiratory activity of the gut microbiota, creating a microoxic periphery around an anoxic center (Figure 1). Because of the small gut diameter, the microoxic zones in the dilated hindgut compartments represent a substantial part of their volume, and the connecting tubular regions are often completely oxic ([6*,9†] and references therein).

Owing to their enormous surface-to-volume ratio, the small guts receive high O2 fluxes per unit volume [6*]. Radiotracer studies performed with the lower termite Reticulitermes flavipes have shown that the influx of O2 via the gut epithelium and its subsequent reduction in the hindgut periphery has a significant impact on carbon and electron flow within the hindgut microbial community [10**]. The substantial accumulation of H2 in the anoxic hindgut lumen and its consumption in the gut periphery give rise to steep gradients directed towards the gut epithelium (Figure 1) and indicate that sources and sinks for H2 are spatially separated [11]. In situ measurements showed that methanogenesis and reductive acetogenesis consume about 4% and 31%, respectively, of the theoretical electron flux through the H2 pool, but there is strong evidence that almost the same proportion of reducing equivalents is consumed directly or indirectly by O2 reduction in the gut periphery [10**].

Surprisingly, the hindgut epithelium of R. flavipes is densely colonized by methanogenic Archaea [12,13]. Methane emission rates of live termites are considerably stimulated by externally added H2 [11], whereas in situ rates of reductive acetogenesis from 14CO2 remain unaffected [10**]. These observations indicate that only the methanogens located at the gut epithelium are H2-limited in situ, which is indirect evidence for a luminal...
Localization of the H$_2$-oxidizing acetogens and supports the hypothesis that the coexistence of methanogens and homoacetogens in this termite is based on the spatial arrangement of the respective populations [6*].

The intestinal flagellates are considered the major source of H$_2$ in lower termites [7*,14]. The gut protozoa are often associated with methanogens and possibly other H$_2$-oxidizing prokaryotes colonizing cytoplasm and exterior surfaces. In contrast to the wall-associated populations, these endobionts and epibionts of the intestinal flagellates are most likely not H$_2$-limited, which implies that the location of the methanogenic populations relative to the H$_2$ source will affect the rate of intestinal CH$_4$ production [5,6*]. It has been postulated that also the inter- and intra-specific differences in H$_2$ emission rates of the termites will depend on the presence and the location of the methanogens (i.e. whether they are attached to the gut wall or associated with the protozoa) [15].

Apart from the radial organization of the gut and its microbiota, the axial differentiation of the hindgut also needs to be considered. While many of the phylogenetically higher termites consume wood and litter at different stages of decay and humification, more than half of all termite genera are humivorous (i.e. they feed on soil organic matter) [2]. The hindgut of soil-feeding termites is highly compartmentalized and characterized by pronounced axial dynamics of the O$_2$ and H$_2$ partial pressure, and intestinal pH (Figure 2). Consequently, it is not surprising that the microbial processes such as H$_2$ production, methanogenesis, and reductive acetogenesis are also unevenly distributed [9*,16*]. Methanogenesis represents an important electron sink, and CH$_4$ emission by termites contributes significantly to the global methane budget [1]; however, recent results underline that the reoxidation of CH$_4$ by microorganisms in the nest material needs to be taken into account [17].

At the low H$_2$ partial pressures in the posterior hindgut, methanogens should outcompete homoacetogens for this electron donor, which would explain the apparent predominance of methanogenesis over reductive acetogenesis in soil-feeding termites [14]. Nevertheless, there is evidence for a coexistence of both metabolic groups in the same compartments [9*,16*], and it has been pointed out that the proximity of the different gut segments in situ (Figure 3) would allow a cross-epithelial diffusion of H$_2$ from the anterior (ms–P3) to the posterior (P4a–P5) gut regions, which would create microhabitats favorable for H$_2$-dependent acetogenesis.
Except in the fungus-cultivating Macrotermiteinae, the anterior hindgut compartments of higher termites are generally quite alkaline [18]. Gut alkalinity in the soil-feeding Termitinae exceeds pH 12 (Figure 2) and belongs to the highest pH values ever encountered in biological systems [19]. Although hindgut alkalinity is not necessarily an adaptation to soil feeding per se [18], it has been demonstrated to enhance solubilization and chemical oxidation of soil organic matter [20•].

**Hindgut carbon and electron flow**

A concrete model of the metabolic processes exists only for the hindgut of wood-feeding lower termites. The results obtained with suspensions of hindgut protozoa and with the few pure cultures of the fastidious flagellates ever available indicate that the anaerobic protozoa ferment polysaccharides to acetate, CO₂, and H₂. On the basis of the high potential activities of H₂-dependent acetogenesis from CO₂ in wood-feeding termites, it was initially assumed that the combined metabolic activities of the flagellates and the hydrogenotrophic acetogens render the digestion of lignocellulose in these termites an overall homoacetogenic process [5,14]. However, microinjection of radiolabeled metabolites into intact, agarose-embedded hindguts of R. flavipes showed that the in situ rates of reductive acetogenesis represent only 10% of the total carbon flux in a living termite, whereas 30% of the carbon flux proceeds via lactate [10••]. The rapid turnover of the intestinal lactate pool consolidates the presence of lactic acid bacteria with the low lactate concentrations in the hindgut fluid of R. flaveipes [21,22•].

Since the gut protozoa of lower termites are essential for termite survival [5,7•], it was initially assumed that the cellulose-degrading ability of all termites is conferred by cellulolytic microorganisms. However, the only lower termites harbor anaerobic protozoa, and many of the smaller flagellate species do not ingest particles and may be involved only in the fermentation of soluble substrates [7•]. There is also no indication for a presence of larger numbers of cellulolytic bacteria in higher termites. Instead, there is now compelling evidence that cellulases are endogenously formed by the salivary glands or the midgut epithelium of lower [23,24] and higher termites [25], respectively. The eukaryotic origin of the endoglucanases has been firmly established [26•,27••].

Consequently, it has to be assumed that the depolymerization products (glucose, cellobiose) are absorbed by the midgut epithelium and never reach the hindgut. This may explain the relatively low potential rates of glucose turnover in ruptured or intact hindguts of Nasutitermes walkeri [28] and R. flaveipes [10••], but raises questions regarding the substrate(s) of the hindgut microbiota. In view of the general lack of pyruvate dehydrogenase activity in termite tissues [5,28] and the high rates of pyruvate turnover by the hindgut contents, it has been suggested that N. walkeri may secrete pyruvate into the hindgut, which is subsequently converted to acetate by the gut microbiota [28]. Small activities of pyruvate dehydrogenase were recently detected in gut-free extracts of the lower termite Coptotermes formosanus [29].

There is strong evidence that the hindgut microbiota of Reticulitermes speratus may be at least partly fueled by hemi-celluloses, since the bulk of the xylanolytic activity in this termite is provided by the symbiotic flagellates [23]. Also lignin-derived aromatic compounds are likely to be substrates for aerobic bacteria present in the hindgut of Reticulitermes santonensis and other termites [30]. However, there is still no conclusive evidence that the hindgut microbiota degrades aromatic compounds under anoxic conditions or that insoluble, polymeric lignin is mineralized to a significant extent (see [5]).

On the basis of the unique hindgut specialization of soil-feeding termites and in view of the low cellulose content of soil organic matter, it has been postulated that soil-feeding species do not thrive on plant polysaccharides but on humus components as the principal source of nutrition [2,6•]. Feeding experiments with specifically radiolabeled humic model compounds indicated that soil-feeding termites mineralize the peptide component but not the polyphenolic fraction of humic substances [31•,32]. Spectroscopic methods such as 13C NMR [33] and stable-isotope techniques (see [34] for a review) may help to further elucidate the carbon source(s) of soil-feeding termites and to identify important metabolic activities in their intestinal tracts.

To understand the functional ecology of the termite gut, all important metabolic activities and corresponding carbon and electron fluxes within the system must be identified and
quantitated. Only in the case of rate-limiting steps (e.g. in polymer degradation) do the transformation rates or enzyme activities exhibited by gut homogenates or in the extracellular gut fluid suffice to estimate the relevance of the respective process. Whenever the substrate concentration limits the turnover rate, in situ techniques are necessary to correctly determine metabolic fluxes.

**Hindgut microbial diversity**

The hindgut of *Reticulitermes flavipes*, which is probably the best-investigated termite from a microbiological viewpoint, harbors at least six species of flagellates [35] and 20–30 different bacterial morphotypes [5]. This is not astonishing, since a high diversity of niches in the hindgut microbial community would lead one to expect an equally large diversity among the microorganisms filling those niches. However, when direct microscopy counts of the microorganisms in the *R. flavipes* hindgut are compared with the sum of all viable counts of the lactic acid bacteria, enterococci, strict aerobes, and methanogens, which predominate in plate counts or liquid serial dilutions [12,21], it is apparent that about 90% of all prokaryotes in the hindgut escape cultivation. A similar (or even worse) situation is seen in other termites.

A clear indication of hindgut biodiversity can be obtained also with molecular methods that allow the detection and identification of microorganisms without the need for cultivation (i.e. analysis of 16S rRNA genes [36•]). Typically, the diversity of bacterial 16S rRNA genes in termite guts is high; the major groups that were detected comprise Proteobacteria, spirochetes, the Bacteroides group, and the low G + C Gram-positive bacteria ([37]; for a summary of several earlier papers, see [38]). However, studies performed at the domain level often lack resolution of diversity, especially if only a small number of clones is investigated.

A higher resolution can be achieved by using group-specific primers prior to cloning, as demonstrated by Lilburn et al. [39**], who targeted the intestinal spirochetes of *R. flavipes* with a specific PCR assay. Spirochetes are a morphologically diverse group and may account for as much as 50% of all prokaryotes in some termites. The 12–15 spirochete morphotypes in *R. flavipes* were paralleled by 21 different spirochete phyotypes, which were assigned to two major clusters of treponemes within the phylogenetic radiation of spirochetes [39**•]. Highly diverse *Treponema*-related clones were also recovered from a variety of other termites [39**,40].

Recently, Leadbetter et al. [41••] were the first to isolate two gut spirochetes from *Zootermopsis angusticollis* in pure culture. These isolates group with the *Treponema* branch that contains mainly spirochetal 16S rRNA gene sequences obtained from termite guts [39**•]. Surprisingly, both isolates were capable of H2-dependent acetogenesis from CO2 [41••]. This finding is most important, because it represents the first clue regarding the so far unknown metabolic function of the spirochetes colonizing the hindgut lumen and the surfaces of many intestinal protozoa. It is tempting (but probably still too early) to conclude that spirochetes are responsible for the large potential activities of H2-dependent acetogenesis encountered in most wood-feeding termites [14].

Lactic acid bacteria are typical and numerically significant carbohydrate-utilizing microorganisms in the guts of many wood- and soil-feeding lower and higher termites [21,22••]. Their presence in *R. flavipes* was confirmed by 16S rRNA fingerprinting of the total hindgut community [22•], but no sequences of lactic acid bacteria were present among those from 16S rDNA clone libraries of the gut community of *R. speratus* [42]. Isolates obtained from the hindguts of *R. flavipes* and *Thoracotermes macrothorax* show a considerable genetic diversity, and comprise strains belonging to the genera *Enterococcus* and *Lactococcus* [22•]. All isolates proved to be aerotolerant and exhibit high potential rates of O2 reduction in the presence of fermentable substrates, which may explain why they are regularly encountered in the intestinal tracts of termites and other insects [22•].

Also sulfate-reducing bacteria seem to be common inhabitants of the intestinal tracts of many different termite species [43,44]. All isolates are related to free-living sulfate reducers of the genus *Desulfovibrio* and show high rates of O2 reduction in the presence of H2 [43], a phenomenon previously reported for *Desulfovibrio* species isolated from sediments. Since members of this genus are metabolically quite versatile, also other potential functions of these bacteria within the gut habitat have been proposed [43].

Another abundant group of microorganisms in termite guts is methanogenic Archaea. Because of their typical autofluorescence of coenzyme F420, they are easily visualized by epifluorescence microscopy. They are usually attached to the gut wall or associated with the anaerobic protozoa of lower termites, but also occur free within the gut fluid [5]. So far, however, only three species of methanogens from termites guts have been isolated in pure culture. All isolates are from the hindgut of *R. flavipes*, where they form large populations attached to the luminal side of the gut epithelium, and were identified as members of the genus *Methanobrevibacter* [12,13]. They grow best with H2 and CO2, and seem to be somewhat O2-tolerant due to the presence of catalase activity, which may explain their ability to colonize the microoxic gut periphery [12].

Methanogens are among the few groups of organisms where one can infer metabolic information from the 16S rRNA gene sequence. Clonal 16S rRNA gene sequences of archaeal gut symbionts were retrieved from wood-feeding lower termites such as *Cryptotermes domesticus* [57], *Nasutitermes takasagonensis* [45], and *Reticulitermes speratus* [46], the fungus-cultivating *Odontotermes formosanus* [45], and the higher soil-feeding termite *Pericapritermes nitobei* [45]. Archaeal diversity was surprisingly low, which may be related to the fact that only hindgut content was sampled, and would, therefore, exclude any cells adhering to the gut epithelium [37]. Most sequences detected were affiliated
with the methanogenic taxa *Methanobacteriaceae*, *Methanosarcinaceae*, and *Methanomicrobiaceae*, although in one study three clones grouped within the phylogenetic radiation of the non-methanogenic *Thermoplasmatales*. [46].

The large discrepancy between the high diversity of microbial phylotypes and the few numerically and metabolically relevant species isolated underscores that new concepts for cultivation are necessary. As a prerequisite, the physicochemical environment within the termite gut needs to be characterized in more detail, from which important clues for cultivation strategies can be obtained.

**Linking community structure and function**

Since microbial activities are confined to certain locations in the termite gut, a spatial organization of the microbial populations has to be expected. Berchtold et al. [47•] demonstrated significant differences in the density and radial distribution among the microbiota in the major hindgut regions of *Mastotermes darwiniensis*. Fluorescent in situ hybridization (FISH) with group-specific rRNA-targeted probes indicated that the thick gut wall of the posterior region was colonized preferentially by Gram-positive cocci and rod-shaped and filamentous bacteria of the Cytophaga–Flexibacter–Bacteroides (CFB) phylum, whereas α-Proteobacteria occurred free in the lumen. Microorganisms in the anterior, thin-walled region of the paunch were morphologically and phylogenetically different and were mostly associated with the flagellates, which were estimated to represent almost 95% of the total colonizable surface area in the anterior paunch [47•]. It has been shown that bacteria may either adhere loosely to or firmly attach at specific contact sites on the flagellate surface [48]. An elegant study by Fröhlich and König [49•] demonstrated that the endobionts and epibionts associated with the intestinal flagellates can be mechanically isolated with a micromanipulator-aided micropipette and subsequently identified by single-cell PCR.

In order to assign functions to the major populations within a microbial community, information on diversity and physiological characteristics have to be combined with localization and characterization of their respective microhabitats. The relative location of a microorganism within a metabolite gradient will determine which one of its different metabolic capacities is actually expressed. For example, both lactic acid bacteria and sulfate-reducing bacteria have the potential to reduce O₂, yet it is essential to know their location relative to the steep O₂ gradient to be able to predict whether they will actually do so in situ.

A general problem with assigning a function to individual microbial populations in a community (i.e. defining their ecological niche) is the fact that physiological properties of a phylotype usually cannot be inferred from the 16S rRNA gene sequence. Genes whose products are directly involved in metabolism, however, can be used as molecular markers for a potential function and, provided that the marker is conserved enough, may even allow phylogenetic inferences. Using the *nifH* gene, encoding dinitrogenase reductase, Ohkuma et al. [50,51•] detected a remarkable diversity of potentially nitrogen-fixing microbial symbionts in the guts of various termites.

The mere presence of a gene, however, does not necessarily reflect that it is actively expressed. It is well known that nitrogenase activity (determined via the acetylene reductase assay) in living termites shows wide interspecific and intraspecific variations and — as shown recently — also seasonal variations [52]. It has to be expected that the expression of nitrogenase by any gut microorganism will be strictly regulated by the intestinal ammonium concentration. In an elegant analysis of *nifH* expression in the gut of *Neotermes koshunensis*, Noda et al. [53••] were indeed able to demonstrate that only few genes of an alternative nitrogenase (*anf*) were preferentially transcribed, whereas most of the *nifH* genes were not.

Many important tools for a comprehensive structural and functional analysis of microbial communities are available, but may need to be refined for studying the termite gut habitat. Cryosectioning techniques have to be improved to conserve the structural integrity of the gut and its luminal content. New types of microsensors will become available, and can be combined with other in situ methods [54•]. A recently developed method that combines fluorescent in situ hybridization with rRNA-targeted oligonucleotide probes and microautoradiography [55•] might allow the direct linking of identity and metabolic activity of individual cells in the hindgut microbial community. Likewise, in situ activities can be assigned to individual microbial cells by targeting mRNAs of functional marker genes by using in situ reverse transcription (RT) techniques [56].

**Conclusions**

Long considered simply as anoxic fermentors, termite guts are in fact axially and radially structured habitats with numerous microniches created by a combination of host and microbial activities. The small size of the guts, their various yet sharply defined microhabitats, and the high specific activities of the microbial populations make termite guts excellent model systems for studying functional interactions within highly organized microbial communities.

To further advance our understanding of termite gut microecology, it is essential to proceed beyond a mere description of the microbial communities by their phylogenetic diversity. An integrative analysis of community structure and function will require the description of the environmental conditions, the localization of individual populations, and the characterization of their major metabolic activities — all in situ and at high resolution.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
**of outstanding interest


A short review article that summarizes the new concept of the termite hindgut as a highly structured environment characterized by steep gradients of metabolites. It contains further references to recent literature.


The latest monograph on termite biology. It contains several excellent chapters on the microbiota of wood-feeding, litter-feeding, and soil-feeding termites, and also the latest information on the taxonomy and phylogeny of lower and higher termites.


The first study where fluxes of carbon and electrons were measured within intact termite hindguts by microinjection of radiotracers. Lactate does not accumulate in the hindgut fluid, but turned out to be a major intermediate on the route to acetate. The presence of O2 has a significant impact on hindgut metabolism in situ.


Using a microinjection technique, it was shown that only the posterior, methanogenic hindgut compartments catalyzed H2-dependent CO2 reduction to acetate. Since reductive acetogenesis is severely hydrogen-limited under in situ conditions, coexistence of methanogens and homoacetogens in the same compartments may depend on cross-epithelial transfer of H2 from the anterior gut regions.


The extremely alkaline P1 segment of the hindgut shows high rates of, most likely chemical, O2 consumption, which is accompanied by a significant decrease of the molecular weight of humic acids extracted from this compartment. The results suggest that chemical oxidation and solubilization of soil organic matter in the anterior may be one of the keys to understanding the basis of humivory in termites.


A polyphasic approach in which the termite intestinal populations of lactic acid bacteria were characterized by both molecular and culture-dependent techniques. By 16S rRNA gene fingerprinting via DGGE, it was found that a Lactococcus strain isolated from the hindgut of Reticulitermes flavipes was closely related to one of the major phytophages in the bacterial community of this termite.


The first report of isolation and characterization of a cellulase gene from insects. The results ultimately confirm the endogenous origin of the endo-β-1,4-glucanase in the salivary glands of R. speratus.


The presence of EGase mRNA in the columnar cells of the midgut epithelium was demonstrated by in situ hybridization probing. In R. speratus, EGase mRNA was detected only in the salivary glands.


31. Ji R, Kappler A, Brune A: Transformation and mineralization of synthetic 14C-labeled humic model compounds by soil-feeding termites. Soil Biol Biochem 2000, 32:in press. Feeding experiments with chemically identical synthetic humic acids (HA), radiolabeled in either their proteinaceous or their aromatic building blocks, showed that pesticidal components of humic substances are selectively digested, whereas aromatic components are apparently not an important food source for soil-feeding termites.


39. Liburn TG, Schmidt TM, Breznak JA: Phylogenetic diversity of termite gut spirochaetes. Environ Microbiol 1999, 1:331-345. In-depth characterization of termite gut spirochaetes with culture-independent molecular tools. All clones were affiliated with the genus Treponema, forming two separate clusters: one containing all of the previously known species and some clonal sequences obtained from termite guts, and the other containing the majority of the termite gut phyotypes and only two free-living spirochaetes formerly assigned to the genus Spirochaeta. This is one of the few recent studies on microbial diversity in termite guts in which the number of analyzed clones per termite (n = 98) was sufficient to allow statistical evaluation.


41. Leadbetter JR, Schmidt TM, Gruber JR, Breznak JA: Acetogenesis from H₂ plus CO₂ by spirochaetes from termite guts. Science 1999, 283:686-689. The authors were the first to succeed in isolating several of the extremely abundant gut spirochaetes from the termite Zootermopsis angusticollis in pure culture. Their diligence was rewarded by being able to demonstrate reductive acetogenesis via the acetyl-CoA pathway as a mode of energy metabolism previously unknown for members of this phylum.


47. Berchtold M, Chatzinotas A, Schönhuber W, Brune A, Amann R, Hahn D, König H: Differential enumeration and in situ localization of microorganisms in the hindgut of the lower termite Mastotermes darwiniensis by hybridization with rRNA-targeted probes. Arch Microbiol 1998, 172:420-418. The first in situ localization of microbial symbionts in different fractions (wall, lumen, and flagellate) of different termite hindgut compartments using FISH with labeled rRNA probes. This study emphasizes the importance of an integrated approach for study of the termite gut ecosystem involving localization and enumeration of microbial symbionts relative to the gut environment.


49. Fröhlich J, König H: Rapid isolation of single microbial cells from mixed natural and laboratory populations with the aid of a micromanipulator. System Appl Microbiol 1999, 22:249-257. A micromanipulator-aided micropipette allows the retrieval of single-cell clones from environmental samples. Even microorganisms that are hard to isolate may be brought into pure culture or at least identified by molecular analysis, such as single-cell PCR. A valuable tool for termite gut microbiology since it will facilitate characterization of the flagellate-associated symbionts.


51. Okhuma M, Noda S, Kudo T: Phylogenetic diversity of nitrogen fixation genes in the symbiotic microbial community in the gut of diverse termites. Appl Environ Microbiol 1999, 65:4926-4934. The 16S rRNA sequences formed several distinct phylogenetic clusters, some of them unique to termites, which differed among termites from different families. The results indicate that each of the termite species investigated harbors diverse potentially diazotrophic microbial populations.


