Chapter 4
Termite Gut Microbiome

Navodita Maurice and László Erdei

Contents

Abstract Termites depend on their gut microbes for digestion of complex polysaccharides of wood into simpler molecules. Cellulose is a major polymeric carbohydrate present in the wood which is broken down to simpler byproducts through metabolic steps by the hindgut microbes. Termite gut microbes also produce gases during the cellulose degradation process, of which methane is the major product. Gut microbes belong to three major groups, namely, bacteria, archaea and protozoa. They show a mutualistic relationship and typically convert 95% of cellulose into simple sugars within 24 h. More than 200 species of microbes form this community,

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producing different types of wood-busting enzymes, mainly cellulases, cellubiases, hemicellulases, glucosidases and gluconases, during wood degradation. Studies suggest that lower termites utilize both endogenous and protozoal enzymes for cellulose digestion, while higher termites acquire enzymes from their diet instead of protozoal enzymes. Some termite species change their feeding habits with seasonal variations. These affect gut microbes population and therefore are responsible for enhancing their survival under changed environmental conditions.

Keywords Gut • Microbes • Cellulose • Enzymes

4.1 Introduction

Termites are among the most efficacious groups of insects on Earth that colonize most landmasses except Antarctica, with colonies assorting from a couple of hundred to several million individuals (Cranshaw 2013). Lower termites predominantly harbour species of oxymonads, trichomonads and hypermastigote flagellates as symbionts in the paunch of hindgut, that aid in wood ingestion. Higher termites (Termitidae) consist of about 75% of all termite species and show instead low protozoan populations (Sanderson 1996). The symbiotic associations in termites are crucial due to the presence of three groups of microorganisms. These are mainly bacteria, protozoa (inhabiting the hindgut) and fungi cultivated as 'fungus gardens' or 'fungus combs' in some species (Darlington 1994). Most termite species reside in the tropical, subtropical and warmer temperate zones of the world and subsist on a diet rich in cellulose either in the form of living or dead wood, woody tissue of plants, humus or dung (Higashi and Abe 1997). They, therefore, play an important role as terrestrial decomposers (Brune and Friedrich 2000).

4.2 Termite Gut

The digestive system of termites consists of three main parts: the foregut, midgut and hindgut (Ptacek et al. 2013; Noirot and Noirot-Timothee 1969). Foregut includes the crop and muscular gizzard and contributes in the secretion of digestive enzymes as well as absorption of soluble nutrients. Hindgut also aids in digestion and absorption of nutrients (Breznak 1994). It may be divided into five successive segments, namely: proctodeal segment, enteric valve, paunch (abundant in symbiotic microorganisms), colon and rectum. Enteric valve prevents return of paunch contents back into the midgut or foregut. Malpighian tubules enter the gut at the junction of the midgut and first proctodeal segment, just in front of the proctodeal valve. Some

higher termites have an elongated midgut known as the 'mixed segment' or mesenteron, due to prolongation of one of the intestinal tube faces (Fig. 4.1).

The hindgut, being the largest part of the intestinal tract, is anaerobic. Here the symbiotic gut microbes depolymerize cellulose and hemicellulose and ferment resulting carbohydrates into short-chain fatty acids, which are later absorbed and oxidized by the host (Brune and Friedrich 2000) (Fig. 4.2). Guts of termites maintain anoxic conditions due to their steep oxygen gradients at the oxic-anoxic interface, which drives a continuous influx of O_2 into the gut. According to microsensor studies, O_2 can travel at a rate of 50–200 μm in the gut, therefore creating a microoxic periphery around an anoxic centre. In the lower termite *Reticulitermes flavipes,* radiotracer studies show an influx of O_2 through gut epithelium which gets reduced at the periphery of the hindgut (Boga and Brune 2003). The microbe-filled paunch region of lower and higher termites has a microoxic periphery where pH and redox potential undergo significant transitions along the guts anterior to posterior axis (Bignell 1994). However, the paunch region measurements are circumneutral and anoxic with a comparatively low redox potential of −150 to -250 mV. The pH values of hindguts also show neutrality from 6.2 to 7.6, but it can undergo alterations with environment changes (Abe et al. 2000).

Soil-feeding termites exhibit extreme pH changes along the gut axis with pH values as high as 11. This is an evolutionary adaptation to their diets, rich in tannins or other polyphenolic constituents as it prevents precipitation of digestive enzymes and enhances solubility of dietary proteins (Breznak 1994). The high gut alkalinity separates organic nutrients from the organo-mineral aggregates, as soil-feeding ter-
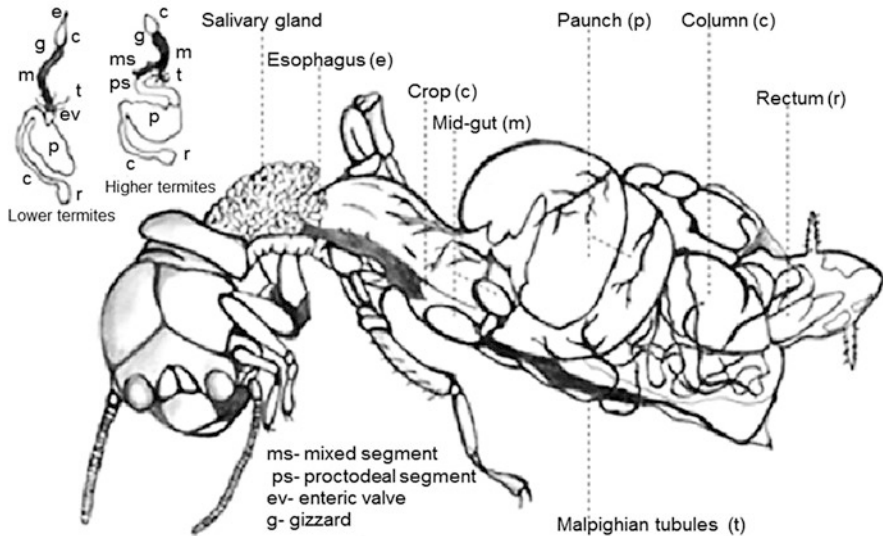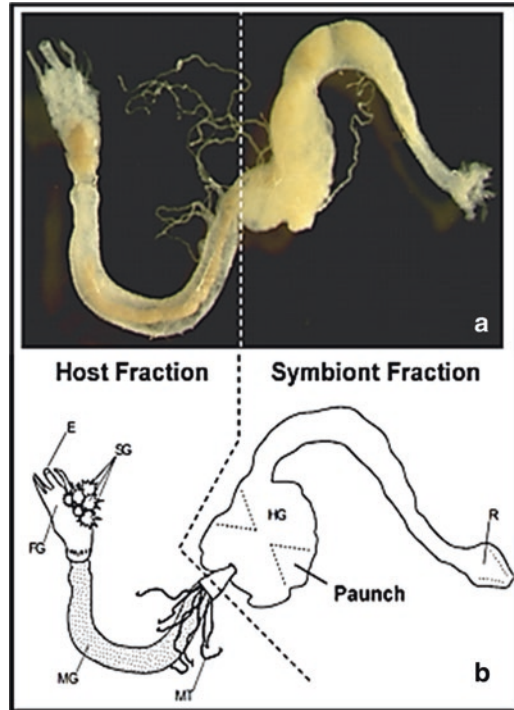

Fig. 4.1 Gut segments of a typical termite (worker) (Ptacek et al. 2013)

Fig. 4.2 Collaboration of
the host and symbionts in
lignocellulose digestion in
R. flavipes (**a**) and different
components of the
digestive tract (**b**) showing
the oesophagus (E),
salivary glands (SG),
foregut (FG), midgut
(MG), Malpighian tubules,
(MT) hindgut (HG) and
rectum (R) (Scharf et al.
2011)

mite ingests organic matter from soil (Kappler 1999). The guts of the wood-feeding
and soil-feeding termites are characterized by noticeable axial dynamics of O_2 and
H_2 partial pressure followed by intestinal pH. These differences bring uneven distri-
bution of H_2 production, methanogenesis and reductive acetogenesis by gut microbes
(Brune and Friedrich 2000).

Numerous prokaryotes have been isolated in pure culture, and many of them are
unique to the termite gut habitat (Graber et al. 2004), including oxygen reduction by
anaerobic bacteria, such as lactic acid bacteria (Bauer et al. 2000), homoacetogenic
bacteria (Boga and Brune 2003) and sulphate-reducing bacteria (Frohlich et al.
1999). Lower and higher termites differ in their gut microflora. Bacteria and archaea
inhabit guts of both higher and lower termites. However, cellulolytic flagellates par-
ticipating in wood digestion are present only in lower termites (Dietrich et al. 2014)
that without them starve (Breznak and Brune 1994). Some higher termites exhibit
symbiosis with the fungus *Termitomyces*, or *Bacteroidetes* and *Firmicutes*, while
Spirochaetes and *Fibrobacteres* outnumber the guts of other higher termites with a
cellulose-rich diet (He et al. 2013; Breznak 1994).

The 'mixed segment' present only in higher termites is composed of a mesen-
teric epithelium which occupies half of the gut wall, while the remaining area is

covered by the proctodeal epithelium. The mixed segment has an elevated pH (Brune and Kuhl 1996) along with an alkaline fluid rich in potassium ions. Symbiotic bacteria occupy the mesenteric side of the mixed segment (Tokuda et al. 1997). A peritrophic membrane separates bacteria from wood particles present in the lumen as shown by in situ hybridization and electron microscopy studies. Due to their non-uniform distribution around the peritrophic membrane, the lumen is not a nutritional source for these bacteria. Electron microscopy indicates a close relationship between bacteria and mesenteric epithelium suggesting that symbiotic bacteria utilize some substances secreted by the mesenteric tissue of the mixed segment. Midgut cells are very quickly replaced in cockroaches (Dow 1986) and termites via endocytosis of the neighbouring epithelial cells, which are later digested by phagolysosomes (Yamaoka and Nagatani 1980). Similar phagosomes have been observed throughout the midgut columnar cells, including the mixed segment of *Nasutitermes takasagoensis* where the symbionts trapped among the microvilli of old cells can be endocytosed by young columnar cells and digested along with old cells. Since bacteria cannot pass through the peritrophic membrane, it is believed that they infect termites only when their peritrophic membrane is intact (Tellam et al. 1999). Phylogeny and distribution studies of the symbiotic bacteria dwelling in the mixed segment suggest that these bacteria have a significant role in the termites gut physiology. Microflora of the mixed segments of soil-feeding and wood-feeding termites are different; therefore, it is possible that these symbionts bear a close relationship with the feeding habits, gut physiology and phylogeny of their hosts (Tokuda et al. 2000).

The neutral pH value of the midgut of the wood-eating higher termite *N. nigriceps* increases in the mixed segment and reaches to 10.23 ± 0.46 in the first proctodeal segment, while a decrease in oxygen concentration in the mixed segment occurs, which ultimately becomes zero in the first proctodeal segment (Brune et al. 1995). The space between the gut wall and peritrophic membrane of the mixed segment is termed the 'ectoperitrophic space' and is the shelter home for the bacterial species. Cocci and rods have been identified in the wood-eating termite *N. exitiosus* (Czolij et al. 1985), while spirochaetes and actinomycete-like bacteria populate the guts of the soil-feeding termites *Cubitermes severus* and *Procubitermes aburiensis* (Bignell and Eggleton 2000). The symbiotic bacteria present in the mixed segment resemble the *Clostridium* group but differ from the bacteria of wood-eating lower termite *Reticulitermes speratus* (Ohkuma and Kudo 1996) as well as from the clostridia isolated from higher termites (Hethener et al. 1992). However, no low-GC-content Gram-positive bacteria have been yet discovered from the gut of the drywood-eating lower termite *Cryptotermes domesticus* (Kudo et al. 1998). *Clostridium* species are usually obligate anaerobes, while some are facultative anaerobes appearing as rods, short rods or cocci (Rieu-Lesme et al. 1996). Some of them are able to degrade polysaccharides by producing acetone, alcohol, acetate, lactate, CO_2 and hydrogen (Rainey et al. 1996), while others can ferment nitroge-

nous or lipidic compounds playing, therefore, an important role in the nutritional physiology of termites (Hethener et al. 1992).

4.3   Termite Gut Microbiome

Termite gut unveils one of the most complex microbial communities entailing diverse microbes from the three domains of life (Bacteria, Archaea and Eukarya) (Ohkuma and Kudo 1996). Detritivorous subsocial cockroaches believed as termite ancestors (Inward et al. 2007) can digest wood with the help of cellulolytic flagellates (Engel et al. 2009; Ohkuma et al. 2009). The evolution of termites coincides with three major events associated with dietary diversification and microbial symbiosis: (i) cellulolytic, flagellate protozoa inhabit the hindguts of all five lower termite families except Termitidae, (ii) an ectosymbiotic relationship with basidiomycete fungi in the litter-feeding Macrotermitinae and (iii) independent evolution of soil-feeding behaviour among the three higher termite subfamilies Nasutitermitinae, Apicotermitinae and Termitinae (Ohkuma and Brune 2011). The role of the cellulolytic flagellates in lower termites is well known, but the exact functions of uncultivated bacterial symbionts in digestion, especially in flagellate-lacking higher termites, need further research (Hongoh 2011). Subterranean termites primarily feed on woody tissue containing lignocellulose as their diets are deficient in vitamins and essential components for protein and fat synthesis. These termites produce their own cellulases (Inoue et al. 1997), but in order to meet their nutritional requisites, the association with symbionts becomes crucial and is achieved by coprophagy and trophallaxis (Grimaldi 2001). These symbionts display a mutualistic beneficial relationship by augmenting nutrients and energy to the termites and by gaining a stable food supply as well as protection under the constant gut environment (Nalepa et al. 2001). Each worker termite acquires an initial inoculum of symbionts from the parents or nest mates, right after hatching as well as after each moult (Thorne 1997).

The gut microbiota varies markedly among termite species comprising many unique phylogenetic clusters (Hongoh et al. 2006). The presence of microbes in the guts of cockroaches (Schauer et al. 2012) and termites (Noda et al. 2009) suggests that the bacterial microbiota of both originated from a common dictyopteran ancestor (Dietrich et al. 2014) (Table 4.1). Termites rely predominantly upon the symbiotic protozoa (metamonads) and flagellate protists for cellulose digestion as well as absorption of the end products (Ikeda-Ohtsubo and Brune 2009). Gut protozoa, such as *Trichonympha*, further rely on the symbiotic bacteria rooted on their body surfaces for the production of the necessary digestive enzymes. Termites of the family Termitidae, although able to produce their own cellulases, depend on bacteria as well (Li et al. 2013).

Table 4.1 Microbiota of termite gut

Domain	Genus, Species	Brief description/Function of microbes
Bacteria	*Treponema*	Swim freely in the gut or attached to the protest; acetogenic, carry out acetogenesis
	Bacteroides, *Bacteroides termitidis*	Fermentative, acidogenic; increase N source by recycling uric acid waste
	Desulfovibrio	Sulphate-reducing bacteria; transfer hydrogen as H_2 donor
	Citrobacter, *Citrobacter freundii*, *Enterobacter*, *Enterobacter agglomerans*	Nitrogen-fixing bacteria
	Enterococcus, *Lactococcus*	Lactic acid bacteria
Archaea	*Methanobrevibacter*	Methanogens, associated with protists as symbionts; carry out methanogenesis
Protists	*Trichonympha*, *Mixotricha*, *Dienympha*, *Eucononympha*	Degrade endocytosed cellulose and produce H_2 plus CO_2. Anaerobic, occur on mitochondria in the cells

4.3.1 Bacteria

Microscopic cell counts approximate presence of 10^6 to 10^7 bacteria in comparison with the protozoa ($4 \cdot 10^4$) in the gut of *R. flavipes* where the prokaryote groups principally consist of methanogens (Archaea), *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Bacteroidetes* and *Spirochaeta* (*Eubacteria*) (O'Brien and Slaytor 1982). Three species of methanogenic Archaea placed in the genus *Methanobrevibacter* have already been cultured from *R. flavipes* gut (Leadbetter et al. 1998). The culture-independent sequencing of archaeal 16S rRNA genes also indicates the presence of methanogens in the gut of *R. speratus* (Shinzato et al. 2001). Some methanogen species are both ecto- and endosymbionts of protozoa (Tokura et al. 2000). Methanogenesis is followed by acetogenesis in the wood-feeding termites and acetogenic bacteria compete with the methanogens for hydrogen (Brauman et al. 1992).

The hindgut of xylophagous lower termite harbours both protozoa and bacteria. However, due to their mutualistic relationship with termites and cellulose-degrading activity, more research has been done on protozoa, although prokaryotes are also important for the termites' survival (Mauldin 1977). The presence of bacteria is necessary for the persistence of protozoa as well as for normal termite nutrition (Breznak 1975). Nitrogen fixation by some bacteria shows a role in nitrogen utilization by termites, as many species exist in intimate physical association with the gut epithelium, thereby reflecting biochemical interactions (Thayer 1976). An anaerobic, cellulolytic actinomycete (*Micromonospora propionici*) from the guts of *Amitermes minimus* was isolated, but it turned out to be of limited importance in situ (Hungate 1946). *Anacanthotermes ahngerianus* and *A. turkestanicus* harbour $1.3 \cdot 10^6$–$4.3 \cdot 10^9$ viable bacterial cells per hindgut, depending on the developmental stage or caste of termite (Husseneder 2010; Krasil'nikov and Satdykov 1970).

The intestinal bacterial microflora of *R. speratus* contains taxa related to the enteric bacteria, such as *Enterobacter*, *Citrobacter*, *Desulfovibrio*, *Treponema*, *Bacteroides* and *Clostridium* which are either strict or facultative anaerobes and have been frequently isolated from animal intestines (Brauman et al. 1992). Apart from *Citrobacter freundii*, *Enterobacter agglomerans*, *Clostridium* and *Desulfovibrio* spp. also partake in nitrogen fixation in several termite species (Fuhrman et al. 1993). Species of *Sporomusa*, *Acetonema* and *Clostridium* act as CO_2-reducing acetogens within the termite guts (Kane and Breznak 1991). *Bacteroides* species (*Bacteroides termitidis*) participates in the uric acid metabolism (Potrikus and Breznak 1980). The termite *Desulfovibrio* cluster points out the presence of significant quantity of the sulphate-reducing bacteria in situ within gut, functioning in interspecies hydrogen (H_2) transfer as H_2 donors by utilizing small organic compounds. These include, for example, pyruvate, lactate and sugar monomers as oxidizable electron donors in comparison to the H_2 acceptors oxidizing sulphur compounds.

Streptococcus sp. occurs as the major bacterial species in several termites (Breznak and Brune 1994). The gut microbes enrich the termites with the required amount of carbon, nitrogen and energy to such an extent that survival of termites becomes almost impossible without them (Brune and Friedrich 2000). A predominance of five bacterial groups, viz., *Cytophaga-Flexibacter-Bacteroides* (CFB) group, low-G + C Gram-positive bacteria, *Proteobacteria*, *Spirochaeta* and the newly discovered 'termite group I' (TG I), has been found in many species (Hongoh et al. 2003). However, spirochaetes occur more in the gut of *R. speratus* as compared to the gut contents of *Nasutitermes lujae*, followed by the *Bacteroides*-related, clostridial and TG I clones which form the second most dominant group (Husseneder 2010; Paster et al. 1996).

Spirochaetes comprise a monophyletic group of motile bacteria with a characteristic spiral or wavy shape (Paster et al. 1991) and a coiled or undulate protoplasmic cylinder confined by a cell wall-cytoplasmic membrane complex, containing genomic DNA, ribosomes and other cytoplasmic constituents. An outer membranous sheath surrounds the protoplasmic cylinder. Between the outer sheath and the protoplasmic cylinder one or more periplasmic flagella are present (Canale-Parola 1984), functioning as organelles of motility (Charon et al. 1992). *Spirochaetes* are widely distributed in nature for example, as free-living forms in freshwater, marine and hypersaline waters or associated with invertebrate and vertebrate hosts, through commensalism, mutualism and parasitism. *Spirochaetes* were first documented by Joseph Leidy (1874–1881, 1877) in the hindgut of the eastern subterranean termite, *Termes* (now *Reticulitermes*) *flavipes*. *Spirochaetes* account for up to 50% of all prokaryotes in the hindgut of some termites and about a dozen of different morphological types can be identified on the basis of cell length, width, wavelength and amplitude or pitch (Paster et al. 1996; Leadbetter et al. 1999).

The nucleotide sequence of MDS1clone of the Australian termite, *Mastotermes darwiniensis* (family Mastotermitidae) (Berchtold et al. 1994), *R. speratus* (family Rhinotermitidae) (Ohkuma and Kudo 1996) and *Cryptotermes domesticus* (family Kalotermitidae) (Ohkuma 1998) indicates that the spirochaetes residing within the

guts of these species are either anaerobes or microaerophiles (Breznak 1994). Termite gut spirochaetes are placed into two phylogenetic clusters namely *Treponema* clusters I and II. Cluster I contains diverse phylotypes of the gut spirochaetes containing the strains isolated from termite gut, while cluster II is smaller, belonging to the *Treponema bryantii* subgroup.

Spirochaetes can exist freely in the gut fluid or are attached as ectosymbionts on the cell surface of gut protists (Iida et al. 2000). They have specialized attachment sites on the protists as shown by ultrastructural observations (Bloodgood and Fitzharris 1976). *Treponema* strains ZAS-1 and ZAS-2 have been isolated from the dampwood termite *Zootermopsis angusticollis* (family Termopsidae) (Leadbetter et al. 1999; Breznak 2002). Recently, ectosymbiotic spirochaetes (*Treponema* sp. strains ZAS-1 and ZAS-2) associated with *Zootermopsis angusticollis* have been clustered with the major cluster I sequences, with function as H_2 and CO_2 consumers by environment absorbance from inside the protist. The other major ectosymbiotic spirochaetes (cluster II) placed in the *T. bryantii* subgroup can enhance the cellulolytic activity of other microbes (Ohkuma and Kudo 1996). Members of cluster II are ectosymbiotic spirochaetes of oxymonad protists as identified in the termite species *R. speratus*, *Hodotermopsis sjoestedti* and *Neotermes.* However, various species of devescovinid (*Devescovina* sp.), calonymphid and hypermastigote protists (*Holomastigotoides mirabile* in *Coptotermes formosanus*), in addition to the oxymonad protists, also harbour dense populations of ectosymbiotic spirochaetes (Iida et al. 2000).

The unique ultrastructure of spirochaete-protist attachment sites is known for several protists of termites and wood-feeding cockroaches, and two types of attachment structures have been identified in the symbiotic protists of *R. flavipes*, *R. tibialis* and *Cryptocercus punctulatus*. One structure is a narrow nose-like appendage making direct contact with the plasma membrane of the host cell, while the other is the flattened end of the spirochaete in contact with the protistan membrane, with a thick layer of electron dense material. The ectosymbiotic spirochaetes are involved in the maintenance of 'motility symbiosis' (Kitade et al. 1997). The termite gut bacteria are deliberated to be vertically transmitted from generation to generation via proctodeal trophallaxis known for the gut symbiotic protists in lower termites (Inoue et al. 2000). However, horizontal transfer among congeneric termites is also known for the bacterial community profiles of *Microcerotermes* species M1 and M2. Ambient temperature, food quality and humidity also affect variation in bacterial gut microbiota within congeneric termites (Donovan et al. 2004).

Gut epithelium is also an important habitat for the dense colonization of the symbionts. Methanogenic archaea utterly occupy the gut wall of *R. flavipes*, while in other termite species, they are located within some protist cells (Leadbetter and Breznak 1996). The methanogenic species present on the gut wall, as well as within the protists cells in *R. speratus* and *Hodotermopsis sjoestedti*, are phylogenetically different (Tokura et al. 2000). This is due to the radical changes in the physico-chemical conditions, especially fluctuations of oxygen and hydrogen partial pressures, found inside the gut (Brune and Friedrich 2000).

Bacterial communities also diversify at the phylum or phylotype levels within the gut wall and gut luminal fractions, where bacteria residing on the gut wall are believed to be more dynamic than those populating the gut lumen (Noda et al. 2003). Divergence index, rarefaction curve and Chao1 richness estimators of biodiversity also confirm that a wide variety of bacteria are able to colonize the gut wall, directly or indirectly. However, the populations on the gut wall are five times lower in comparison to those on the gut luminal fraction (Iida et al. 2000).

Bacterial groups densely colonizing the gut wall fraction include species of *Actinobacteria*, *Bacteroidales*, *Clostridiales* and *Lactococcus.* Many of them are nonmotile species. However, gut lumen shows abundance of highly motile *Spirochaetes* and *Desulfovibrio* species (Wenzel et al. 2003). In order to resist fluid flow within the gut, bacterial symbionts also subordinate with the protists, for example, spirochaetes and TG1 bacteria that form associations with the gut protists. The central part of the gut is anoxic as oxygen penetrates the gut via its wall, thereby maintaining a steep oxygen gradient near the gut wall (Brune and Friedrich 2000). This affects the gut metabolism as already displayed by the isolated lactic acid (Tholen and Brune 1997) or sulphate-reducing bacterial strains (Kuhnigk et al. 1996) highlighting the presence of strict aerobic communities inside the gut (Moriya et al. 2003).

Bacteroidales endosymbionts dominate the gut bacterial community of *C. formosanus* and *Pseudotrichonympha* sp. by living intracellularly within eukaryotic cells, while few *Bacteroidetes* or the *Cytophaga-Flavobacterium-Bacteroides* (CFB) phylum bacteria occur as intracellular endosymbionts of eukaryotic cells (Bandi et al. 1995). Intracellular *Blattabacterium* sp. dwell within the bacteriocytes of fat bodies of cockroaches and *Mastotermes darwiniensis*. Intracellular acanthamoebae bacteria affiliated as *Flavobacterium* or '*Candidatus Amoebophilus asiaticus*' resemble with the endosymbionts of a tick and a whitefly through a novel phylogenetic lineage (Horn et al. 2001).

The ectosymbiotic *Bacteroidales* carry a typical Gram-negative cell wall along with inner and outer membranes. However, endosymbionts have lost the cell wall as they localize within the cytoplasm of the host protist. Ectosymbionts colonizing the protist cell surface are incorporated into vacuoles of the host cytoplasm, as structures required for attachment with the host cell membrane occur in the vacuoles (Stingl et al. 2004).

Endomicrobia (formerly termite group 1) represent a deep branching clade of the uncultivated bacteria of the phylum *Elusimicrobia* with single isolate, *Elusimicrobium minutum* which occurs as intracellular symbiont of termite gut flagellates and transferred vertically. Many genes of *E. proavitum* are highly similar to the closely related '*Candidatus Endomicrobium trichonymphae*' strain Rs-D17 (Brune 2014).

Bacteria of the *Cytophaga/Flexibacter/Bacteroides* (CFB) phylum predominate the guts of *Macrotermes michaelseni*, fungus-cultivating (*Macrotermes gilvus*) (Hongoh et al. 2006), wood-feeding (Ohkuma et al. 2004) and soil-feeding termites (Schmitt-Wagner et al. 2003). Many CFB phylum bacteria are capable of degrading plant fibres and proteins which form the ingredients of the termite diet (Shah 1992). *Proteobacteria*, *Desulfovibrio* sp., *Escherichia hermannii* and E. *senegalensis* also

colonize the gut of *M. gilvus* instead of CFB phylum members (Hongoh et al. 2006). *Desulfovibrio* spp. are strict anaerobes partaking in sulphate reduction and nitrogen fixing. Clostridia are also present in the mixed segment of *Nasutitermes takasagoensis* (Shiraki) (Tokuda et al. 2000) and *M. gilvus* (Hongoh et al. 2006) where they degrade polysaccharides and produce acetone, alcohol, acetate, lactate, CO_2 and hydrogen (Chen 1995). However, *Clostridium mayombei* present in the mixed segment of *Cubitermes speciosus* brings about acetogenesis (Kane et al. 1991). The intestinal bacteria in termites aid in the production of short-chain fatty acids from carbohydrates or amino acid synthesis, just like they do in humans (Cummings and Macfarlane 1997).

The *Anaerobaculum-Thermoanarovibrio*, also denoted as the 'Synergistes group', shows coevolution with termites although the exact role of these members in the termite guts is unknown. It is believed that they are anaerobic amino acid degraders participating in the amino acid turnover in natural anaerobic ecosystems (Godon et al. 2005). The 'Synergistes' strains *Aminomonas paucivorans* (Baena et al. 1999a), *Thermoanaerovibrio acidaminovorans* (Baena et al. 1999b), *Dethiosulfovibrio* sp. (Surkov et al. 2001), *Aminobacterium mobile* (Baena et al. 2000) and *Aminobacterium colombiense* (Baena et al. 1998), for example, degrade amino acids, but some strains such as *Thermanaerovibrio velox* (Zavarzina et al. 2000) and *Anaerobaculum* sp. can utilize carbohydrates as well (Rees et al. 1997).

The population of spirochaete-like cells in the gut contents of fungus-cultivating termites is generally low, ranging 2–3% in *Pseudacanthotermes*, 6–10% in *Odontotermes* sp. (Liu et al. 2013), 11–19% in *Ancistrotermes* and 22–29% in *Microtermes* sp. (Makonde et al. 2013).

Firmicutes and *Bacteroidetes*, placed in the *Treponema* I lineage, form the major fraction of the bacterial community in the Macrotermitinae (Dietrich et al. 2014) although their actual is not clear, but several of them resemble isolates from the lower termite hindguts. These species either partake in fermentation of mono- and oligosaccharides by producing acetate and other products or are homoacetogenic (Droge et al. 2008). Uncultured *Treponema* lineages in higher termites can carry out reductive acetogenesis from $H_2 + CO_2$ (Warnecke et al. 2007). Methanogenesis dominates the reductive acetogenesis as hydrogen sink in Macrotermitinae. Therefore, *Treponema* shows a faint representation which is consistent with the reduced acetogenesis observed in the guts of *Macrotermes mulleri*, *Postelectrotermes militaris* and *Pseudacanthotermes spiniger* (Brauman et al. 1992). The reason behind this is, however, unknown (Hongoh 2011). Variation in the microbiotas of Macrotermitinae reflects their ecological differences which is governed by the plant diet and *Termitomyces* association.

Diet shapes the gut communities of wood-feeding termites (Huang et al. 2013), but such variation in the Macrotermitinae is generally not very clearly understood (Hongoh 2010). *Macrotermes* spp., with the exception of *Macrotermes malaccensis*, are primarily leaf litter feeders (Hyodo et al. 2000). *Odontotermes* and *Ancistrotermes* sp. predominantly feed on wood, and *P. militaris* feeds on both leaf litter and wood (Hyodo et al. 2003). The role of *Termitomyces* also varies among different termite hosts (Nobre et al. 2011). Although this needs more attention,

some termite species mainly gain access to cellulose via the lignolytic activity of *Termitomyces* (Hyodo et al. 2000). Some species exploit *Termitomyces* as a protein-rich food source (Hyodo et al. 2003), while other species extract cellulases and xylanases from *Termitomyces* for the decomposition of plant substrate (Rouland et al. 1991). Therefore, differences in gut communities among the termite hosts may be the result of specific diets or symbiont functions.

The role of the bacterial community in lignocellulose breakdown in the Macrotermitinae is not fully elucidated. Metagenomic studies suggest their possible contributions in the cellulose digestion in the wood-feeding termites (He et al. 2013). Glycosyl hydrolases showing cellulase activity in the P3 lumen metagenome of the wood-feeding *Nasutitermes* sp. are now taxonomically confined to the phyla *Fibrobacteres* and *Spirochaetes (*Warnecke et al. 2007). These phyla, along with the TG3 phylum, show high abundance in wood-feeding termites of the subfamilies Nasutitermitinae (Mikaelyan et al. 2014) and Termitinae (Dietrich et al. 2014).

4.3.2   Protozoa

The symbiotic associations between termites and their gut microorganisms are continuously under study since the beginning of the century, when earlier work was centred on the intestinal protozoa of lower termites and their role in digestion. Currently more attention is given indeed to the bacterial and archaeal populations, their metabolic activities, structure and function.

Intestinal protozoa provide shelter to the prokaryotes in the hindgut of lower termites by forming intimate associations with them. Previously, it was difficult to obtain protozoan cultures in vitro and maintain pure cultures of prokaryotic symbionts, so only a morphological description of the different associations was known. With advanced molecular biology tools, informations regarding the symbiotic associations between prokaryotes and termite gut flagellates have now become clear.

Symbiotic flagellates exclusively inhabit the lower termites and closely related cockroaches (*Cryptocercus*), whereas higher termites largely harbour prokaryotic microbiota. Molecular phylogeny of termites and *Cryptocercus* clarified that gut protists share a common ancestor (Lo 2003). The beneficial nature of these peculiar symbionts came into light for the first time by the studies of Lespes in 1856 (Leidy 1874–1881), who classified them as parasites, while Cleveland (1926) pointed out that termites cannot live without the gut flagellates. The symbionts heavily populate the hindgut paunch, with a fresh weight that may account for more than 50% of the fresh termite weight (Katzin and Kirby 1939). Phylogenetically, gut flagellates are extremely diverse, and almost 450 distinct species, from about 200 termite species, are into account till date (Yamin 1979).

Termite gut flagellates were earlier considered as primitive, primarily amitochondriate eukaryotes. Recent molecular data indicated that they share two separate eukaryotic lineages and can be placed under three distinct taxa, namely, trichomonads, hypermastigids and oxymonads (Yamin 1979). Phylogenetic studies conducted

by 18S rRNA gene sequence analysis confirmed that majority of termite gut flagellates fall within two classes of the phylum Parabasalia, i.e. Trichomonadea and Hypermastigea (Gerbod et al. 2004). On the other hand, the phylogeny of Oxymonadea was under dark for a long time, but comparative sequence analysis indicated that they are a sister taxon of unidentified protists which are now classified in the phylum Loukozoa (Stingl et al. 2005).

Hypermastigea are exclusive termite gut symbionts, while Oxymonadea and Trichomonadea inhabit other habitats as well, such as the intestinal tract or body cavities of other animals, including humans (Cavalier-Smith 2002). Out of the 440 species of amitochondriate protists belonging to *Trichomonadida*, *Hypermastigida* and *Oxymonadida* are exclusively gut symbionts of wood-eating termites (Mastotermitidae, Kalotermitidae, Hodotermitidae, Termopsidae, Rhinotermitidae and Serritermitidae) and the wood-eating roach, *Cryptocercus*.

Bacterial symbionts associate either on the cell surface or in specific cytoplasm regions of the protozoan, as confirmed by morphology-based descriptions. Trichomonads and other amitochondriate protist symbionts of wood-eating termites and *Cryptocercus* can be easily identified by the presence of their motility structures (mastigont) while in Parabasalia (trichomonads, hypermastigids) by the parabasal body (Golgi complex). The key characters include number and arrangement of flagella (undulipodia), presence and shape of accessory structures (e.g. costa and cresta) and arrangement of connected microtubular structures, axostyle and pelta. The parabasal body bears a distinct shape as well as size and can branch or spirally coiled around the axostyle. It is connected to the mastigont in the trichomonads and arranged in multiple copies in the hypermastigids. Every termite species inhabits a characteristic community of gut protists. Therefore, in general the protist species are not restricted to one termite species only (Simpson 2006).

The phylum Parabasalia has a monophyletic but complex assemblage of diverse species of flagellated protists characterized by a unique parabasal apparatus (Golgi complex associated with striated fibres), closed mitosis with an external spindle (cryptopleuromitosis) and anaerobic energy-generating organelles (hydrogenosomes) (Brugerolle 2005). On the basis of their morphological characters, more than 80 genera and 400 parabasalid species are now known (Yamin 1979). Most parabasalids occur in the digestive tract of animal hosts either as commensals, parasites or symbionts, whereas the symbiotic parabasalids present in the gut of termites and wood-eating cockroaches have a key role in cellulose digestion (Brune and Ohkuma 2011). This symbiotic relationship between termites and parabasalids also provides an evidence for the evolution of social behaviour in the hosts and also carries an ecological significance for plant litter decomposition in terrestrial ecosystems (Ohkuma 2003).

Mixotricha paradoxa, a protozoan species with multiple bacterial symbionts living in the gut of the Australian termite species, *Mastotermes darwiniensis*, was first described in 1933 by Australian biologist J.L. Sutherland (Cleveland and Grimstone 1964). *Mixotricha* bears four anterior flagella which are used for steering rather than for locomotion. Locomotion is performed by cilia-like movements of approximately 250,000 hairlike *Treponema* spirochaetes attached to the cell surface. *Mixotricha*

also shows the presence of rod-shaped bacterial *Bacteroides*-like species, arranged in an ordered pattern on the cell surface. Both these bacterial types (*Spirochaetes* and *Bacteroides*) are ectosymbionts. Apart from the ectosymbionts, *Mixotricha* has spherical bacteria inside the cell (endosymbionts) functioning as mitochondria, which are absent in this protozoan. Just like in its relatives, including *Trichonympha*, these bacteria help in cellulose digestion, as without *Mixotricha* the host termites fail to survive (Radek and Nitsch 2007).

Pseudotrichonympha (class Hypermastigea and order Trichonymphida) carries *Hoplonympha*, *Barburanympha*, *Urinympha* and *Staurojoenina* as ectosymbionts that form a monophyletic lineage. The endosymbionts of *Pseudotrichonympha* are distinct (Ohkuma et al. 2005) as they closely resemble the ectosymbionts of *Devescovina* protists (order Cristamonadida; class Trichomonadea). Two other protist species, *Holomastigotoides mirabile* and *Spirotrichonympha leidyi* also dwell in the gut of *C. formosanus*. Endosymbionts rarely occur within the cells of *H. mirabile* as this protist carries attached spirochaetes (ectosymbionts) of the cluster I of termite treponemas (Noda et al. 2003). The concentration of ectosymbiotic spirochaetes is less than 200 cells per *H. mirabile* cell. However, cells of *Spirotrichonympha leidyi* have an endosymbiotic methanogen (*Methanobrevibacter*) and only 300 cells of the methanogen occur within a single cell (Shinzato et al. 2005).

4.3.3 Fungi

The nests of the fungus-growing macrotermitine termites can occupy high volumes and can persist for decades, with million sterile helper individuals produced by a single queen (Shellman-Reeve 1997). This agricultural symbiosis with fungi has enabled this termite species to occupy niches loaded with abundant resources that were previously inaccessible (Waller 1988).

The fungal symbionts of Macrotermitinae produce sexual fruiting bodies (Katoh et al. 2002). Symbiotic relationship has a significant role in termite evolution, as it involves a wide range of intestinal microorganisms (Bignell 2000). Macrotermitinae is the only example of a single Termitidae subfamily displaying a mutualistic ecto-symbiosis with a white-rot fungi, *Termitomyces* [tribe Termitomyceteae (Julich) Singer, family Tricholomataceae Roze, Basidiomycotina]. The fungus is capable of digesting lignin and provides food, either directly, when termites consume fungal nodules containing asexual spores, or indirectly, when they ingest partially degraded fungal biomass (Bignell 2000). The fungus is cultivated on a specialized structure within the nest, known as the fungus comb, which is maintained via continuous addition of predigested plant substrate. However, the older comb material is also consumed (Rouland-Lefevre et al. 2002). Approximately 40 species of *Termitomyces* symbiont are known till date, in Macrotermitinae (Kirk et al. 2001).

Just like most *Basidiomycetes*, *Termitomyces* shows homokaryotic and hetero-karyotic stages in the life cycle, with one and two genetically different types of haploid nuclei (De Fine Licht et al. 2005). The fungus mycelium is heterokaryotic

in all natural colonies, which emphasizes that the incipient termite colonies must contain at least two compatible and genetically different sexual spores, with a relatively short-lived homokaryotic stage (Aanen et al. 2007). The cultivation is beneficial to both the termites and the fungus in a number of ways, for example, the fungus is able to chemically degrade complex substances (e.g. lignin) in an easily accessible form for the termites and also the fungal component increases the termite diet N/C ratio, enabling the use of more diverse cellulose sources. On the other hand, the fungus gains advantages by accessing plant material for easier penetration, along with an increased surface area, in a suitable microclimate optimal for establishment, and, last but not the least, termite secretions prevent spread of microbial infections (Darlington 1994).

The Macrotermitinae nests exhibit an expanded thermoregulation by maintaining constantly high temperatures along with high relative humidity (Korb and Linsenmair 2000a, b). This provides an optimal microclimate for fungal cultivation (Wood and Thomas 1989) as well as a platform for the synergistic interaction of the complementary enzyme systems (enzymes derived from the termite and fungus) for cellulose digestion (Veivers et al. 1991).

The morphospecies of *Termitomyces* and its fruiting bodies form a monophyletic group intimately associated with the termite nests (Rouland-Lefevre et al. 2002), suggesting a single evolutionary origin of mutualistic symbiosis with termites. Cospeciation and specificity patterns are, however, consistent with the fungal symbiont transmission from host to host, outside the vertical host lineage [horizontal transmission] (Frank 1996).

Horizontal fungal transmission occurs in most of the Macrotermitinae-*Termitomyces* associations where the fungus produces fruiting bodies (basidiocarps) bearing sexual spores which are carried towards the newly formed nests by the first workers of the new colony through their first foraging trips (Darlington 1994). Laboratory trials confirm that alates (winged sexuals) fail to establish a colony unless provided with external fungal spores (Sieber 1983) and also the fruiting bodies of the fungus are in synchronization with the emergence of first fully developed foraging workers (Johnson 1981). Few Macrotermitinae termites also show vertical, uniparental symbiont transmission, in which reproductive units of termite and fungus are aligned together. *Microtermes* sp. and *Macrotermes bellicosus* alates (either male or female) carry in their foregut a bolus of conidia (asexual spores) from the fungus combs of the parent colony for inoculation of first fungus combs in their newly formed colonies (Wood and Thomas 1989). In *M. bellicosus* males transmit the fungus, while females transmit the fungus in *Microtermes* sp. However, fungal fruiting bodies have never been identified in these termite species (Darlington 1994). Phylogenetic and molecular investigations have supported this difference in sex-specificity in fungus transmission, pointing out an independent origin of uniparental and vertical transmission in these termite species. Termites with vertical transmission do not form a monophyletic group but fall in two unrelated clades, suggesting horizontal transmission as ancestral mode of transmission of which uniparental transmission is a derived trait, having two independent origins (Aanen et al. 2002).

Like most basidiomycetes, *Termitomyces* also have a heterothallic (i.e. outcrossing) life cycle where germinating spores form a monokaryon, and all cells carry a single nucleus. Later, two monokaryons of same species with different mating types fuse to form a stable dikaryon, and all cells have two nuclei, one derived from each monokaryon. This dikaryon can form fruiting bodies by meiosis, finally forming a spore. Macrotermitinae fruiting symbionts showing horizontal transmission follow this pattern, while some fruiting *Termitomyces* fungi also have a homothallic (i.e. non-outcrossing) mating system, in which only a single fungal spore completes the life cycle.

Two main models have been proposed for the successive evolution of the fungus in social insects. The first model is the traditional 'consumption first' model where consumption is followed by cultivation and the transmission fungi become essential component of the insect's diet (vertical fungus transmission). The second model is the alternative 'transmission first' model, in which transmission is preceded by consumption, resulting in the cultivation of a specialized fungus which is dispersed by the insect. Insects cultivate the fungus by the addition of substrate (Mueller et al. 2001).

Xylaria (*Ascomycotina*, *Xylariales*) occupies a wide variety of habitats including dead or live plant material as endophytic in living plants (Davis et al. 2003) and also a vast number of fungus-growing termite nests, where the termites actively control species composition by continuous excretion of antimicrobial peptides (Fuller 2007). This active suppression of spore germination or mycelial growth by termites results in a patchy distribution of *Xylaria* across fungus combs. *Xylaria* species can degrade lignin. Therefore, they cause white rot in wood and plant debris (Osono and Takeda 1999). Termites bring the inocula of *Xylaria* into their nests by their foraging activities. Certain *Xylaria* species (*X. escharoidea*, *X. furcate* and *X. nigripes*) show coevolution with termites as they carry smaller spores (Rogers et al. 2005) that can be easily ingested or carried by insects, enhancing the chances of dispersal (Rogers 2000). Termite-associated *Xylaria* act like 'sit-and-wait saprotrophs', foliar endophytes latently present on the leaf and start degrading processes only when the leaf falls from the tree (Herre et al. 2007).

4.4 Physiological Roles of the Microorganisms

The termite gut symbionts carry out the following physiological roles:

4.4.1 Nitrogen Fixation

Nitrogen fixation is a fundamental aspect of symbiosis in termites and is controlled by the gut microbes due to low nitrogen content of the termite diet. The rate of nitrogen fixation greatly varies among and within the same termite species (Braun et al. 1999). A nitrogenase complex catalyses the biological nitrogen fixation (Dean

and Jacobson 1992) with the help of a molybdenum (Mo)-containing nitrogenase enzyme, encoded by the *nif HDK* operon. The cofactors of Mo-independent nitrogenases coordinate either with vanadium or lack Mo/vanadium (alternative nitrogenase) which in turn is encoded by the *vnfH-vnf DGK* and *anfH DGK* operons. The *nifH*, *vnfH* and *anfH* genes bear a high degree of sequence conservation (Widmer et al. 1999). Although there is a simultaneous regulation of genes within the single operon, the three operons (*nif*, *vnf* and *anf*) are differentially regulated. Availability of fixed nitrogen strictly regulates transcription of all the three nitrogenase operons, while Mo availability differentially affects expression of nitrogenase genes during transcription (Ohkuma and Kudo 1996).

The *nif* operon is repressed in the absence of Mo, but *vnf* and *anf* operons are repressed in the presence of Mo. A high nitrogen fixation activity, where more than half of the fixed nitrogen comes from the atmospheric N_2, is known in the drywood termite, *Neotermes koshunensis* (Tayasu et al. 1994). A wide diversity of *nifH* genes has been documented in the gut of *N. koshunensis* and other termite species as well (Ohkuma et al. 1999). In the symbiotic microbial community of *N. koshunensis*, the *anf* gene, connected with termite *anf*-methano cluster I, is the most critical gene responsible for fixation. It is believed that inadequate amount of Mo in the diet of this termite species is responsible for the expression of *anf* genes as the ordinary Mo-dependent nitrogenases require Mo as a cofactor for nitrogen-fixing activity. Termite species lacking *anf* genes in the gut community are possibly able to obtain sufficient amounts of Mo from their food (Ohkuma et al. 1996).

Some methanogenic archaea have *nifH* genes associated with the *anf*-methano group of *nifH* genes, but their *nifD* genes are phylogenetically grouped with Mo-dependent nitrogenases (Kirshtein et al. 1991). Mo-independent regulation of *anf* gene expression suggests encoding of a Mo-dependent ordinary enzyme, just like in methanogens. The *anfH*, *anfD* and *anfG* in the *anf* gene cluster encode both Mo- and V-independent alternative nitrogenases.

Gene organization and sequence features of the termite *anf* gene distantly resemble with those of well-characterized organisms. For example, in the Archaea domain, a Mo-independent alternative nitrogenase and *anfD* and *anfG* orthologous genes have not been reported yet, but the *anfH* gene resembles bacterial *anfH* gene. The Archaea domain including all diazotrophic methanogenic archaea contains two ORFs between *nifH* and *nifD* genes. In *Clostridium cellobioparum*, a sequence homologous to ORF105 has been recently identified, but the nucleotide sequence of the DNA region corresponding to ORF122, or even existence of ORF122-like genes, has not been documented yet. Presence of ORF105 in the genome of the bacteria domain indicates that it is not a characteristic of the Archaea domain only. The two ORFs of the termite *anf* gene cluster closely resemble the methanogen, *Methanosarcina barkeri* (Chien and Zinder 1996).

N_2 fixation by the gut microbes contributes to about 60% of the total nitrogen in some termite colonies (Breznak 2000). Several strains of spirochaetes residing in termite gut also fix N_2 (Tayasu et al. 1994), and recently ZAS strains, along with their two homologs of *nifH*, also exhibit nitrogenase activity, in which ZAS-9 shows greatest specific activity (100-fold greater) than ZAS-1 and ZAS-2 (Lilburn et al.

2001). ZAS-9 also fixes $^{15}N2$. The NifH amino acid sequences of several spirochaetes along with the ZAS strains are identical or nearly identical to various NifHs observed in termite guts (Noda et al. 1999), indicating their spirochaete origin (Lilburn et al. 2001).

4.4.2   Acetogenesis

The symbiotic protozoa and bacteria present in the hindgut of *Reticulitermes flavipes* carry out homoacetic fermentation of cellulose. Cellulolytic protozoa first hydrolyse cellulose by fermenting every C (CO_2) and H_2 (Breznak 1984):

$$C_6H_{12}0_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2$$

CO_2-reducing acetogenic bacteria then convert H_2 and CO_2 to an additional acetate molecule (Breznak and Switzer 1986):

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O$$

The three acetates formed per glucose monomer are absorbed and oxidized by the termite to supply 100% of the insect's respiratory requirement (Odelson and Breznak 1983):

$$3CH_3COOH + 6O_2 \rightarrow 6CO_2 + 6H_2O$$
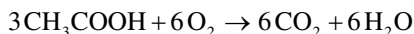
H_2 and CH_4 (methane is formed by reduction of CO_2 by methanogenic bacteria) are also emitted by termites. The rate of CO_2 reduction to acetate in the wood- and grass-feeding termites is greater in comparison to the fungus-growing or soil-feeding termites, while the rate of CH_4 emission by soil-feeding and fungus-growing termites (lesser extent) is greater than wood-feeding termites. Three strains of CO_2-reducing acetogenic bacteria from the guts of a higher and a lower wood-feeding and a higher soil-feeding termite have been described (Kane and Breznak 1991). Each one was a novel and different bacterial species capable of fermenting a variety of organic substrates for energy, including methoxylated aromatics (components of lignin). One of these isolates, *Sporomusa termitida*, is mixotrophic deriving energy by simultaneous usage of organic and inorganic (H_2 + CO_2) substrate mixtures (Breznak and Blum 1991). Mixotrophy enhances the ability of acetogens to outcompete methanogens for CO_2 reduction in wood- and grass-feeding termites.

Termite emissions can be considered as a significant source of total annual global CH_4 production, ranging from <5% to >40%. The hydrogenotrophic activity of acetogenic hindgut bacteria of the wood- and grass-feeding termites produces <10% CH_4, while fungus-growing and soil-feeding termites do not have significant levels of bacterial acetogenesis from H_2 + CO_2 so are more potent sources of CH_4 emission (Khalil et al. 1990).

H$_2$/CO$_2$ acetogenesis by termite gut spirochaetes is already known (Breznak 1975). The spirochaetes in higher termites and protozoa in lower termites display the highest H$_2$ concentration (up to 50,000 ppmv) in the luminal region, as confirmed by microelectrode-determined radial profiles (Ebert and Brune 1997). H$_2$-consuming methanogens (nonspirochetal prokaryotes) in *R. flavipes* lie on or near the hindgut epithelium (Leadbetter and Breznak 1996) and maintain low H$_2$ concentrations (Tholen and Brune 2000). The attachment of spirochaetes on the surface of hindgut protozoa as well as their ability to grow by H$_2$/CO$_2$ acetogenesis is responsible for 'motility symbiosis' (Cleveland and Grimstone 1964) allowing access to major sites of H$_2$ production. Recently, fluorescent rRNA-targeted oligonucleotide probes showed that only few distinct phylogenetic types of spirochaetes attach to protozoa (Iida et al. 2000).

Treponema strains ZAS-1 and ZAS-2, although capable of H$_2$/CO$_2$ acetogenesis but not restricted to the substrate like many so-called homoacetogens (anaerobic microbes producing acetate as major fermentation product), also ferment various mono- or disaccharides either alone or by H$_2$ consumption. ZAS-2 also carries out homoacetogenesis by using methyl groups of methoxylated aromatic compounds (Graber and Breznak 2000). Therefore, spirochaetes contribute to demethoxylation of lignin (Esenther and Kirk 1974) as well as other methoxylated aromatic components of termite food, but all termite gut spirochaetes are not homoacetogens. The *Treponema* strain ZAS-9 ferments sugars and produces acetate and other products, including H$_2$, but cannot conduct H$_2$/CO$_2$ acetogenesis (Graber and Breznak 2000).

4.4.3   Lignin Degradation

Lignin degradation in the gut of insects is carried out by the diversified microbial community. Cellulose degradation in insect guts is very well known (Breznak and Brune 1994), but the fate of lignin needs further research (Brune 2007) as it is widely accepted that insect gut systems lack the capability to degrade lignin (Ohkuma 2003). Apart from this fact, many previous studies have indicated that several wood-feeding insects can overcome the lignin barrier either by feeding on pre-degraded wood (Kukor et al. 1988) or via exosymbiotic relationships with wood-degrading fungi (Johjima et al. 2006) and that a few insect species can feed on the inner wood of alive healthy trees (Taprab et al. 2005). The major products of undegraded wood are 3,4-dimethoxybenzaldehyde (G4) from guaiacyl (G) lignin and 3,4,5-trimethoxybenzaldehyde (S4) from syringyl (S) lignin. The fungal lignin degradation has three main reactions: (i) propyl side-chain oxidation/cleavage, (ii) ring hydroxylation and (iii) demethylation (Filley 2003). Side-chain oxidation is responsible for Cα–Cβ cleavage/depolymerization of lignin in the white-rot fungi. The oxidative alteration of lignin propyl side chain produces higher amounts of 3,4-dimethoxybenzoic acid (G6) and 3,4,5-trimethoxybenzoic acid (S6), increasing the G6/G4 and S6/S4 ratios (Filley et al. 2000). Ring hydroxylation of guaiacyl units brings hydroxylation of either intact or side-chain-oxidized lignin. The

diastereomeric pair of the enantiomers 1-(3,4-dimethoxy)-1,2,3-trimethoxypropane represents integral lignin. Syringyl lignin can be more easily degraded and depolymerized (Chiang and Funaoka 1990).

The recent metagenome sequencing of the hindgut microbe community of the higher termite species *Nasutitermes corniger* strengthens the view that termites can degrade lignin although no genes encoding lignin-degrading enzymes are yet known (Warnecke et al. 2007). It is presumed that hindgut microbes are also anaerobic and can degrade lignin. The lignin modification by the lower termite *Cryptotermes brevis* showed only minor changes in the molecule, without modifying the side chains (Katsumata et al. 2007). Lignin biodegradation in *Zootermopsis angusticollis*, a lower termite feeding on coniferous trees, shows side-chain oxidation along with demethylation. All three reactions (side-chain oxidation, ring hydroxylation and demethylation) in this termite gut are possibly related to the brown-rot fungal decay, as brown and white fungi are already known to be associated with termite guts. Excluding the fungal components, aromatic degrading bacteria (actinomycetes) in termite guts are also known. However, they participate in evading lignin barrier although their actual biochemical abilities are not well defined (Delalibera et al. 2005). Actinomycetes plays similar aromatic degrading role in the gut of *Anoplophora glabripennis* (Schloss et al. 2006).

The well-coordinated cooperation between termites and fungi is responsible for the efficient utilization of lignocellulose. Old workers collect plant litter, while young workers masticate and ingest the collected plant litter which passes down the termite gut without digestion in the form of faecal pellets (primary faeces) used in the formation of fungus comb for the growth of the symbiotic fungi. The fungi form mycelia as well as fungus nodules, and lignin content progressively decreases, with the maturation of the fungus comb (Shary et al. 2007). The in vitro digestibility of cellulose in a matured fungus comb is approximately three times higher as compared to that in a newly formed one. Fungus nodules are consumed by young workers while old workers consume old combs for producing final faeces, but it is almost impossible to observe final faeces which suggest a highly efficient decomposition as well as complete biorecycling of plant litter. It is now very well known that symbiotic fungi degrade lignin bringing easy degradation of cellulose in comparison to the cellulase produced by the termites (Filley et al. 2006).

Lignocellulose digestion needs efficient cellulases along with glycoside hydrolases for the degradation of the cellulose and hemicellulose present in the plant cell wall, in addition to a mechanism for handling lignin barrier which is actually a combined effort of termite and symbionts (Brune 2014). Both higher and lower termites produce enzymes for the first stage of the tricarboxylic acid (TCA) cycle but lack an enzyme capable for the conversion of pyruvate to acetyl CoA or acetate (Breznak and Brune 1994).

Cellulose hydrolysis starts with endoglucanases released by the termites (salivary glands secrete endoglucanases in lower termites while they are secreted within

the midgut epithelium in higher termites). Numerous exoglucanases, exogluca-
nases, β-glucosidases and numerous other glycoside hydrolases are produced by the
gut flagellates in lower termites. Higher termites also produce many cellulases,
xylanases and other glycoside hydrolases. Metaproteomic studies of the hindgut of
N. corniger have shown the presence of almost a quarter of the 886 proteins identi-
fied as enzymes of which 36 are glycoside hydrolases (Burnum et al. 2011). These
findings suggest that these enzymes are important for the symbiotic relationship of
the hindgut microbes and termites. The metaproteomic and metatranscriptomic
analysis carried out by He et al. (2013) on *N. corniger* and *Amitermes wheeleri*
(both higher termites with different diets and habitats) displayed differences in the
abundance of certain bacteria in the guts due to their varied diets, but both species
contained many glycoside hydrolases for cellulose degradation. No lignin degrada-
tion genes were identified in hindguts of the two species (Fig. 4.3). (The details of
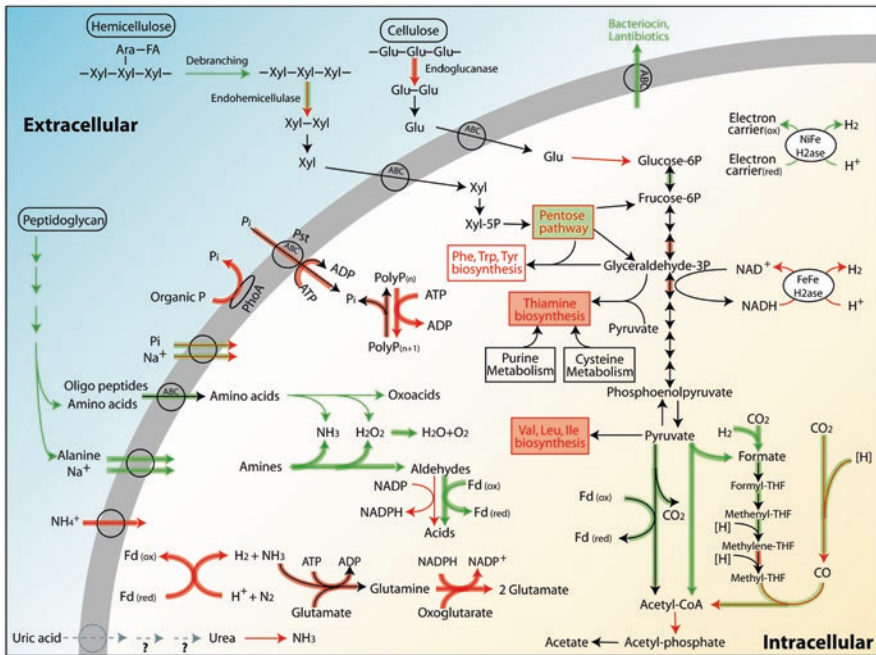the cellulose degradation will be discussed in another chapter.)

Fig. 4.3 Major metabolic differences between two higher termites from the metatranscriptomic
study of He et al. (2013) (Green lines indicate the genes more abundant in *A. wheeleri* while red to
N. corniger)

4.5 Conclusion

Termites play a vital role in recycling waste material important for the maintenance of the ecological balance. They produce about 11% of the atmospheric methane via cellulose breakdown. Symbiotic protozoa, as well as other flagellate protists residing in their guts, carry out the cellulose degradation. Gut protozoa (*Trichonympha*) are dependent on symbiotic bacteria for the production of necessary digestive enzymes. The higher termites (family Termitidae), however, produce their own cellulase enzymes. Some termite species practice fungiculture by maintaining 'fungal gardens' of specialized fungi *Termitomyces*, which are being nourished by their excrements. Researchers are trying to understand the details of the relationship between the termite digestive tract and the microbial endosymbionts.

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