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Diversity and Lignocellulolytic Activities of Cultured Microorganisms

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11.1

Introduction

Termites thrive between the 47° latitude north and 47° latitude south, which accounts for 68% of the Earth's landscape. Their main area of distribution is in tropical and subtropical regions (Krishna 1970; Myles 1999). The total population is estimated to be 2.4×10^{17} individual termites (Zimmermann et al. 1982). Termites are assigned to 282 genera. The intestinal microbiota of the higher termites including 71% of 2761 species lack flagellates. Termites (Isoptera) and ants (Formicidae) represent 80% of the individual insects and 30% of the total animal biomass near Manaus in Brazil (Fittkau and Klinge 1973). They process 28% of the Earth's net primary production. Termites release large amounts of gases and they are estimated to produce about 1.5×10^8 tonnes methane (total emission: up to 12.1×10^8 tonnes), 2×10^8 tonnes hydrogen, 4.6×10^{10} tonnes of carbon dioxide, 1×10^7 tonnes of carbon monoxide and 7×10^5 tonnes of dimethyl disulfide per year (Zimmermann et al. 1982; Hackstein and Stumm 1994; Hackstein et al. 1996).

Because of their intestinal flora termites are among the most important wood- and litter-feeding insects (Wood and Sands 1978). In amber containing the Miocene termite *Mastotermes electrodomenicus*, a 20 million-year-old fossil microbial community consisting of protists, spirochetes and other bacteria has been observed (Wier et al. 2002). The gut microbes play an indispensable role in the digestion of food. The dense gut microbiota can include a variety of microorganisms from the domains Bacteria, Archaea, flagellates (formerly named Archaezoa) and also yeasts and fungi (cf. König et al. 2002). Because of the interesting microbial symbionts and their ecological importance in the global carbon cycle, termites have attracted the interest of many scientists from different disciplines. The microbial flora is not distributed randomly in the gut, but plays certain roles in the degradation of lignocellulose and occupies distinct microhabitats. The degradation of lignocellulose in the oxygen-limited hindgut paunch

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formally occurs in three steps: a hydrolytic step, an oxidative/fermentative step and methanogenic/acetogenic step.

In recent years, a large portion of the involved microorganisms was characterized and their phylogenetic position was determined. Although, several hundred microbial strains have been obtained in pure culture from the termite gut, still a major portion of the microbial species has not been isolated yet. The cultured species will be described in this chapter, while the non-cultured microbial clones identified by SSU rRNA sequence analysis is dealt with in Chapter 12.

11.2 Flagellates

Unique groups of flagellates (formerly named Archaezoa), which have branched off very early in the evolution of the eukaryotes, are found in the termites gut. These species seem not to occur elsewhere in nature except in wood-eating roaches of the genus *Cryptocercus*. All protozoa of the termite gut belong to the oxymonads, trichomonads and the hypermastigotes (Honigberg 1970; Yamin 1979; Radek and Hausmann 1993; Brugerolle et al. 1994; Viscogliosi et al. 1993; Keeling et al. 1998; Kitade and Matsumoto 1998; Moriya et al. 1998; Brugerolle 2000; Brugerolle and König 1995; Dacks and Redfield 1998; Ohkuma et al. 1998; Brugerolle and Lee 2000a,b; Delgado-Viscoliosi et al. 2000). From 205 examined termite species 434 species of flagellates were described up to 1979. The flagellates occur in high number in the paunch (10^3 – 10^7) and they can occupy more than 90% of the paunch volume (cf. König et al. 2002). Only three species of the flagellate flora have been obtained in culture: *Trichomitopsis termopsidis* (Yamin 1978, 1980), *Trichonympha sphaerica* (Yamin 1981) from *Zootermopsis* sp. and *Trichomitus trypanoides* from *Reticulitermes santonensis* (Berchtold et al. 1995). Whether lower termites obligatory depend on the flagellates is a matter of debate. We observed one colony of *Zootermopsis angusticollis* and one colony of *Kaloterme flavicollis*, which lived without flagellates.

11.3 Bacteria

The total number of prokaryotes in the hindgut of termites lies within the range of 10^7 – 10^{11} ml⁻¹ (Krasil'nikov and Satdykov 1969; Bignell et al. 1980b; To et al. 1980; Tholen et al. 1997; Berchtold et al. 1999). The bacterial groups from the termite gut identified by the 16S rDNA approach

mainly belong to the spirochetes, proteobacteria, Gram positives and *Bacteroides/Flavobacterium* branch (Berchtold et al. 1994, 1999; Berchtold and König 1996; Paster et al. 1996; Kudo et al. 1998; Ohkuma et al. 1999a–c; Tokuda et al. 2000; Watanabe et al. 2003). Differences in domain-level profiles were correlated with the termite diet, e.g. methanogenic bacteria were more abundant in soil-feeding termites compared to wood-feeding species (Braumann et al. 2001). The predominant bacterial flora in the hindgut contents is represented by spirochetes (Berchtold et al. 1994; Berchtold and König 1996; Ohkuma et al. 1999b; Lilburn et al. 2000). Up to now three species have been obtained in pure culture (Leadbetter et al. 1999; Graber et al. 2004). Most isolates obtained in pure culture belong to the branch of Gram positive bacteria and proteobacteria, but also isolates from the *Bacteroides/Fusobacterium* branch were obtained (Table 11.1). Intestinal bacteria are involved in the degradation of cellulose, hemicellulose and aromatic compounds as well as nitrogen fixation. They also contribute to the redox status of the gut.

11.4 Archaea

All Archaea identified in the termite gut belong to the families Methanobacteriaceae, Methanosarcinaceae and Methanomicrobiales and most identified species were assigned to the genus *Methanobrevibacter* (Braumann et al. 2001; Fröhlich and König 1999b; Tokura et al. 2000). Three species of the genus *Methanobrevibacter* have been obtained in pure culture from the termite *Reticulitermes flavipes*: *Mbr. cuticularis*, *Mbr. curvatus* and *Mbr. filiformis* (Leadbetter and Breznak 1996; Leadbetter et al. 1998).

11.5 Yeasts and Fungi

A variety of yeasts were isolated from the gut of the lower termites *Mastotermes darwiniensis*, *Zootermopsis angusticollis*, *Zootermopsis nevadensis*, *Neotermes jouteli*-related termite, *Reticulitermes santonensis*, *Heterotermes indicola* and the roach *Cryptocercus punctulatus* (Prillinger et al. 1996). Between 10^7 and 5×10^8 yeast cells were found per ml gut contents in *Zootermopsis angusticollis* and *Neotermes castaneus*. The isolates were assigned to 13 different species, which belonged to the genera *Candida*, *Cryptococcus*, *Debaryomyces*, *Pichia* and *Sporothrix*. Some isolates were able to hydrolyse xylan or cellulose (Schäfer et al. 1996; Wenzel et al. 2002). Some filamentous fungi (*Alternaria alternater*, *Aspergillus awamuri*, *As-*

Table 11.1. Bacterial isolates from the termite gut

| Species | Termites | References |
|------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------|
| <u>Branch:</u> Gram-positive Bacteria | | |
| <u>Subbranch:</u> Gram positive bacteria with high G + C content | | |
| <u>Order:</u> Actinomycetales | | |
| <i>Arthrobacter</i> sp. | <i>Nasutitermes nigriceps</i> (h) <i>Reticulitermes hesperus</i> (l) <i>Reticulitermes santonensis</i> (l) | Kuhnigk et al. (1994) |
| <i>Aureobacterium</i> sp. | <i>Nasutitermes nigriceps</i> (h) <i>Reticulitermes santonensis</i> (l) | Kuhnigk et al. (1994) |
| <i>Cellulosimicrobium variabile</i> | <i>Mastotermes darwiniensis</i> (l) | Bakalidou et al. (2002) |
| <i>Cellulomonas cellulans</i> | <i>Zootermopsis angusticollis</i> (l) <i>Nasutitermes nigriceps</i> (h) | Kuhnigk (1996) |
| <i>Cellulomonas</i> sp. | <i>Neotermes castaneus</i> (l) <i>Schedorhinotermes intermedius</i> (l) <i>Heterotermes indicola</i> (l) <i>Mastotermes darwiniensis</i> (l) <i>Zootermopsis angusticollis</i> (l) | Kuhnigk (1996); Wenzel et al. (2002) |
| <i>Kocuria varians</i> | <i>Zootermopsis angusticollis</i> (l) | Wenzel et al. (2002) |
| <i>Microbacterium</i> sp. | <i>Zootermopsis angusticollis</i> (l) | Wenzel et al. (2002) |
| <i>Micrococcus luteus</i> | <i>Reticulitermes santonensis</i> (l) | Kuhnigk (1996) |
| “ <i>Micromonospora acetofornici</i> ” | <i>Reticulitermes lucifugus</i> | Sebald and Prévot (1962) |
| <i>Micromonospora propionici</i> | <i>Amitermes minimus</i> (h) | Hungate (1946) |
| <i>Rhodococcus equi</i> | <i>Zootermopsis angusticollis</i> (l) | Kuhnigk (1996) |
| <i>Nocardia</i> sp. | <i>Reticulitermes santonensis</i> (l) | Kuhnigk and König (1997); Schäfer et al. (1996) |
| <i>Streptomyces</i> sp. | <i>Nasutitermes nigriceps</i> (h) <i>Cubitermes severus</i> (h) <i>Heterotermes indicola</i> (l) <i>Mastotermes darwiniensis</i> (l) <i>Proclubitermes aburiensis</i> (h) <i>Zootermopsis angusticollis</i> (l) | Bignell et al. (1980b); Kuhnigk and König (1997); Mannesmann and Piechowski (1989); Schäfer et al. (1996) |
| <u>Subbranch:</u> Gram-positive bacteria with low G + C content | | |
| <u>Order:</u> Bacillales | | |
| <i>Bacillus brevis</i> | <i>Anacanthotermes ahngerianus</i> (h) <i>Zootermopsis angusticollis</i> (l) | Krasil'nikov and Satdykov (1969); Wenzel et al. (2002) |

Table 11.1. (continued)

| Species | Termites | References |
|--------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------|
| <i>Bacillus cereu</i> | <i>Anacanthotermes ahngerianus</i> (h) <i>Nasutitermes nigriceps</i> (h) <i>Neotermes castaneus</i> (l) <i>Reticulitermes hesperus</i> (l) <i>Reticulitermes santonensis</i> (l) | Krasil'nikov and Satdykov (1969); Kuhnigk and König (1997); Thayer (1976); Schäfer et al. (1996) |
| <i>Bacillus cereus</i> -related isolate | <i>Zootermopsis angusticollis</i> (l) | Wenzel et al. (2002) |
| <i>Bacillus circulans</i> -related isolate | <i>Zootermopsis angusticollis</i> (l) | Wenzel et al. (2002) |
| <i>Bacillus coagulans</i> | <i>Mastotermes darwiniensis</i> (l) | Schäfer et al. (1996) |
| <i>Bacillus firmus</i> | <i>Reticulitermes santonensis</i> (l) | Kuhnigk (1996) |
| <i>Bacillus licheniformis</i> | <i>Reticulitermes santonensis</i> (l) | Kuhnigk and König (1997); Kuhnigk et al. (1994); Schäfer et al. (1996) |
| <i>Bacillus megaterium</i> | <i>Anacanthotermes ahngerianus</i> (h) <i>Mastotermes darwiniensis</i> (l) <i>Zootermopsis angusticollis</i> (l) | Krasil'nikov and Satdykov (1969); Kuhnigk (1996); Mannesmann and Piechowski (1989); Wenzel et al. (2002) |
| <i>Bacillus mycoides</i> | <i>Anacanthotermes ahngerianus</i> (h) | Krasil'nikov and Satdykov (1969) |
| <i>Bacillus oleronius</i> | <i>Reticulitermes santonensis</i> (l) | Kuhnigk et al. (1995) |
| <i>Bacillus</i> sp. | <i>Schedorhinotermes intermedius</i> (l) <i>Coptotermes acinaciformis</i> (l) <i>Coptotermes formosanus</i> (l) <i>Heterotermes indicola</i> (l) | Eutick et al. (1978); Kuhnigk (1996); Mannesmann and Piechowski (1989) |
| <i>Bacillus sphaericus</i> | <i>Odontotermes distans</i> (h) <i>Odontotermes obesus</i> (h) <i>Reticulitermes santonensis</i> (l) <i>Zootermopsis angusticollis</i> (l) | Kuhnigk (1996); Kuhnigk and König (1997); Schäfer et al. (1996) |
| <i>Bacillus subtilis</i> | <i>Anacanthotermes ahngerianus</i> (h) <i>Reticulitermes santonensis</i> (l) | Krasil'nikov and Satdykov (1969); Kuhnigk and König (1997); Schäfer et al. (1996) |
| <i>Brevibacillus brevis</i> | <i>Zootermopsis angusticollis</i> (l) | Wenzel et al. (2002) |
| <i>Listeria</i> sp. | <i>Reticulitermes santonensis</i> (l) <i>Zootermopsis angusticollis</i> (l) | Kuhnigk et al. (1994) |
| <i>Paenibacillus macerans</i> | <i>Mastotermes darwiniensis</i> (l) | Schäfer et al. (1996) |
| <i>Paenibacillus</i> sp. | <i>Zootermopsis angusticollis</i> (l) | Wenzel et al. (2002) |

Table 11.1. (continued)

| Species | Termites | References |
|-------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| Order Clostridiales | | |
| <i>Acetonema longum</i> | <i>Pterotermes occidentis</i> (l) | Kane and Breznak (1991) |
| <i>Clostridium mayombeii</i> | <i>Cubitermes speciosus</i> (h) | Kane et al. (1991) |
| <i>Clostridium</i> sp. | <i>Mastotermes darwiniensis</i> (l) <i>Nasutitermes nigriceps</i> (h) | Kuhnigk et al. (1994) |
| <i>Clostridium termitidis</i> | <i>Nasutitermes lujae</i> (h) | Hethener et al. (1992) |
| <i>Clostridium beijerinckii</i> | <i>Coptotermes formosanus</i> (l) | Taguchi et al. (1993) |
| <i>Sporomusa aerivorans</i> | <i>Thoracotermes macrothorax</i> | Boga et al. 2003 |
| <i>Sporomusa termitida</i> | <i>Nasutitermes nigriceps</i> (h) | Breznak et al. (1988) |
| Order Lactobacillales | | |
| <i>Enterococcus avium</i> | <i>Mastotermes darwiniensis</i> (l) | Kuhnigk (1996) |
| <i>Enterococcus faecalis</i> | <i>Anacanthotermes ahngerianus</i> (h) <i>Mastotermes darwiniensis</i> (l) <i>Reticulitermes flavipes</i> (l) | Bauer et al. (2000); Krasil'nikov and Satdykov (1969); Kuhnigk (1996) |
| <i>Enterococcus faecium</i> | <i>Mastotermes darwiniensis</i> (l) | Schäfer et al. (1996) |
| <i>Enterococcus</i> sp. | <i>Reticulitermes flavipes</i> (l) | Tholen et al. (1997) |
| <i>Lactobacillus</i> sp. | <i>Reticulitermes flavipes</i> (l) | Schultz and Breznak (1978) |
| <i>Lactococcus cremoris</i> | <i>Reticulitermes flavipes</i> (l) | Schultz and Breznak (1978) |
| <i>Lactococcus lactis</i> | <i>Reticulitermes santonensis</i> (l) <i>Reticulitermes flavipes</i> (l) <i>Thoracotermes macrothorax</i> (h) | Bauer et al. (2000); Kuhnigk (1996); Schultz and Breznak (1978) |
| <i>Staphylococcus saprophyticus</i> | <i>Reticulitermes santonensis</i> (l) | Kuhnigk (1996) |
| <i>Staphylococcus</i> sp. | <i>Nasutitermes exitiosus</i> (h) <i>Nasutitermes graveolus</i> (h) <i>Nasutitermes walkeri</i> (h) | Eutick et al. (1978) |
| <i>Streptococcus</i> sp. | <i>Nasutitermes exitiosus</i> (h) <i>Coptotermes lacteus</i> (l) <i>Cryptotermes primus</i> (l) <i>Heterotermes ferox</i> (l) <i>Mastotermes darwiniensis</i> (l) <i>Schedorhinotermes intermedius</i> (l) <i>Reticulitermes flavipes</i> (l) | Eutick et al. (1978); Kuhnigk (1996); Potrikus and Breznak (1980) |

Table 11.1. (continued)

| Species | Termites | References |
|-------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------|
| <u>Branch:</u> Proteobacteria | | |
| <u>Division:</u> α -Proteobacteria | | |
| <u>Order:</u> Rhizobiales | | |
| <i>Ochrobactrum anthropi</i> | <i>Heterotermes indicola</i> (l) <i>Nasutitermes nigriceps</i> (h) <i>Neotermes castaneus</i> (l) <i>Mastotermes darwiniensis</i> (l) <i>Reticulitermes santonensis</i> (l) | Kuhnigk (1996); Kuhnigk et al. (1994); Schäfer et al. (1996) |
| Rhizobium-related isolate | <i>Nasutitermes nigriceps</i> (h) | Kuhnigk et al. (1994) |
| Rhizobium etli-related isolate | <i>Zootermopsis angusticollis</i> (l) | Wenzel et al. (2002) |
| <u>Order:</u> Sphingomonadales | | |
| <i>Sphingomonas</i> sp. | <i>Zootermopsis angusticollis</i> (l) <i>Mastotermes darwiniensis</i> (l) | Fröhlich and König (1999b); Kuhnigk (1996); Wenzel et al. (2002) |
| <u>Division:</u> β -Proteobacteria | | |
| <u>Order:</u> Burkholderiales | | |
| <i>Alcaligenes faecalis</i> | <i>Reticulitermes santonensis</i> (l) | Kuhnigk and König (1997) |
| <i>Alcaligenes</i> sp. | <i>Reticulitermes hesperus</i> (l) | Thayer (1976) |
| <i>Alcaligenes xylooxidans</i> | <i>Mastotermes darwiniensis</i> (l) | Kuhnigk (1996) |
| <i>Burkholderia cepacia</i> | <i>Coptotermes formosanus</i> (l) <i>Nasutitermes nigriceps</i> (h) <i>Zootermopsis angusticollis</i> (l) <i>Reticulitermes santonensis</i> (l) | Mannesmann and Piechowski (1989); Schäfer et al. (1996) |
| <i>Burkholderia</i> sp. strain VE22 | <i>Coptotermes formosanus</i> | Harazono et al. (2003) |
| <i>Burkholderia</i> sp. | <i>Heterotermes indicola</i> (l) | Kuhnigk (1996) |
| <i>Comamonas acidovorans</i> | <i>Nasutitermes nigriceps</i> (h) | Kuhnigk and König (1997); Kuhnigk et al. (1994) |
| <u>Division:</u> γ -Proteobacteria | | |
| <u>Order:</u> Pseudomonadales | | |
| <i>Acinetobacter baumannii</i> | <i>Mastotermes darwiniensis</i> (l) <i>Nasutitermes nigriceps</i> (h) | Kuhnigk and König (1997); Kuhnigk et al. (1994); Schäfer et al. (1996) |
| <i>Acinetobacter</i> sp. | <i>Nasutitermes nigriceps</i> (h) | Kuhnigk and König (1997) |

Table 11.1. (continued)

| Species | Termites | References |
|----------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|
| <i>Pseudomonas aeruginosa</i> | <i>Kaloterme flavicollis</i> (l) <i>Mastoterme darwiniensis</i> (l) <i>Nasutiterme nigriceps</i> (h) <i>Reticuliterme santonensis</i> (l) | Kuhnigk and König (1997); Kuhnigk et al. (1994); Mannesmann and Piechowski; (1989) Schäfer et al. (1996) |
| <i>Pseudomonas cepacia</i> | <i>Reticuliterme santonensis</i> (l) | Kuhnigk and König (1997) |
| <i>Pseudomonas citronellolis</i> | <i>Mastoterme darwiniensis</i> (l) | Kuhnigk and König (1997) |
| <i>Pseudomonas fluorescens</i> | <i>Kaloterme flavicollis</i> (l) | Mannesmann and Piechowski (1989) |
| <i>Pseudomonas luteola</i> | <i>Reticuliterme lucifugus</i> (l) | Mannesmann and Piechowski (1989) |
| <i>Pseudomonas putida</i> | <i>Nasutiterme nigriceps</i> (h) <i>Reticuliterme lucifugus</i> (l) | Kuhnigk et al. (1994); Mannesmann and Piechowski (1989) |
| <i>Pseudomonas</i> sp. | <i>Coptoterme formosanus</i> (l) <i>Kaloterme flavicollis</i> (l) <i>Nasutiterme nigriceps</i> (h) <i>Reticuliterme lucifugus</i> (l) <i>Odontoterme obesus</i> (h) <i>Odontoterme distans</i> (h) | Kuhnigk (1996); Mannesmann and Piechowski (1989) |
| <u>Order: Aeromonadales</u> | | |
| <i>Aeromonas</i> sp. | <i>Kaloterme flavicollis</i> (l) <i>Nasutiterme nigriceps</i> (h) | Mannesmann and Piechowski (1989) |
| <u>Order: Enterobacteriales</u> | | |
| <i>Citrobacter freundii</i> | <i>Coptoterme formosanus</i> (l) <i>Neoterme jouteli</i> -related (l) | Kuhnigk (1996); Mannesmann and Piechowski (1989) |
| <i>Citrobacter</i> sp. | <i>Nasutiterme nigriceps</i> (h) <i>Mastoterme darwiniensis</i> (l) <i>Neoterme castaneus</i> (l) <i>Odontoterme obesus</i> (h) <i>Odontoterme distans</i> (h) <i>Reticuliterme flavipes</i> (l) <i>Reticuliterme santonensis</i> (l) | Kuhnigk (1996); Mannesmann and Piechowski (1989); Potrikus and Breznak (1980); Schultz and Breznak (1978) |
| <i>Citrobacter amalonaticus</i> | <i>Neoterme castaneus</i> (l) | Mannesmann and Piechowski (1989) |

Table 11.1. (continued)

| Species | Termites | References |
|---------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------|
| <i>Citrobacter diversus</i> | <i>Coptotermes formosanus</i> (l) <i>Neotermes castaneus</i> (l) <i>Neotermes jouteli</i> -related (l) <i>Reticulitermes lucifugus</i> (l) | Mannesmann and Piechowski (1989); Schäfer et al. (1996) |
| <i>Citrobacter</i> sp. | <i>Coptotermes formosanus</i> (l) | Harazono et al. (2003) |
| <i>Enterobacter aerogenes</i> | <i>Mastotermes darwiniensis</i> (l) <i>Coptotermes formosanus</i> (l) <i>Anacanthotermes ahngerianus</i> (h) | Krasil'nikov and Satdykov (1969); Kuhnigk et al. (1994); Mannesmann and Piechowski (1989); Schäfer et al. (1996) |
| <i>Enterobacter agglomerans</i> | <i>Coptotermes formosanus</i> (l) | Mannesmann and Piechowski (1989); Potrikus and Breznak (1977) |
| <i>Enterobacter cloacae</i> | <i>Anacanthotermes ahngerianus</i> (h) <i>Coptotermes formosanus</i> (l) <i>Mastotermes darwiniensis</i> (l) <i>Nasutitermes nigriceps</i> (h) <i>Odontotermes obesus</i> (h) <i>Odontotermes distans</i> (h) <i>Reticulitermes lucifugus</i> (l) <i>Reticulitermes santonensis</i> (l) | Krasil'nikov and Satdykov (1969); Kuhnigk (1996); Mannesmann and Piechowski (1989) |
| <i>Enterobacter</i> sp. | <i>Coptotermes acinaciformis</i> (l) <i>Coptotermes lacteus</i> (l) <i>Heterotermes ferox</i> (l) <i>Mastotermes darwiniensis</i> (l) <i>Nasutitermes graveolus</i> (h) <i>Reticulitermes flavipes</i> (l) <i>Reticulitermes santonensis</i> (l) <i>Schedorhinotermes intermedius</i> (l) | Eutick et al. (1978); Krasil'nikov and Satdykov (1969); Kuhnigk et al. (1994); Schultz and Breznak (1978) |
| <i>Enterobacter sakazaki</i> | <i>Reticulitermes santonensis</i> (l) | Schäfer et al. (1996) |
| <i>Escherichia coli</i> | <i>Anacanthotermes ahngerianus</i> (h) | Krasil'nikov and Satdykov (1969) |
| <i>Hafnia alvei</i> | <i>Coptotermes formosanus</i> (l) | Mannesmann and Piechowski (1989) |
| <i>Hafnia</i> -related sp. | <i>Odontotermes distans</i> (h) <i>Odontotermes obesus</i> (h) | Kuhnigk (1996) |
| <i>Klebsiella oxytoca</i> | <i>Coptotermes formosanus</i> (l) | Mannesmann and Piechowski (1989) |

Table 11.1. (continued)

| Species | Termites | References |
|-----------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| <i>Klebsiella pneumoniae</i> | <i>Mastotermes darwiniensis</i> (l) | Kuhnigk et al. (1994); Mannesmann and Piechowski (1989); Schäfer et al. (1996) |
| <i>Klebsiella</i> sp. | <i>Coptotermes formosanus</i> (l) | Mannesmann and Piechowski (1989) |
| <i>Serratia ficaria</i> | <i>Reticulitermes santonensis</i> (l) | Kuhnigk et al. (1994); Schäfer et al. (1996) |
| <i>Serratia marcescens</i> | <i>Coptotermes formosanus</i> (l) <i>Mastotermes darwiniensis</i> (l) <i>Neotermes jouteli</i> -related (l) <i>Reticulitermes hesperus</i> (l) | Kuhnigk et al. (1994); Mannesmann and Piechowski (1989); Thayer (1976) |
| <i>Serratia</i> sp. | <i>Reticulitermes lucifugus</i> (l) | Mannesmann and Piechowski (1989) |
| <u>Order: Xanthomonadales</u> | | |
| <i>Xanthomonas maltophilia</i> | <i>Nasutitermes nigriceps</i> (h) <i>Kalotermes flavicollis</i> (l) <i>Reticulitermes lucifugus</i> (l) <i>Odontotermes obesus</i> (h) | Kuhnigk (1996); Mannesmann and Piechowski (1989) |
| <i>Xanthomonas</i> sp. | <i>Zootermopsis angusticollis</i> (l) | Kuhnigk (1996) |
| <u>Division: δ-Proteobacteria</u> | | |
| <i>Desulfovibrio termitidis</i> | <i>Heterotermes indicola</i> (l) <i>Cryptocercus punctulatus</i> (roach) | Fröhlich et al. (1999); Trinkerl et al. (1990) |
| <i>Desulfovibrio intestinalis</i> | <i>Mastotermes darwiniensis</i> (l) | Fröhlich et al. (1999) |
| <i>Desulfovibrio desulfuricans</i> | <i>Cubitermes speciosus</i> (h) <i>Reticulitermes santonensis</i> (l) | Braumann et al. (1990); Kuhnigk et al. (1996) |
| <i>Desulfovibrio giganteus</i> | <i>Cubitermes speciosus</i> (h) | Braumann et al. (1990) |
| <u>Branch: Bacteroides/Flavobacterium</u> | | |
| <u>Order: Bacteroidales</u> | | |
| <i>Bacteroides termitidis</i> | <i>Reticulitermes flavipes</i> (l) | Potrikus and Breznak (1980) |
| <u>Order: Flavobacteriales</u> | | |
| <i>Flavobacterium menigosepticum</i> | <i>Reticulitermes lucifugus</i> (l) | Mannesmann and Piechowski (1989) |

Table 11.1. (continued)

| Species | Termites | References |
|----------------------------------|------------------------------------------------------------------------------|------------------------------------------------|
| <i>Flavobacterium</i> sp. | <i>Mastotermes darwiniensis</i> (l) <i>Reticulitermes santonensis</i> (l) | Eutick et al. (1978); Schäfer et al. (1996) |
| <u>Order:</u> Fusobacteriales | | |
| <i>Fusobacterium</i> sp. | <i>Reticulitermes flavipes</i> (l) | Schultz and Breznak (1978) |
| <u>Order:</u> Sphingobacteriales | | |
| <i>Spirosoma</i> -related sp. | <i>Zootermopsis angusticollis</i> (l) | Wenzel et al. (2002) |
| <u>Branch:</u> Spirochetes | | |
| <i>Treponema primitia</i> | <i>Zootermopsis angusticollis</i> (l) | Graber et al. (2004) |
| <i>Treponema azotonutricium</i> | <i>Zootermopsis angusticollis</i> (l) | Graber et al. (2004) |

Designation of the branches according to Woese et al. (1990). Designation of the orders according to Madigan et al. (2001): l, lower termite; h, higher termite

pergillus clavatus, *Aspergillus flavus*, *Aspergillus nidulans*, *Cladosporium* sp., *Paecilomyces fusiporus*, *Rhizopus stolonifer*) have also been isolated from gut fluids of termites (Rajagopal et al. 1979, 1981). Whether they grow with mycelia in the termite gut has yet to be demonstrated.

11.6 Microhabitats

The digestive system of termites consists of the foregut with the crop and the gizzard, the midgut and the hindgut (Fig. 11.1; Noirot 1995; Noirot and Noirot-Thimotheé 1969). Despite their small volumes of about 0.5–10 μ l, the hindguts of termites are morphologically complex systems. The hindgut consists of five segments (P1–P5): the proctodeal segment, the enteric valve, the paunch, the colon and the rectum. The paunch is the microbial fermentation chamber, but the midgut and colon also contain microorganisms.

The termite gut can be described as an anaerobic gradient system, which is constantly supplied with oxygen via the epithelium. Examinations using microelectrodes have shown a pronounced spatial differentiation of termite hindguts with respect to pH (Noirot and Noirot-Thimotheé 1969; Brune and Köhl 1996) and axial or radial gradients of oxygen or hydrogen concentrations (Veivers et al. 1980, 1982; Brune 1998; Brune et al. 1995a,b; Ebert and Brune 1997; Schmitt-Wagner and Brune 1999). In the case of *Mastotermes darwiniensis* oxygen diffusion gradients could be detected up to 100 μ m below the epithelium (Berchtold et al. 1999).

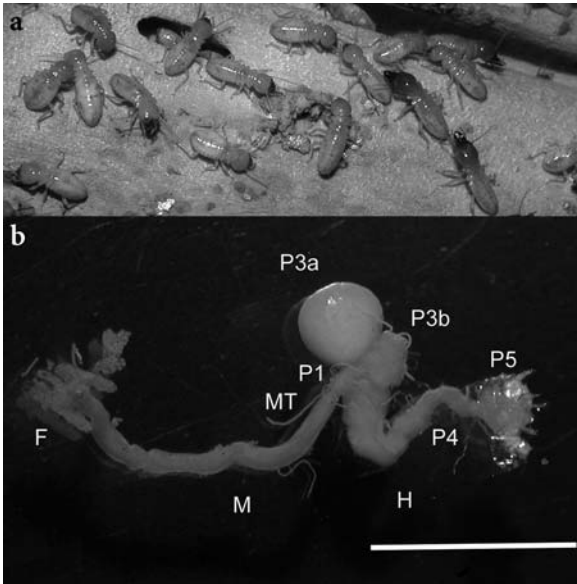


Fig. 11.1. The intestinal system of the Australian termite *Mastotermes darwiniensis*. **a** Workers and soldiers of *Mastotermes darwiniensis*. **b** Intestine. *F*=foregut with salivary glands, esophagus, crop and gizzard; *M* midgut; *MT* Malpighian tubules; *H* hindgut; *P1* proctodeal segment of the hindgut followed by the enteric valve; *P3a* thin-walled part of the paunch; *P3b* thick-walled part of the paunch; *P4* colon; *P5* rectum; *bar* 5 mm

The pH of the midgut is about neutral and that of the hindgut is in the range between 6.0 and 7.5. In higher termites such as *Odontotermes obesus* parts of the gut have an alkaline pH up to 10.4 (Noirot and Noirot-Timotheé 1969; Bignell and Anderson 1980; Paul et al. 1986). The soil-feeding termites in particular possess more elongated and compartmentalized hindguts than wood-eating termites. Microscale pH measurements with microelectrodes demonstrated alkaline pH values of up to 12 in parts of the gut (Brune and Köhl 1996).

The paunch of termites is about 10^8 times smaller than a rumen resulting in a 500 times larger oxygen influx per unit volume (Brune 1998). Because of the small size the passage time of the food through the intestinal tract is only about 24 h (Breznak 1984). These facts have significant influences on the composition of the microbial flora. In the hindgut of termites strictly aerobic and microaerophilic bacteria could be isolated (Kühnigk et al. 1994; Kühnigk and König 1997; Wenzel et al. 2002).

In the termite gut four distinct microhabitats can be distinguished: the gut lumen, the surface and the cytoplasm of the flagellates and the gut epithelium. Electron microscopy studies of termite guts have shown that

prokaryotes occur either suspended in the contents, located within or on the surface of flagellates, or they are attached to the gut wall (Breznak and Pankratz 1977; Czolij et al. 1984–1986). Electron microscopic examinations of the gut of *Reticulitermes flavipes* and *Coptotermes formosanus* revealed that a heterogenous assemblage of bacteria colonize the gut (Breznak and Pankratz 1977; Schultz and Breznak 1978). Most morphotypes were located close to the epithelium. The midgut was scarcely colonized, but did possess distinctive cuboid-shaped, endospore-forming bacteria between the microvilli of the epithelium. Similar observations were made with other termites (Bignell and Anderson 1980; Bignell et al. 1980a; Czolij et al. 1986). Direct microscopic counts of bacteria ranged from 10^6 to 10^7 per gut and that of protozoa up to 4×10^4 per gut of *Reticulitermes flavipes*, while about 10^6 colony forming units per gut were obtained (Breznak 1982; O'Brien and Slaytor 1982).

Combining the rRNA approach with confocal laser scanning microscopy and oxygen microelectrode measurements, the gut microbial community within the hindgut of the wood-feeding Australian termite *Mastotermes darwiniensis* was examined (Berchtold et al. 1999). The anterior part of the paunch (P3a region) of *Mastotermes darwiniensis* is tightly packed with large flagellates (1285 ± 244 cells of *Deltotrichonympha operculata*, *Deltotrichonympha nana*, *Koruga bonita*, and *Mixotricha paradoxa* per termite) (Berchtold et al. 1999). From the combined volume of the larger flagellates it can be estimated that 95% of the anterior part of the paunch (P3a region) is occupied by flagellate protozoa. In *Mastotermes darwiniensis* approximately 90% of the DAPI-stained cells were associated with the protozoa in the P3a region, only 2% were attached to the gut wall and the rest were found in the residual liquid volume of the lumen fraction. In contrast, the flagellate population in the P3b/P4 region was much smaller. The flagellate cells represented only 10% of the total volume. The potentially colonizable surface area provided by the flagellates in the P3a region exceeds that of the wall by a factor of 18. In contrast to the P3a region about 85% of the prokaryotes of the P3b region are attached to the wall. The prokaryotic cell density on the P3b/P4 epithelium ($2 \times 10^6 \text{ mm}^{-2}$) is considerably higher than that on the P3a surface ($3 \times 10^4 \text{ mm}^{-2}$). The concentration of non-attached cells in the residual volume is higher in the P3a region ($7 \times 10^9 \text{ cells ml}^{-1}$) than in the P3b/P4 region ($1 \times 10^9 \text{ cells ml}^{-1}$). The flagellates preferentially colonize the paunch, while low numbers are found in the colon (P4 region) (Breznak and Pankratz 1977; Yoshimura et al. 1992; Berchtold et al. 1999).

It is surprising that strictly anaerobic bacteria such as methanogens (Leadbetter and Breznak 1996; Leadbetter et al. 1998), sulfate reducers (Berchtold et al. 1999) and clostridia (Tokuda et al. 2000) occur also attached to the aerated gut epithelium. Beside the gut epithelium (Leadbetter

and Breznak 1996; Leadbetter et al. 1998) methanogens occur as ecto- and endosymbionts of flagellates (Fröhlich and König 1999a,b,c; Tokura et al. 2000). Ectosymbiotic bacteria of flagellates can easily be detected by electron microscopy (Radek et al. 1992, 1996; Dyer and Khalsa 1993; Radek and Tischendorf 1999) or after staining the cells with ethidium bromide (Fröhlich and König 1999a).

Ectosymbiotic spirochetes have been identified on the surface of flagellates (Iida et al. 2000; Noda et al. 2003). *Mixotricha paradoxa*, a trichomonad from the hindgut of the Australian termite *Mastotermes darwiniensis* Froggatt, is a rare example of a movement symbiosis between eukaryotic and prokaryotic microorganisms (Cleveland and Grimstone 1964). The surface of *Mixotricha paradoxa* is covered with spirochetes and a rod-shaped bacterium. The four flagella at the anterior end seem only to alter the direction of movement, while the ectosymbiotic spirochetes propel the flagellate cells. Based on a 16S rDNA sequence analysis after a semi-specific PCR and subsequent fluorescence in situ hybridization applying helper oligonucleotides and a denaturing step of the 16S rRNA, three different spirochete clones could be clearly identified on the surface of the protozoal cells (Wenzel et al. 2003). They belonged to the *Treponema* cluster. The rod shaped bacterium showed highest 16S rDNA sequence similarity to *Bacteroides*-related species. Due to its low phylogenetic relationship to its next relatives in the data base it should represent a so far undescribed species.

11.7

Lignocellulose Degradation

The microbiology of the gut ecosystem of termites has been summarized in several review articles (Breznak 1982; O'Brien and Slaytor 1982; Breznak and Brune 1994; Varma et al. 1994). Formally, the microbial degradation of lignocellulose can be divided into three stages (Table 11.2; cf. König et al. 2002); a hydrolytic stage, a fermentative/oxidative stage and a methanogenic/acetogenic stage. These stages have been proved by microbial isolates (cf. König et al. 2002). The molecular aspect of cellulose degradation in the termite gut is described in Chapter 9.

11.7.1

The Hydrolytic Stage of Lignocellulose Degradation

Termites have different feeding habits. They can feed on wood, grass, dung, humus and soil. The passage of the food through the digestive tract needs 24 h (Breznak 1984). Therefore, this symbiotic system degrades wood much

more efficiently than fungi. The symbiosis between termites and the gut flora can be described as a synergistic interaction of a mechanical rasp provided by the termites and of microbial enzymes located in the gut. The fermentation products are acetic acid, propionic acid and butyric acid (mol%; 94, 3.3, 2.3 in *Reticulitermes flavipes*; 98.6, 1.3, 0.0 in *Zootermopsis angusticollis*) and the gases CO₂, H₂ and CH₄ (Odelson and Breznak 1983).

Wood consists of cellulose, hemicellulose, and lignin (Fengel and Wegener 1984). Since the 1920s it has been known that termites hydrolyse cellulose (Cleveland 1924; Beckwith and Rose 1929; Dickmann 1931) The extent of cellulose degradation is between 59 and 99% (Seifert and Becker 1965; Esenther and Kirk 1974; Mishra 1979). In lower termites wood particles are endocytosed by the archaezoa (Honigberg 1970). The isolated flagellates *Trichomitopsis termopsidis* and *Trichonympha sphaerica* from *Zootermopsis* need cellulose for growth (Yamin 1980, 1981; Odelson and Breznak 1985). These protozoa have significant cellulose and hemicellulose-degrading activities. Cellulase genes in flagellates have been identified (Othoko et al. 2001; Li et al. 2003). Cellulose is fermented to acetate, CO₂, and H₂. Acetate is mainly used by the termites as an energy source and is oxidized to CO₂.

A different situation was found in the case of the flagellates of the most primitive Australian termite *Mastotermes darwiniensis* (Li et al. 2003). Two endoglucanases with similar apparent molecular mass of approx. 36 kD have been isolated. Surprisingly, the N-terminal sequences of these cellulases exhibited significant homology to cellulases of termite origin, which belong to glycosyl hydrolase family 9. The corresponding genes were detected not in the mRNA pool of the flagellates, but in the salivary glands of *Mastotermes darwiniensis*. This showed that cellulases isolated from the flagellate cells originated from the termite host. Using a PCR-based approach DNA encoding cellulases belonging to glycosyl hydrolase family 45 were obtained from micromanipulated nuclei (Fröhlich and König 2000) of the flagellates *Koruga bonita* and *Deltotrichonympha nana*. These results indicated that the intestinal flagellates of *Mastotermes darwiniensis* take up the termite's cellulases from gut contents, probably attached to wood particles. *Koruga bonita* and *Deltotrichonympha nana* possess at least their own endoglucanase genes, which are still expressed, but without significant enzyme activity in the nutritive vacuole. These findings give the impression that the gut flagellates of *Mastotermes darwiniensis* are heading towards a secondary-loss of their own endoglucanases and they use exclusively termite cellulases.

Cellulose digestion by bacteria was discussed controversially (Thayer 1978; Slaytor 1992). Several cellulolytic bacteria were isolated from termites (Wenzel et al. 2002; cf. König et al. 2002). In contrast to the rumen, where strictly anaerobic cellulolytic bacteria belonging to the genera

Table 11.2. Intestinal isolates involved in the different stages of lignocellulose degradation in the termite gut

| Substrate | I. Stage Hydrolytic microorganisms | II. Stage Oxidative/fermentative microorganisms | III. Stage Acetogenic, methanogenic, sulfate-reducing bacteria | |
|---------------------------------------------------------------------------|---------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------|
| Wood mechanical degrada- tion to μm- particles by termites | Cellulose (34–62%) | Cellulase Flagellates: <i>Trichomitopsis termopsidis</i> <i>Trichonympha sphaerica</i> <u>Bacteria</u> : <i>Alcaligenes</i> , <i>Azospirillum</i> , <i>Bacillus</i> , <i>Brevibacillus</i> , <i>Cellulomonas</i> -related spp., <i>Clavibacter</i> , <i>Clostridium</i> , <i>Corynebacterium</i> , <i>Klebsiella</i> , <i>Kocuria</i> , <i>Paenibacillus</i> , <i>Microbacterium</i> , <i>Micromonospora</i> , <i>Nocardioforme</i> , <i>Rhizobia</i> , <i>Ochrobactrum</i> , <i>Paenibacillus</i> , <i>Sphingomonas</i> , <i>Spirosoma</i> -related spp., <i>Streptomyces</i> Yeasts (<i>Cryptococcus</i> , <i>Filobasidium</i>) | <i>Actinobacteria</i> , <i>Arthrobacter</i> , <i>Au-reobacterium</i> , <i>Bacillus</i> , <i>Citrobacter</i> , <i>Enterobacter</i> , <i>Enterococcus</i> , <i>Klebsiella</i> , <i>Lactococcus</i> , <i>Nocardia</i> , <i>Ochrobactrum</i> , <i>Pseudomonas</i> , <i>Rhizobia</i> , <i>Rhodococcus</i> , <i>Serratia</i> , <i>Streptomyces</i> | <u>Food source</u> <u>of termites</u> : acetate, propionate, butyrate, microbial cells |
| | Hemicellulose (14–32%) | 1.4-β-Xylanase 1.3-β-Galactanase | β-D-Galactosidase α-L-Arabinosidase β-L-Xylosidase | 2 H ₂ +O ₂ ⇒H ₂ O Lactate⇒Acetate+CO ₂ <i>Desulfovibrio</i> |

| | | | |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <p><i>Actinobacteria, Acinetobacter, Bacillus, Flavobacterium</i>-related spp., <i>Ochrobactrum, Paenibacillus, Pseudomonas, Streptomyces</i></p> <p>Yeasts (<i>Candida, Debaryomyces, Pichia, Sporothrix</i>)</p> <p>Aromatic compounds (5%)</p> <p>Lignin (18–39%)</p> | <p><i>Actinobacteria, Acinetobacter, Arthrobacter, Aureobacterium, Bacillus, Burkholderia, Citrobacter, Cellulomonas, Enterobacter, Escherichia, Klebsiella, Nocardia, Ochrobactrum, Pseudomonas, Paenibacillus, Rhizobium, Serratia, Staphylococcus, Streptomyces</i></p> <p>Yeasts</p> <p><i>Acinetobacter, Arthrobacter, Alcaligenes, Aureobacterium, Bacillus, Burkholderia, Citrobacter, Comamonas, Enterobacter, Klebsiella, Listeria, Nocardia, Ochrobactrum, Pseudomonas, Rhizobia, Serratia, Streptomyces</i></p> | <p>$4\text{H}_2 + 2\text{CO}_2 \Rightarrow \text{Acetate} + 2\text{H}_2\text{O}$ <i>Acetone, Clostridium, Sporomusa, Spirochetes</i></p> <p>$4\text{H}_2 + \text{CO}_2 \Rightarrow \text{CH}_4 + 2\text{H}_2\text{O}$ <i>Methanobrevibacter</i></p> | <p>CO_2, H_2, CH_4</p> <p>Microbial cells</p> <p>Excreted: modified lignin</p> |
| N_2-fixation | <p><i>Enterobacter, Desulfovibrio, Citrobacter, Rhizobia, Klebsiella, Spirochetes</i></p> | | |

Ruminococcus, *Butyrivibrio* and *Bacteroides* are present, the cellulolytic bacteria in the termite gut are facultatively anaerobic or microaerophilic bacteria. Species of the genera *Bacillus* are predominant with titers of up to 10^7 ml⁻¹ gut contents (Wenzel et al. 2002). Cellulolytic yeasts have also been isolated from *Zootermopsis angusticollis* and *Mastotermes darwiniensis* (Wenzel et al. 2002). The isolates from *Zootermopsis angusticollis* belong to the genus *Sporothrix*. Fungus-growing termites of the subfamily Macrotermitinae culture the fungus *Termitomyces* sp. in their nests. Together with the fungal nodules they probably consume fungal cellulolytic enzymes (Martin 1991; Rouland et al. 1991; cf. Chap. 17). Termites themselves produce also cellulases (Yokoe 1964). Recently the corresponding gene was sequenced (Watanabe et al. 1998; Tokuda et al. 1997, 1999; Li et al. 2003). In *Reticulitermes speratus* the main portion of the cellulolytic activities (cellulase, glucosidase) were found in the salivary glands, while the xylanolytic activities (xylanase, xylosidase) were found in the anterior part of the hindgut (Inoue et al. 1997).

Hemicelluloses are chemically complex and include different heteropolysaccharides such as arabinans, galactans, glucans, mannans, and xylans. The most common polyose is xylan. The complete degradation of xylan requires a synergistically acting set of enzymes: endo- β -1,4-xylanase, β -xylosidase, α -glucuronidase, α -L-arabinosidase, acetylsterase and feruloyl- or *p*-coumaroyl esterase (Fengel and Wegener 1984; Varma et al. 1994). The full set of enzymes required for release of all constituents of xylan has not been found in a single bacterial isolate from the termite gut (Saxena et al. 1993; Schäfer et al. 1996). Hemicellulose is degraded through synergistic activities of the glycolytic enzymes. An effective hemicellulose-degrading community of microbes is found in the termite gut (Breznak and Brune 1994; Varma et al. 1994; Schäfer et al. 1996). Hemicelluloses are degraded between 49 and 78% (Mishra 1979). The xylan degrading bacterial isolates mainly belong to the actinomycete- and *Clostridium*-branch of Gram positive bacteria as well as enterobacteria and *Pseudomonas*, *Acinetobacter* and *Ochrobactrum* species (Schäfer et al. 1996).

Based on the analysis of termite faeces some authors reported between 5% and 83% of lignin degradation (Seifert and Becker 1965; Butler and Buckerfield 1979; Cookson 1988). The involvement of gut microbes was not determined. In order to approach the problem of microbial lignin degradation in the gut the degradation of lignin monomers, other aromatic compounds, and dimeric lignin model compounds by the intestinal flora was studied (Kuhnigk et al. 1994; Brune et al. 1995a; Kuhnigk and König 1997). From the lower termites *Mastotermes darwiniensis* and *Reticulitermesantonensis* and from the higher termite *Nasutitermes nigriceps* anaerobic, facultatively anaerobic and aerobic bacterial strains were isolated in media containing lignin monomers or other aromatic compounds. Most of the

monomeric aromatic compounds and dimeric model compounds were degraded in the presence of oxygen by the mixed gut flora and pure cultures. Under anaerobic conditions the side chains of the aromatic compounds were only modified, but the aromatic ring was not split. Decarboxylation and reduction of the double bond in the side chain of phenyl propane derivatives were obtained in the absence of oxygen. Synthetic dehydrogenative lignin was not degraded under anaerobic or aerobic conditions. The results suggested that in the termite hindgut the breakdown of the aromatic ring systems requires oxygen (Kuhnigk et al. 1994), which is most probably supplied via the aerated paunch epithelium.

Besides stomodeal and proctodeal food exchange termites feed also on their own faeces. During passage of wood particles through the digestive tract they are inoculated with microorganisms. Members of bacterial genera, especially of Gram positive bacteria, belonging to the actinomycete branch, such as *Streptomyces* sp. (Pasti and Belli 1985; Pasti et al. 1990; Schäfer et al. 1996) which are believed to attack lignin, also were isolated. However, the breakdown activities of the gut isolates seems to be negligible, except in *Nasutitermes takasagoensis*. In this termite lignin components were found to be degraded in significant amounts (28%; Kato et al. 1998). The microorganisms may start to attack lignin near the aerated gut epithelium and they continue their activity outside of the gut. The faecal pellets may also be inoculated by fungi in the nest. Therefore, repeated recycling of faecal material may increase the efficiency of the digestion of wood particles by a termite colony. This procedure may be a kind of extracorporal digestion. In fungus combs of the fungus-growing termite *Macrotermes gilvus* the mutualistic fungus *Termitomyces* sp. was shown to progressively degrade lignin and thus enhance the digestibility of cellulose for the termites (Hyodo et al. 2000). Laccase activity acquired probably from fungi, was only found in fungus-growing termites of the genera *Macrotermes*, *Odontotermes* and *Pseudacanthotermes* (Mora and Lattaud 1999). Wood contains a large number of extractable low-molecular weight compounds such as phenols, tannins and terpenoid (Fengel and Wegener 1984), which may be used by aromatics-degrading bacteria. The degradation of these extractives may not only provide fatty acids as carbon sources for the host, but represents also a detoxification, since these compounds are known to be harmful to insects.

The majority of termite species are considered to be humivorous. In feeding experiments with ^{14}C -labeled humic model compounds labeled either in their proteinaceous or aromatic building blocks as substrates, it was shown that the peptide moiety were selectively digested, but the aromatic components seemed not to be an important food source of humivorous termites such as *Cubitermes orthognathus*, *Cubitermes umbratus* and *Thoracotermes macrothorax* (Ji et al. 2000).

11.7.2

The Oxidative/Fermentative Stage of Lignocellulose Degradation

A diverse variety of bacterial strains, belonging mainly to the Gram positive bacteria and proteobacteria have been isolated. They are able to degrade monosaccharides and oligosaccharides, which are produced during the hydrolysis of cellulose and hemicellulose. Oligosaccharide-hydrolyzing enzyme activities of the isolates have been determined (Schäfer et al. 1996).

11.7.3

The Methanogenic/Acetogenic Stage of the Lignocellulose Degradation

In the third step of lignocellulose degradation three physiological prokaryotic groups are involved: methanogenic bacteria, homoacetogenic bacteria and sulfate reducing bacteria. Symbiosis between animals and intestinal methanogens is widespread (Hackstein und Stumm 1994; Hackstein et al. 1996). High amounts of methane are released into the atmosphere by termites (Zimmermann et al. 1982; Martius et al. 1993; Rouland et al. 1993). Acetogenesis and methanogenesis were considered to be the most important terminal electron-accepting processes in the hindgut of termites (Braumann et al. 1992). Methanogens detected in the termite gut belong to the families Methanobacteriaceae, Methanosarcinaceae and Methanomicrobiales (Braumann et al. 2001; Ohkuma et al. 1999c). At least for xylophagous termites, acetate is the major end product of microbial fermentation in the gut (Breznak and Switzer 1986). Acetate can be used by the termites as an energy and a carbon source. Relatively high concentrations of acetate (58–81 mmol l⁻¹) have been found (Odelson and Breznak 1983). The homoacetogens belong to the genera *Acetonema*, *Clostridium*, and *Sporomusa*. Spirochetes occur free living and attached to the surface of protozoa. One homoacetogenic isolate has been described (Leadbetter et al. 1999).

Until now, all sulfate-reducing bacteria isolated from the termite gut belong to the genus *Desulfovibrio* (Braumann et al. 1990; Trinkerl et al. 1990; Fröhlich et al. 1999b; Kuhnigk et al. 1996) and have been assigned to four species (*D. desulfuricans*, *D. giganteus*, *D. intestinalis*, *D. termitidis*). Up to 10⁷ living cells per ml paunch content were counted in *Mastotermes darwiniensis* and *Reticulitermes santonensis* (Kuhnigk et al. 1996). When termites were fed with cellulose powder moistened with a Na₂SO₄ solution, the total number of sulfate reducers in the gut contents increased up to 10⁸ ml⁻¹ in *Reticulitermes santonensis* and *Heterotermes indicola*. Typical features of *Desulfovibrio* are the incomplete oxidation of electron donors to acetate, and the capacity of hydrogen consumption (Cypionka 2000). This genus is also able to reduce oxygen with hydrogen (Knallgas reaction).

Microaerophilic conditions, with a constant inflow of oxygen via the epithelium, are found near the gut epithelium (Brune et al. 1995b). Sulfate reducing bacteria, alike methanogens, have been found attached to the aerated gut epithelium (Berchtold et al. 1999). The sulfate concentration in the termite gut ($0.3\text{--}0.7\text{ mmol l}^{-1}$) is higher than in fresh water sediments (Kuhnigk et al. 1996). Under hydrogen limitation sulfate reducers may outcompete acetogenic and methanogenic bacteria. The nutritional versatility of acetogens may be one of the reasons, why they outcompete methanogens in xylophagous termites (Breznak and Blum 1991). Experiments with *Cubitermes* spp. showed a difference in the spacial activity of methanogens and acetogens (Tholen and Brune 1999) depending on the hydrogen concentrations. The anterior regions of the gut of *Cubitermes* spp. accumulated hydrogen, whereas the hydrogen concentration in the posterior hindgut was below the detection limit (Schmitt-Wagner and Brune 1999). All three groups may principally co-exist, although differences in the number of methanogens and acetogens have been found in wood-feeding and soil-feeding termites (Braumann et al. 1992).

Sulfate-reducing *Desulfovibrio* species are thus able to catalyze all reactions of a complete sulfur cycle (Cypionka 2000). *Desulfovibrio termitidis* has been found in the termite *Heterotermes indicola* and in the wood-feeding roach *Cryptocercus punctulatus*, which live in Asia and North America (Fröhlich et al. 1999). Likewise, *Desulfovibrio intestinalis* exists in geographically remote hosts, *Mastotermes darwiniensis* from Australia and *Odontotermes obesus* from India. Two species, *Desulfovibrio desulfuricans* and *Desulfovibrio giganteus*, were found in the termite *Cubitermes speciosus* (Braumann et al. 1990). Based on the fact that members of the same *Desulfovibrio* species can be found in phylogenetically and geographically remote hosts, it may be speculated that *Desulfovibrio* species have repeatedly and independently colonized the intestine or were already present in termites before the separation of the continents. Belonging to the physiological group of sulfate reducers, members of the genus *Desulfovibrio* are anaerobes, although some exhibit strong oxygen tolerance (Cypionka 2000). Termites benefit from the *Desulfovibrio* species, which produce acetate, and thus provide nutrition for their hosts. In co-culture *Desulfovibrio desulfuricans*, from the gut used the fermentation products of other fermentative gut bacteria and produced mainly acetate. They also contribute to the anoxic milieu of the gut by producing hydrogen sulfide and removing oxygen with hydrogen or low molecular weight organic or reduced sulfur compounds. The isolates were able to oxidize sulfur compounds such as sulfide, thiosulfate and sulfite. Sulfide was oxidized to sulfate as indicated by the amount of sulfide to oxygen consumed (molar ratio: 1:2). Performing a complete sulfur cycle the desulfovibrios can balance the redox status of the gut contents. This activity would allow sulfate recovery and keep

a complete sulfur cycle running. Sulfide reoxidation could prevent toxic effects of H_2S . Only substrates that are of no direct use for the termites (H_2 , formate) were oxidized completely, while others were transformed to acetate. Rates of oxygen consumption were as high as $1,570 \text{ nmol O}_2 \text{ min}^{-1} (\text{mg protein})^{-1}$ with electron donors in excess, which is also the case in the termite gut. These are the highest respiration rates of sulfate-reducing bacteria ever measured. These rates exceed by far the respiration rates of typical aerobes or facultative anaerobes such as *Escherichia coli* ($300 \text{ nmol O}_2 \text{ min}^{-1} (\text{mg protein})^{-1}$) (Kuhnigk et al. 1996).

A sulfate-reducing bacterium was also enriched from the gut of the rosechafer *Pachnoda marginata*, which showed the highest 16S rDNA sequence identity (93%) to *Desulfovibrio intestinalis* and *Desulfovibrio* strain STL1 (Dröge et al. 2005). Compared to *Mastotermes darwiniensis* (1×10^7 cells of SRB per ml gut contents), sulfate reducing bacteria occur in higher numbers in the gut contents of *Pachnoda marginata* reaching cell titers of up to 2×10^8 cells per ml gut contents. In vitro sulfate reduction rates were determined with SRB from the gut contents of the termite *Mastotermes darwiniensis* and the beetle *Pachnoda marginata*. Due to the higher cell titer, the sulfate reduction rate of *Pachnoda marginata* was $104 \text{ nmol h}^{-1} \text{ ml}^{-1}$ and, therefore, 21 times higher than that of *Mastotermes darwiniensis*. In addition, in vivo sulfate reduction was detected in *Mastotermes darwiniensis*, which indicates that sulfate reducers play an active role in the sulfur metabolism in the termite gut.

11.8 Nitrogen Fixing Bacteria

Wood is deficient in nitrogen (Fengel and Wegener 1984). Nitrogen fixation has been demonstrated in all families of termites, and atmospheric nitrogen is a considerable nitrogen source for xylophagous termites (Tayasu et al. 1994; Golichenkov et al. 2002). Several bacteria have been isolated with the capability to fix molecular nitrogen. They belong to the genera *Enterobacter* (Potrikus and Breznak 1977), and *Desulfovibrio* (Kuhnigk et al. 1996) as well as *Rhizobium*-/*Sinorhizobium*-related (Wenzel et al. 2002) and *Treponema*-related strains (Lilburn et al. 2000). The nitrogen-fixing bacteria improve the nitrogen balance of their hosts. In the gut of the termite *Neotermes koshunensis* expression of nitrogen fixation genes was detected by direct amplification of the *nifH* cDNA from mRNA (Noda et al. 1999; Ohkuma et al. 1999a). Seasonal patterns of nitrogen fixation in termites was found. The nitrogenase activity was found to be highest in autumn and spring, and lowest in winter and summer (Curtis and Waller 1998).

11.9 Intracellular Symbiosis

While all examined species of cockroaches have been shown to harbour intracellular bacteria in specialized cells (mycetocytes, bacteriocytes) of the fat body, in termites bacteriocytes are restricted to *Mastotermes darwiniensis*. All of these bacteria have been assigned to the same eubacterial lineage, with the intracellular bacteria of *Mastotermes darwiniensis* as the sister group to the cockroach bacteria. (Bandi et al. 1997). They belong to the *Blattabacterium* group of the *Bacteroides/Flavobacterium* branch. These symbionts are thought to have originated from a bacterium that infected an ancestor common to cockroaches and termites. Furthermore, non-mycetocyte intracellular bacteria are widespread in termites, which belong to the *Wolbachia* group (Bandi et al. 1995, 1997).

11.10 Conclusions

The symbiotic ecosystem “termite gut” can be described as a mechanical rasp provided by the host, which grinds wood down to a micron scale and a fermentation chamber of microbial and termite’s own hydrolytic enzymes. Microbial genera containing species with the ability to produce polysaccharide- or oligosaccharide-degrading enzymes are combined in Table 11.2 (stages 1 and 2). The goal of this symbiotic system is to convert lignocellulose mainly to acetate, propionate and butyrate (Odelson and Breznak 1983) and microbial cells. The advantage for the host is the capability to use hardly degradable compounds as a food source, while the microflora has a constant substrate supply from the host. The low chain fatty acids produced by the microbiota are used by the termites as an energy and a carbon source. The gases hydrogen, methane and carbon dioxide are released in significant amounts into the atmosphere. Lignin seems not to be degraded during the passage through the gut, but is excreted. The ecosystem “termite gut” is small, but the microbial flora and the interactions between the microbiota and their host are very complex. Termites play a significant ecological role in the mineralization of organic material, especially of lignocellulose. Further studies of this exciting ecosystem may also lead to novel biotechnological applications of the wood-degrading flora.

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