Methanogens in the Digestive Tract of Termites

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Contents

Abstract Methanogenesis in the enlarged hindgut compartments of termites is a product of symbiotic digestion, fueled by hydrogen and reduced one-carbon compounds formed during the fermentative breakdown of plant fiber and humus. Methanogens are not always the predominant hydrogenotrophic microorganisms, especially in wood-feeding termites, but are restricted to particular microhabitats within the gut. The methanogens in lower termites belong to different lineages of Methanobacteriales that either are endosymbionts of flagellate protists or colonize the periphery of the hindgut, a habitat that is not fully anoxic. The oxygen-reducing capacities of the few isolates so far available indicate that they are well adapted to the continuous influx of oxygen across the gut wall. Higher termites, which lack gut flagellates, often have highly compartmented guts with highly dynamic physicochemical conditions, including redox and pH. The differences between the microenvironments are most pronounced in the soil-feeding species, where each

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compartment houses a characteristic archaeal community, comprising Methanobacteriales, Methanosarcinales, Methanomicrobiales, and a novel, deep-branching lineage of putative methanogens distantly related to the Thermoplasmatales. All clades form distinct phylogenetic clusters unique to the intestinal tract of insects, but with the exception of several *Methanobrevibacter* species, none of these archaea have been isolated in pure culture. The high methane emissions of termites, together with their enormous biomass in the tropics, make them a significant natural source of this important greenhouse gas.

1 Introduction

Most insects thriving on a fiber-rich diet harbor microbial symbionts that participate in digestion, but only termites, cockroaches, and the larvae of scarab beetles have been found to emit methane (Hackstein et al. 2006). Methane is the product of methanogenic archaea, which are the last link in an anaerobic feeding chain of microorganism located in the enlarged hindgut of these insects – a microbial bioreactor that transforms lignocellulosic matter to short-chain fatty acids, the major energy source for the host (Brune 2009b; Fig. 1).

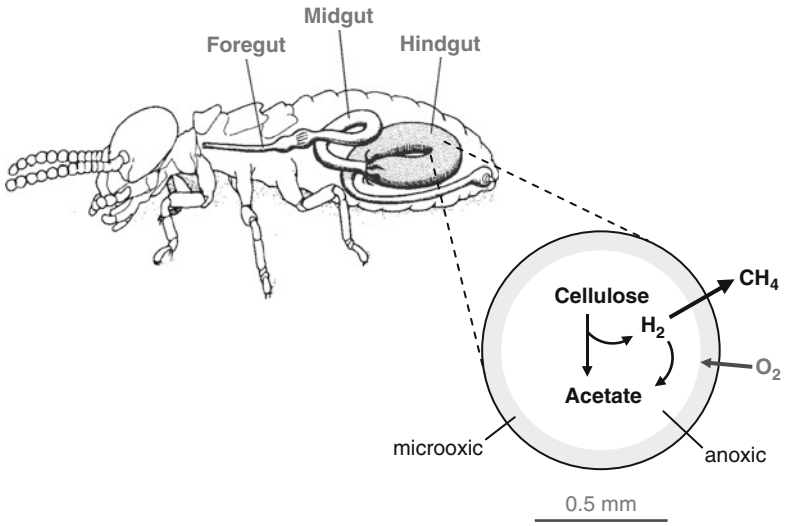

Fig. 1 The hindgut of termites is a microbial bioreactor that transforms lignocellulose to acetate and other short-chain fatty acids, which are the major energy source of the host. Hydrogen is an important intermediate of the microbial fermentations. It is converted either to acetate by homoacetogenic bacteria or to methane by methanogenic archaea. The anoxic status of the hindgut lumen is maintained by the microorganisms colonizing the microoxic hindgut periphery, which consume the oxygen that constantly diffuses into the gut [adapted from Brune and Ohkuma (2010)]

This chapter will provide an overview of the diverse aspects of methanogenesis in termites, including the role of methanogens in symbiotic digestion, the diversity and structure of the methanogenic community in different termite taxa, and recent conceptual advances concerning the interactions of methanogens with other gut microbiota and the physicochemical gut microenvironment. A more detailed treatment of certain aspects can be found in previous reviews of the literature (e.g., Breznak 2000; Brune 2006, 2009a; Purdy 2007; Brune and Ohkuma 2010).

2 Methane as a Product of Symbiotic Digestion

Methane formation in the guts of termites had been suspected already almost 80 years ago. When Cook (1932) studied the respiratory gas exchange of the lower termite *Zootermopsis nevadensis*, he found that the termites continued to form substantial amounts of an unidentified gas when the oxygen in the vessel was depleted. He was not able to analyze the gas, but inspired by the situation in ruminants, proposed that the gas was most likely hydrogen or methane, or a mixture of both. However, it took more than 40 years after Cook's initial observation until methane production in termite guts was finally recognized by Breznak and coworkers. While demonstrating nitrogenase activity in living termites and wood-feeding cockroaches with the acetylene reduction assay, the authors identified methane as an additional peak present in the gas chromatograms (Breznak et al. 1973, 1974) – a classical case of serendipity in science (see Brune 2009a for historical details).

Breznak (1975) had pointed out that the amount of methane produced by termites, if based on body weight, is in the same order of magnitude as that of ruminants. This observation immediately aroused the interest of atmospheric chemists studying the role of methane in radiative forcing of the atmosphere, and termites were identified as a potential source of considerable strength of this greenhouse gas (see also below). In the following years, methane production was found among almost all termite species investigated (e.g., Brauman et al. 1992; Shinzato et al. 1992; Wheeler et al. 1996; Bignell et al. 1997; Sugimoto et al. 1998b), although there were marked differences between taxa that seem to be related to the composition of the diet (wood vs. humus; Fig. 2).

Methane is formed by methanogenic archaea by two fundamentally different processes: (1) the reduction of CO_2 or other C_1 compounds to CH_4 via the C_1 pathway (hydrogenotrophic methanogenesis), and (2) the cleavage of acetate to CH_4 and CO_2 via the acetyl-CoA pathway (aceticlastic methanogenesis) (Hedderich and Whitman 2006; Liu and Whitman 2008). Interestingly, there is no evidence for aceticlastic methanogenesis in termite guts. As in the human gut and in the rumen, it is assumed that the relatively slow-growing aceticlastic species cannot cope with the short retention times of intestinal habitats (Lange et al. 2005; Liu and Whitman 2008). However, this does not explain why they could not avoid washout by attaching to intestinal surfaces (see below).

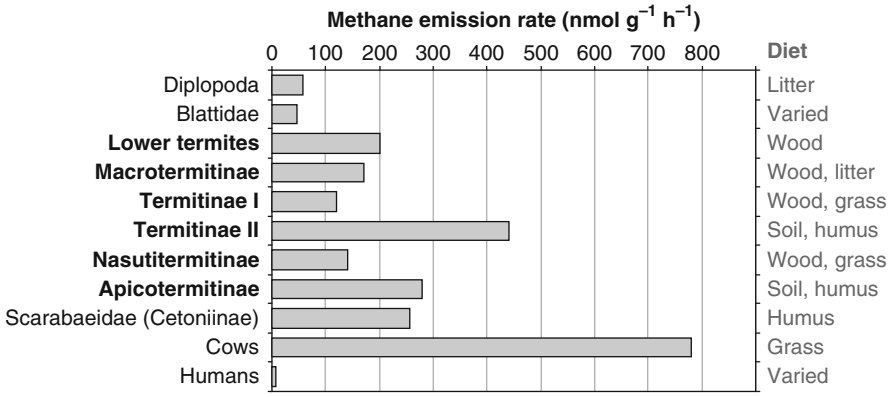

Fig. 2 Methane emission rates of termites (taxa shown in *bold*) in comparison to that of other invertebrates, cows, and humans, and the typical diet of the respective taxon. Termitinae were grouped into wood-feeding (I) and humus-feeding (II) species. Values are averages, based on fresh weight, and were compiled from various sources [for details, see Brune (2009a)]

The most important electron donors of hydrogenotrophic methanogenesis are hydrogen and reduced C_1 compounds, such as methanol and formate, which are formed during the fermentative breakdown of organic matter. In the hindguts of lower termites, hydrogen is a major fermentation product of cellulolytic flagellates and can accumulate to substantial concentrations (Ebert and Brune 1997; Pester and Brune 2007). Although methane production strictly depends on the presence of (hydrogen-producing) gut flagellates (Odelson and Breznak 1983; Rasmussen and Khalil 1983; Messer and Lee 1989), the rates are much lower than one would expect based on the large amount of hydrogen presumably formed by these protists. If the termites are fed with antibacterial drugs, both hydrogen and methane emission rates increase strongly, which suggests that the methanogenic archaea compete with bacteria for the hydrogen formed by the flagellates (Odelson and Breznak 1983). In the phylogenetically higher termites (family Termitidae), which lack gut flagellates, the substrates of methanogens are most likely formed by fermenting bacteria. Also in these taxa, methanogenesis in intact guts or gut homogenates is strongly stimulated by the supply of external hydrogen, as well as by formate (Brauman et al. 1992; Schmitt-Wagner and Brune 1999).

The process responsible for bacterial hydrogen oxidation in termite guts is reductive acetogenesis (Breznak and Switzer 1986). It is a unique feature of termite guts that the bacteria responsible for this activity are members of the Spirochaetes (Leadbetter et al. 1999). While CO_2-reductive acetogenesis seems to predominate over methanogenesis as the hydrogenotrophic process in most wood-feeding termites, the opposite is true for most fungus-cultivating and soil-feeding termite species, both in gut homogenates and in situ (Breznak and Switzer 1986; Brauman et al. 1992; Tholen and Brune 1999, 2000; Pester and Brune 2007). Despite the apparent substrate limitation of methanogenesis in termite guts, termites emit hydrogen in considerable amounts (Zimmerman et al. 1982; Odelson and Breznak 1983; Ebert and Brune 1997;

Sugimoto et al. 1998b; Schmitt-Wagner and Brune 1999; Pester and Brune 2007), which indicates that production and consumption of hydrogen in the hindgut are not tightly coupled (see below).

3 Diversity of Methanogens in Termite Guts

The methanogens in termite guts comprise representatives from almost all major lineages of methanogenic archaea; only Methanococcales seem to be absent (Fig. 4). In view of this considerable diversity, the number of methanogens from termite guts ever isolated in pure culture is quite disenchanting. There are only three described species, all of which belong to the genus *Methanobrevibacter* (Methanobacteriales), and all have been obtained from the same host species, the lower termite *Reticulitermes flavipes* (Leadbetter and Breznak 1996; Leadbetter et al. 1998; Fig. 3). Like other *Methanobrevibacter* species isolated from the human gut or the rumen, the isolates show a very restricted substrate spectrum, growing exclusively on $H_2 + CO_2$ (*Methanobrevibacter cuticularis* also grows, albeit poorly, on formate). The only other methanogen isolated from insect guts is *Methanomicrococcus blatticola* from the cockroach *Periplaneta americana*, the first cultivated representative of a novel lineage of Methanosarcinales that is present also in higher termites (see below). It differs from the *Methanobrevibacter* species in its inability to grow on $H_2 + CO_2$. Instead, it is specialized in the obligately hydrogen-dependent reduction of methanol or methylamines to methane (Sprenger et al. 2000). The strict requirement for hydrogen in methanogenesis is explained

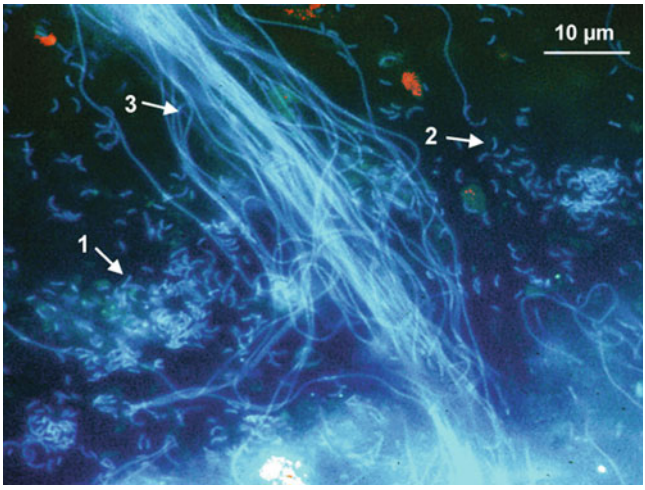

Fig. 3 Methanogens associated with the hindgut wall of *Reticulitermes flavipes*, photographed by the autofluorescence of their cofactor F_{420}. The *arrows* point to the characteristic morphotypes of *Methanobrevibacter cuticularis* (1), *Methanobrevibacter curvatus* (2), and *Methanobrevibacter filiformis* (3). Microphotograph courtesy of J.R. Leadbetter and J.A. Breznak

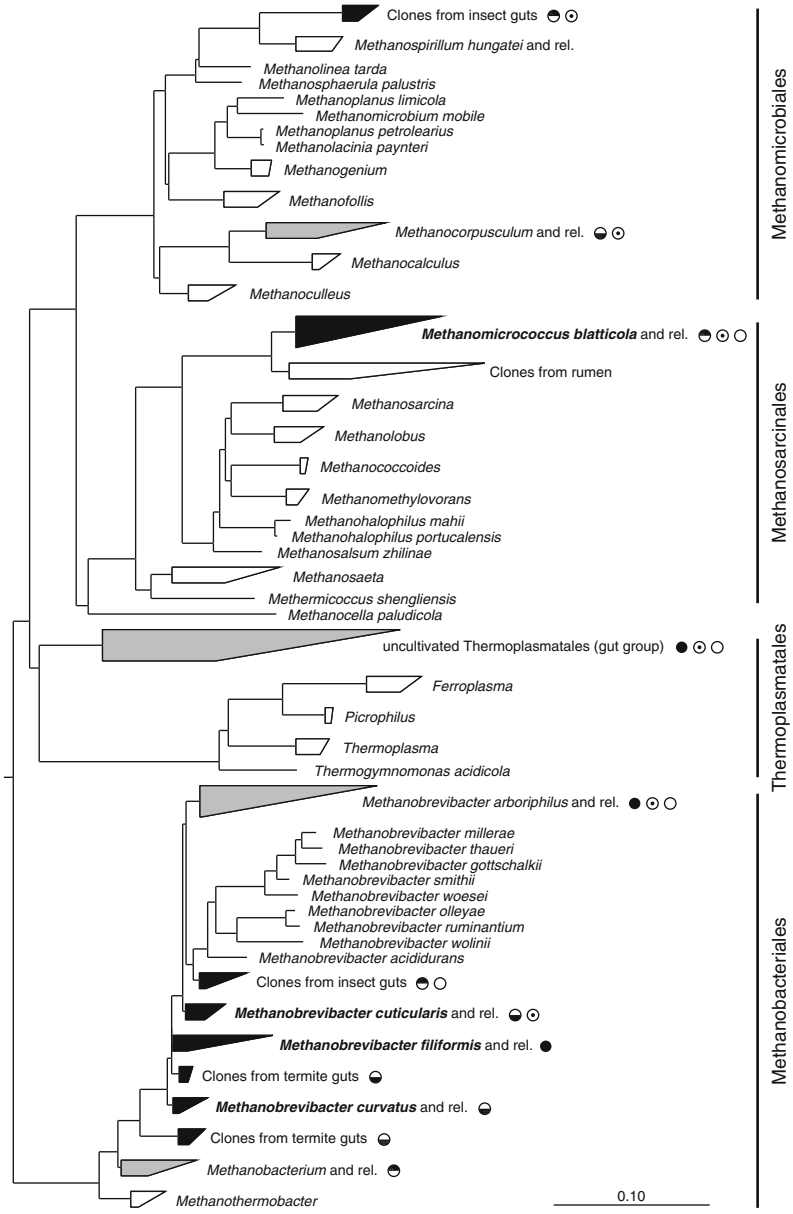

Fig. 4 Major lineages of Euryachaeota harboring representatives of putative methanogens from termite guts. Lineages marked in *black* consist exclusively of clones from insect guts and those marked in *gray* also contain clones or isolates from other environments. *Symbols* indicate the origin of the clones (*Circle with lower half black*, lower termites; *circle with upper half black*, higher termites; *filled circle*, both lower and higher termites; *circled dot*, cockroaches; *open circle*, other insects). The species isolated from insect guts are listed in *bold*

by its inability to oxidize methyl groups to carbon dioxide (Sprenger et al. 2005). The substrate affinities of *M. blatticola* for hydrogen and methanol are higher than those of other methylotrophic methanogens (*Methanosphaera stadtmanae*, *Methanosarcina barkeri*), and since the use of methanol as the terminal electron acceptor is thermodynamically more favorable than the use of carbon dioxide, *M. blatticola* may have a competitive advantage over other methanogens at low hydrogen concentrations (Sprenger et al. 2007).

The methanogens colonizing the hindgut of lower termites belong almost exclusively to the genus *Methanobrevibacter* (Methanobacteriales; Fig. 4). Many lineages of *Methanobrevibacter*-related sequences have been detected by cultivation-independent, 16S rRNA-based surveys (Ohkuma et al. 1995, 1999; Ohkuma and Kudo 1998; Shinzato et al. 1999, 2001), documenting the presence of unique *Methanobrevibacter*-related phylotypes in each lower termite investigated (reviewed by Dighe et al. 2004). Many termites harbor more than one lineage of *Methanobrevibacter*, and selective cloning of archaeal 16S rRNA genes from capillary-picked suspensions of gut flagellates (Tokura et al. 2000) revealed that the phylotypes associated with the flagellates are phylogenetically distinct from those that are attached to the hindgut cuticle or to filamentous bacteria at the gut wall. The *Methanobrevibacter* phylotypes associated with distantly related flagellates form a monophyletic cluster, which indicates that each of the *Methanobrevibacter* lineages within the same termite may have a preference for a particular microhabitat (Tokura et al. 2000; Hara et al. 2004; Inoue et al. 2008).

The methanogenic communities in the hindgut of higher termites are much more diverse. 16S rRNA-based analyses revealed the presence of Methanomicrobiales, Methanosarcinales, and Methanobacteriales in the wood-feeding species *Nasutitermes takasagoensis* (Nasutitermitinae) (Ohkuma et al. 1999; Miyata et al. 2007) and the soil-feeding species *Pericapritermes nitobei* (Ohkuma et al. 1999), *Cubitermes orthognathus* (Friedrich et al. 2001), and *Cubitermes fungifaber* (Donovan et al. 2004) (all Termitinae). The fungus-cultivating *Odontotermes formosanus* (Macrotermitinae) is an exception in that only Methanosarcinales have been recovered (Ohkuma et al. 1999). A general absence of methanogens of other orders from the Macrotermitinae is corroborated by the finding that the amount of rRNA of Methanosarcinales recovered from the gut of the fungus-cultivating *Macrotermes subhyalinus* was in the same range as the total amount of archaeal rRNA obtained from this termite (Brauman et al. 2001). The microbial diversity in representatives of the fourth subfamily of higher termites (Apicotermitinae), which are mostly soil-feeding, has not been investigated. The Methanobacteriales clones obtained from higher termites fall into the radiation of the genus *Methanobrevibacter*, but are phylogenetically distinct from their relatives in the lower termites and other insects. The clones of Methanomicrobiales and Methanosarcinales recovered from higher termites are not represented among the lower termites, but cluster with clones obtained from cockroaches and scarab beetle larvae (e.g., Hara et al. 2002; Egert et al. 2003), including also *M. blatticola* isolated from the cockroach *P. americana* (Fig. 4).

In a comprehensive survey of numerous species of lower and higher termites using dot-blot hybridization with group-specific probes, rRNA of Methanobacteriales was detected in the guts of most termite species studied, regardless of diet or taxonomic classification (Brauman et al. 2001). However, Methanosarcinales were detected only in about half of the species, and a signal for Methanomicrobiales was not obtained. The reasons for these discrepancies are not clear, but the large gap between the combined hybridization signals of the group-specific probes and the archaeal domain probe observed with almost all termites investigated may reflect the presence of other (nonmethanogenic) archaea, which were presumably not covered by the group-specific probes. In several of the clone-based studies of archaeal diversity in termites and other insects (see above), an additional, deep-branching clade of uncultivated archaea only distantly related to cultivated members of the Thermoplasmatales was discovered (Fig. 4); this clade formed a substantial fraction of the clones in the respective libraries and also comprises clones obtained from the guts of mammals. It is not clear whether the clade represents methanogenic or nonmethanogenic archaea.

4 Differences in Methanogenic Activities and Populations

Information on the population sizes of methanogens in insect guts is scarce. Cultivation-based studies indicate that *R. flavipes* harbors about 10^6 methanogens per gut, which would represent about 5% of the total prokaryote cell count (Leadbetter and Breznak 1996; Tholen et al. 1997). Such numbers are not very accurate because of the difficulties in enumeration created by the attachment of methanogens to intestinal surfaces and the uncertainties surrounding the determination of the total cell number of prokaryotes, most of which are intimately associated with the flagellate cells that occupy the bulk of the hindgut volume. Hybridization of RNA extracted from the guts of a wide range of termite species with domain-specific oligonucleotide probes indicated that archaeal rRNA was on average only 1.5% of all prokaryotic rRNA (Brauman et al. 2001). Although not all the archaea in termite guts are necessarily methanogenic (see above), the higher fraction of archaeal rRNA in soil-feeding species ($2.3 \pm 0.5\%$) than in wood-feeding and fungus-cultivating species ($0.9 \pm 0.5\%$) is in agreement with a general trend toward higher methane emission rate among termites with a humivorous lifestyle (Fig. 2).

Since soil-feeding termites, in contrast to their wood- and litter-feeding relatives, digest peptide-rich soil organic matter (Ji and Brune 2006), it is tempting to suggest that these differences are diet related. However, information on the fermentative processes in the hindguts of humivorous insects is sparse, and also the biology of the mostly uncultivated methanogens in higher termites has to be better understood before a reasonable hypothesis can be proposed. Such knowledge may also help to clarify whether the presence of methanogens provides a competitive advantage to their respective hosts. Elimination of methanogens from *Zootermopsis angusticollis* by feeding with bromoethanesulfonic acid (BES) did not affect the survival of the

termites (Messer and Lee 1989). In some species of lower termites, not all colonies were colonized by methanogens, and trends in methane emission among members of the same genus or even species were not always consistent (e.g., Shinzato et al. 1992; Wheeler et al. 1996).

5 Coexistence with Homoacetogens

The predominance of reductive acetogenesis over methanogenesis in most wood-feeding termites has puzzled microbiologists for the longest time. For thermodynamic reasons, methanogens should always outcompete homoacetogens for hydrogen, their common substrate — at least in a well-mixed system. However, the introduction of microsensor techniques into termite gut research led to the recognition that termite guts are spatially structured microenvironments characterized by steep diffusion gradients of metabolites (see Brune 1998; Brune and Friedrich 2000), which brought conceptual advances that allowed the coexistence of methanogens and homoacetogens in this habitat to be explained.

First, it turned out that hydrogen concentrations in termite guts are much higher than originally considered — far above the threshold concentrations at which methanogens can outcompete homoacetogens for hydrogen. At the hydrogen partial pressures observed in the hindgut proper of several lower termites (1–100 kPa; Ebert and Brune 1997; Pester and Brune 2007), both processes would operate at substrate saturation, and a direct competition for hydrogen cannot occur. Therefore, explanations of the predominance of reductive acetogenesis as the hydrogenotrophic process that are based on the ability of homoacetogens to grow mixotrophically on H_2 and other substrates (Breznak 1994) are no longer applicable.

Second, high-resolution profiles of hydrogen concentration in the intestinal tracts of lower and higher termites (Ebert and Brune 1997; Schmitt-Wagner and Brune 1999; Pester and Brune 2007) and rate measurements of reductive acetogenesis by microinjection of radiotracers (Tholen and Brune 1999, 2000; Pester and Brune 2007) documented that sources and sinks are not evenly distributed within the termite gut. The high hydrogen concentrations in the hindgut paunch and the steep hydrogen gradients toward the gut periphery of *Reticulitermes* spp. are in agreement with the location of hydrogen-producing flagellates and (in part) homoacetogenic spirochetes in the gut lumen, and with the absence of any stimulatory effect of externally supplied hydrogen on the in situ rates of reductive acetogenesis. In contrast, the strong hydrogen sink at the hindgut wall of *R. flavipes*, which is clearly caused by an anaerobic process (Ebert and Brune 1997), together with the dense colonization of the cuticle with *Methanobrevibacter* species (Leadbetter and Breznak 1996; Leadbetter et al. 1998), is in agreement with the strong stimulation of methanogenesis by externally supplied hydrogen in this and other termites.

The spatial separation of the two hydrogenotrophic processes – reductive acetogenesis in the hydrogen-rich gut lumen and methanogenesis in the hydrogen-poor gut periphery – precludes any direct competition for hydrogen between

homoacetogens and methanogens (Fig. 5). Although this scenario provides the answer to the original question concerning the basis for the apparent outcompetition of methanogens by homoacetogens for their common substrate, it remains to be explained why the homoacetogens are able to colonize the hydrogen-rich gut lumen, whereas the methanogens (unless associated with gut flagellates) are not. In this context, it is important to recall that the termite gut is unusual not only with respect to the predominance of reductive acetogenesis over methanogenesis but also in the abundance of spirochetal life forms (Lilburn et al. 1999; Breznak 2000). So far, the termite gut is also the only habitat that harbors spirochetes capable of reductive acetogenesis (Leadbetter et al. 1999; Breznak and Leadbetter 2006). Diversity studies and expression analysis of FTHFS genes, the functional markers of reductive acetogenesis, have revealed that termite gut treponemes predominate over homoacetogenic firmicutes in all termites studied (Salmassi and Leadbetter 2003; Ottesen et al. 2006; Pester and Brune 2006; Warnecke et al. 2007). Apparently, these highly motile spirochetes are well adapted to actively maintain their position in the hindgut lumen, whereas methanogens must attach to surfaces to prevent washout – they can colonize the gut lumen only by associating with the gut flagellates or (in higher termites) with cuticular spines protruding from the gut wall into the lumen (Bignell et al. 1980).

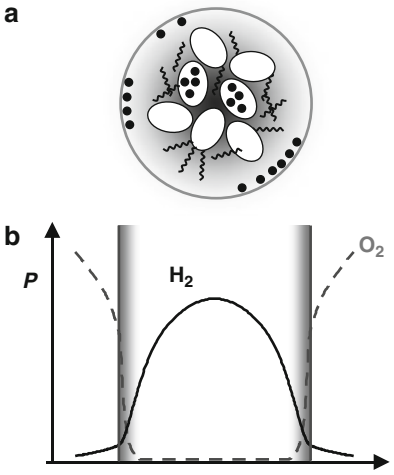

Fig. 5 Schematic cross section (**a**) of an agarose-embedded hindgut (paunch region) of a wood-feeding lower termite (*Reticulitermes* spp.), illustrating the location of methanogens (*filled circles*) attached to the hindgut wall and homoacetogenic spirochetes (*spirals*) within the gut proper. In some species, methanogens are also associated with the gut flagellates (*white ovals*). Radial profiles (**b**) of oxygen and hydrogen partial pressure (P) document that the respiratory activity of the gut microbiota maintains steep oxygen gradients (*dashed line*) within the gut periphery, rendering the center anoxic. Hydrogen (*solid line*) formed by the flagellates accumulates at the gut center but is consumed throughout the entire gut. The strong hydrogen sink below the gut wall is probably caused by methanogens, which prevent larger amounts of H_2 from escaping into the atmosphere [Scheme from Brune and Ohkuma (2010)]

6 Association with Gut Flagellates

In many anoxic environments, methanogens are associated with anaerobic protists (van Hoek et al. 2000; Hackstein et al. 2001; Fenchel and Finlay 2010). The association of methanogens with the gut flagellates of lower termites is a common phenomenon (Odelson and Breznak 1985; Messer and Lee 1989; Shinzato et al. 1992; Hackstein and Stumm 1994; Radek 1994, 1997; Tokura et al. 2000; Hara et al. 2004; Hongoh and Ohkuma 2010), although the hindgut cuticle or the surface of filamentous bacteria colonizing the gut wall remain their typical habitats (Hackstein and Stumm 1994; Leadbetter and Breznak 1996; Leadbetter et al. 1998). Since methanogens located in the gut periphery are clearly hydrogen limited (see above), Sugimoto et al. (1998b) suggested that the rates of hydrogen and methane emission of different termite species may depend on the particular location of methanogens relative to the hydrogen source.

Generally, only the smaller species of termite gut flagellates are associated with methanogens. Lee et al. (1987) investigated the colonization of gut flagellates by methanogens in the hindgut of *Z. angusticollis* by epifluorescence microscopy and reported that only the small trichomonadid flagellates *Trichomitopsis termopsidis*, *Tricercomitus termopsidis*, and *Hexamastix termopsidis* were associated with cells showing the characteristic F_{420} autofluorescence of methanogens. The larger hypermastigotes, which appeared to be the major hydrogen source (Messer and Lee 1989), usually lacked methanogenic symbionts. Similar observations were made by Tokura et al. (2000) with *Reticulitermes speratus*, where the methanogens were regularly associated with the oxymonadid *Dinenympha parva* and a small hypermastigote *Microjoenia* sp., and with *Hodotermopsis sjoestedti*, where the methanogens were associated with *Dinenympha* sp. and *Microjoenia* sp. in large abundance. In all cases, the methanogens seemed to be located within the host cells, which is in agreement also with ultrastructural data reported by Lee et al. (1987).

Odelson and Breznak (1985) were the first to note that a putatively axenic culture of *Trichomitopsis termopsidis*, a gut flagellate isolated from a *Zootermopsis* species, contained a methanogenic symbiont. The symbiosis was not obligate because cultures continued to grow after they were cured of the methanogenic symbiont. Nevertheless, growth yields of *Trichomitopsis termopsidis* increased when the flagellate was cultivated in the presence of the methanogen *Methanospirillum hungatei*, which suggested that the flagellates may benefit in a similar manner from their methanogenic symbiont. There are reports from other environments that indicate that methanogens associated with eukaryotic partner organisms may benefit from an interspecies hydrogen transfer, and the stimulation of fermentative processes by end product removal (hydrogen, formate) may even result in a mutual advantage (see Schink 1997; Worm et al. 2010). However, considering the high hydrogen concentrations throughout the gut lumen of lower termites, it is not clear whether termite gut flagellates indeed benefit from the hydrogen-consuming activity of their methanogenic symbionts under in situ conditions. At the same time, this

would mean that the methanogens associated with gut flagellates are never hydrogen-limited as long as they can maintain their position in the hydrogen-rich gut lumen, no matter whether their particular host is producing hydrogen or not. From that perspective, the association of *Methanobrevibacter* species with gut flagellates may simply serve to maintain a stable position in the anoxic and hydrogen-rich hindgut lumen, an argument that may apply also to other, nonmethanogenic prokaryotes commonly associated with such protists (Brune and Stingl 2005; Hongoh and Ohkuma 2010).

7 Intercompartmental Transfer of Hydrogen

The gut of higher termites is characterized by the absence of cellulolytic flagellates and shows (with the exception of the fungus-cultivating species) also a pronounced compartmentation, which goes hand in hand with a remarkable dynamics of intestinal pH and redox potential (Fig. 6). In soil-feeding *Cubitermes* species, hydrogen production and consumption are spatially separated in different gut compartments (Schmitt-Wagner and Brune 1999; Tholen and Brune 1999). The strong stimulation of both methanogenesis and reductive acetogenesis by external hydrogen added to intact gut compartments led to the hypothesis that hydrogen diffuses across the gut epithelia between hydrogen-producing and hydrogen-consuming gut regions, which are in close contact in situ. Such cross-epithelial transfer of reducing equivalents has been documented in detail in cockroaches and scarab beetle larvae (Lemke et al. 2001, 2003) and would explain the low hydrogen and high methane emissions of such soil-feeding termites. Since methanogenesis in the posterior hindgut is stimulated not only by hydrogen but also by formate, which accumulates to considerable concentrations in other gut compartments, there may also be an intercompartmental transfer of reducing equivalents via the hemolymph (Schmitt-Wagner and Brune 1999).

A detailed analysis of the archaeal community structure in the different gut compartments of *C. orthognathus* showed that the different phylogenetic groups are not evenly distributed among the different compartments (Friedrich et al. 2001). Each of the individual gut compartments harbors a distinct assemblage of euryarchaeota (Fig. 6d). Methanosarcinales colonize the anterior, extremely alkaline hindgut compartment (P1), whereas Methanobacteriaceae and Methanomicrobiales predominate in the posterior compartments (P3 and P4). Members of a deep-rooting clade of putative methanogens distantly related to the Thermoplasmatales (see above) increase toward the rectum (P5). Many of the microbial cells attached to the gut wall or cuticular spines projecting from the hindgut wall into the lumen are putative methanogens based on their characteristic UV-fluorescence (Schmitt-Wagner and Brune 1999), but are yet to be assigned to the different phylogenetic groups.

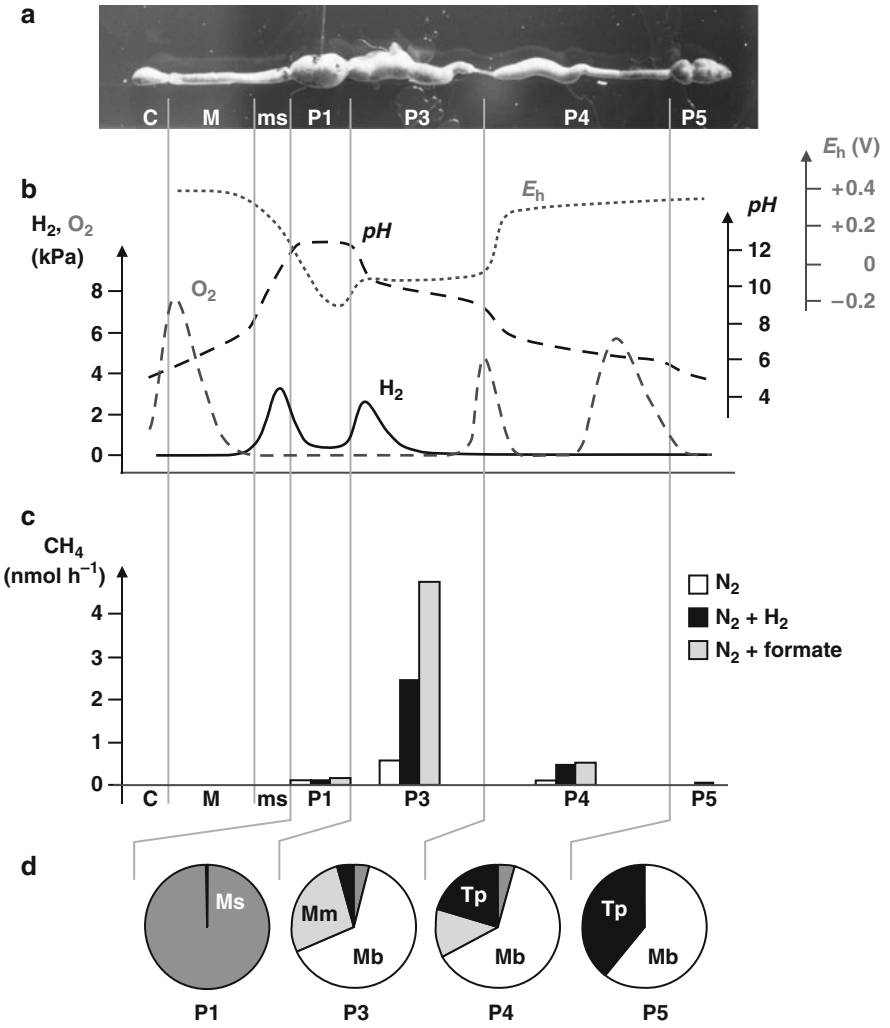

Fig. 6 Gut morphology (**a**) and axial profiles (**b**) of different physicochemical parameters along the gut axis of a soil-feeding termite (*Cubitermes* spp.). Oxygen and hydrogen partial pressures, intestinal pH, and apparent redox potential (against a standard hydrogen electrode) were measured with microsensors. The gut was stretched out and embedded in agarose-solidified Ringer's solution. Methane emission rates (**c**) were determined with isolated gut sections incubated under a N_2 headspace with or without addition of H_2 or formate. Relative abundance of euryarchaeotal clones in 16S rRNA gene libraries of the respective sections (*Ms* Methanosarcinales, *Mb* Methanobacteriales, *Mm* Methanomicrobiales, *Tp* Thermoplasmatales-related clade). The borders between the different gut regions are indicated by the *vertical lines* [scheme based on data from Brune and Kühl (1996), Schmitt-Wagner and Brune (1999), Friedrich et al. (2001) and Kappler and Brune (2002)]

8 Relationship to Oxygen

As obligate anaerobes, the methanogens in termites are restricted to the hindgut, the only gut region characterized by a negative redox potential (Ebert and Brune 1997; Kappler and Brune 2002). It is not clear why they are regularly (in some cases exclusively) located at the hindgut wall, a microhabitat that is characterized by the constant influx of oxygen across the epithelium (Brune 1998). Like all other methanogens, the three *Methanobrevibacter* species colonizing the gut epithelium of *R. flavipes* (Leadbetter and Breznak 1996; Leadbetter et al. 1998) (and also *M. blatticola* colonizing the hindgut epithelium of cockroaches; Sprenger et al. 2000) do not grow in media containing even traces of oxygen and are much more sensitive to oxygen accumulation than the homoacetogenic *Sporomusa* species isolated from termite guts (Boga and Brune 2003). However, *Methanobrevibacter* species remain metabolically active in dense cell suspensions that are exposed to controlled oxygen fluxes as long as the influx of oxygen does not exceed their capacity for oxygen removal (Tholen et al. 2007), whereas reductive acetogenesis of *Sporomusa* species is inhibited even at the lowest oxygen fluxes. It has been proposed that the redirection of electron flow from methanogenesis toward oxygen reduction enables *Methanobrevibacter* species to colonize the hindgut periphery of termites. The mechanisms of tolerance to reactive oxygen species and the biochemistry of oxygen reduction in *Methanobrevibacter* species have been discussed elsewhere in detail (see Brune 2009a).

Nevertheless, the location of methanogens at the gut wall of lower termites, at the unfavorable end of the outwardly directed hydrogen gradient, remains enigmatic. It has been suggested that an attachment to the hindgut cuticle may protect against predation or prevent washout from the gut, which may compensate methanogens for the negative effects of hydrogen limitation and exposure to inflowing oxygen (Breznak 2000). In higher termites, the explanation for the colonization of the hindgut cuticle may lie also in the putative transfer of hydrogen between different compartments. The microorganisms located at the gut wall may be at the bottom end of the radial hydrogen flux from the gut proper, but may benefit from external hydrogen entering the hindgut by cross-epithelial transfer from other compartments (see above).

9 Termites as a Source of Atmospheric Methane

Although the countergradients of methane and oxygen in the hindgut periphery provide seemingly ideal conditions for aerobic methane oxidation (Brune et al. 2000), there is no evidence for the presence of methanotrophic bacteria or their activities in termite guts (Pester et al. 2007). This means that the different methane emission rates of termites from different feeding guilds directly reflect differences in methane production within their intestinal tract. In the past, many attempts were made to extrapolate from the results of laboratory measurements of methane emissions to the contribution of termites to the global methane budget, but even

the most recent estimates are still far from accurate and suffer from numerous biases (see Sanderson 1996; Bignell et al. 1997). Sugimoto and colleagues demonstrated that it is very important to consider methane oxidation in the mound material and the surrounding soil as an important factor mitigating methane production by termites at the environmental level (Sugimoto et al. 1998a, 2000). As a consequence, the net emissions of methane from intact colonies of soil-feeding termites are much lower than those of wood-feeding termites, although the opposite would be predicted from the gross methane emission rates determined with individual termites in the laboratory.

In view of the grossly overestimated contribution of termites to global methane emissions into the atmosphere propagated in the older literature (see Collins and Wood 1984), it is important to note that the most recent estimates place these rates at probably less than 10 Tg per year (1.5–7.4 Tg; Sugimoto et al. 1998b) and almost certainly below 20 Tg per year (a number that is still used in the last global budget published by the IPCC; Denman et al. 2007). Nevertheless, termites remain the second largest natural source of methane on the planet, although their contribution to the total source strength (ca. 600 Tg per year) is certainly dwarfed by the sources under anthropogenic influence (such as the ruminants). A more detailed review of the literature on this subject can be found elsewhere (Brune 2009a).

10 Conclusions

Termites are a significant source of methane in tropical ecosystems. Methane and short-chain fatty acids are formed from lignocellulosic matter by an anaerobic feeding chain of microorganisms located in the highly enlarged hindguts. However, termite hindguts are not purely anoxic fermentors. The gut habitat is characterized by the continuous influx of O_2 across the gut wall and steep hydrogen gradients between gut lumen and periphery. Despite the high hydrogen concentrations in the gut lumen, methanogens are not the predominant hydrogenotrophic microorganisms in lower termites. The ability to attach to biotic or abiotic surfaces or to colonize the cytoplasm of flagellate protists may be an important factor in the successful colonization of the intestinal tract. In higher termites, which lack gut flagellates, the increased methane production is correlated with a dietary shift from wood to humus. The assemblage of methanogenic archaea is more diverse and distributed among several consecutive gut compartments characterized by pronounced axial dynamics of physicochemical parameters, including redox and pH, and (in the case of soil-feeding termites) a cuticle sometimes adorned with cuticular spines. The drivers determining archaeal community structure in the different microhabitats are not clear, but may involve the availability of and competition for methanogenic substrates and differences in adaptation to oxidative stress and other factors imposed by the respective environments. Since many methanogens in termite guts belong to taxa without any cultured representatives, more isolates are sorely needed to address these questions.

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