# Lignocellulose degradation by microorganisms from termite hills and termite guts: A survey on the present state of art

Ajit Varma [a], Bala Krishna Kolli [a], Jaishree Paul [a], Shailendra Saxena [a] and Helmut König [b,*]

[a] *School of Life Sciences, Jawaharlal Nehru University, New Delhi 110 067, India, and* [b] *Angewandte Mikrobiologie, Universität Ulm, 89069 Ulm, FRG*

*Abstract:*

In several aspects termites are a fascinating group of insects having attracted the interest of many researchers. They exhibit a complex social behavior and caste differentiation occurring elsewhere only among the hymenoptera. In an enlarged part of the hindgut, the paunch, termites have established a unique symbiotic association with prokaryotic and eukaryotic microorganisms. A similar flora is also found in wood-eating roaches of the genus *Cryptocercus*. The study of symbiosis between termites and their intestinal microbes is of general interest, because due to this symbiotic interaction termites can feed on complex biopolymers such as wood. Flagellates and bacteria occur in the gut of lower termites, while higher termites possess only bacteria. In particular spirochetes are abundant in the termite gut. Apart from spirochetes and other more common bacteria, actinomycetes, yeasts and fungi have also been isolated from different species of termites. This review summarizes the distinct role of the intestinal flora in degradation of wood components such as cellulose, hemicellulose and lignin.

## Indroduction

Termites are important ecologically and economically and they are of obvious concern. World-wide termite damage and control costs are estimated at 1.2 billion dollars annually [1]. They occur in abundance in the tropical and subtropical parts of the world where their destructive capacity is best known, but they are also found throughout the temperate regions. In natural environment, they play a role in the turnover and mineralization of complex biopolymers such as lignocellulose by degrading dead trees and other cellulose- and hemicellulose-containing materials to more simpler compounds. In view of this ability, termites may also play a hitherto unrecognized role in soil fertilization by replenishing combined organic compounds in the soil. They also increase soil aeration and drainage. This may be particularly significant in tropical regions and semi-arid soils where termites abound.

A fascinating and frequently cited example of

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\* Corresponding author. Tel.: (0731) 502 2707; Fax: (0731) 502 2719

nutritional symbiosis is the association between certain xylophagous termites and their intestinal microflora. Of all the intriguing activities and properties of termites, none seems as widely recognised as their ability to utilize wood as a food source [2–8]. Diet of termites as group is quite diverse, but is basically one rich in cellulose, hemicellulose and lignin or lignin derivatives. Since their diet is relatively poor in combined nitrogen, termites may be thought of as oligonitrotrophic saprovores. This trait places termites in an important position ecologically, particularly in tropical regions, where their activities can dominate the process of decomposition and nutrient cycling. Furthermore, the biomass density of termites can be so large (10–20 g m$^{-2}$) that their impact is similar to, and may even surpass, that of grazing mammals [8].

The termite gut and the termite soils provide a very distinct ecological environment which harbours and promotes very specialized cellulolytic and hemicellulolytic microorganisms. This review provides an up-to-date account of such bacteria, their ecological role in carbon cycling, physiological and biochemical characteristics with the aim to emphasize the fact that they may provide a distinct environment for isolation of several new and more potentially active lignocellulose-degrading bacteria producing extracellular hydrolytic enzymes with far reaching consequence in maintaining nutrient and microbial balance in nature, especially in arid and semi-arid soils. In addition, congregation of large numbers of specialized microorganisms in an ecosystem plays a vital role directly or indirectly in maintaining parasitic and symbiotic balances and regulates the ecology of microorganisms.

Here we summarize the present state of knowledge about the role of the flora of termite gut and termite soil on the degradation of wood components.

## Termites

### General features of termites

Termites, often misnamed white ants, are small to medium-sized, soft-bodied insects which range in colour from dull white to light and dark brown, and belong to the insect order Isoptera [9,10]. Termites are social insects and live in small to large colonies. In some species a colony may have almost a million or more individuals. They also exhibit polymorphism, and a well-defined grouping of individuals into different functional castes, viz. larvae, workers, pseudergates, soldiers, nymphs and reproductives. Of these the first four are non-winged and incapable of reproduction. Pseudergates retain their ability to differentiate and nymphs develop to alated reproductives. Nymphs already have wing buds. Larvae, soldiers, workers and nymphs are sometimes dimorphic and even polymorphic. The castes are morphologically distinguishable and have different functions. The soldiers, which have a strongly chitinised head usually with large mandibles, take active part in the defense of the colony. In the higher developed Nasutitermitinae the soldiers produce a sticky defensive fluid which is secreted from a projection in front of the head. The majority of individuals in a colony are workers; there are few soldiers (up to 10%) and usually only a pair of reproductives. Pseudergates retaining their caste-differentiating ability and replacement reproductives are present in several genera. The reproductives are initially winged and swarm out of a colony, usually once or twice a year, to form new colonies. As a rule, a colony has only a pair of primary reproductives (a male and a female, often termed king and queen) which are generally imprisoned by the workers in a royal cell and their sole function is to reproduce. Fertility is high and queens (e.g. *Odontotermes obesus*) can lay almost one egg per second. The eggs hatch in a few days into whitish larvae which develop into workers, soldiers or winged reproductives as the case may be [9–11].

### Taxonomy of termites

According to Krishna [12], termites are subdivided into six families, all of which comprise wholly social species (Fig. 1). The most primitive and closest relatives to the cockroaches are the Mastotermitidae. They had a world-wide distribution in the tertiary period, but they are today restricted to Australia as the single species *Mas-*
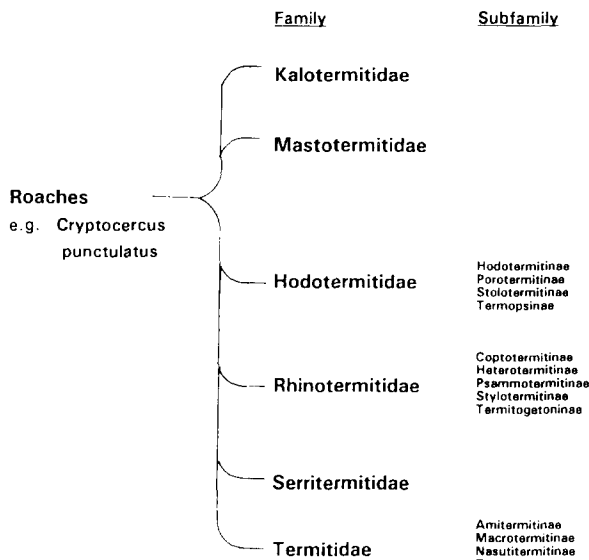
| Family | Subfamily |
|---|---|
| Kalotermitidae | |
| Mastotermitidae | |
| Hodotermitidae | Hodotermitinae<br>Porotermitinae<br>Stolotermitinae<br>Termopsinae |
| Rhinotermitidae | Coptotermitinae<br>Heterotermitinae<br>Psammotermitinae<br>Stylotermitinae<br>Termitogetoninae |
| Serritermitidae | |
| Termitidae | Amitermitinae<br>Macrotermitinae<br>Nasutitermitinae<br>Termitinae |

Roaches e.g. Cryptocercus punctulatus

Fig. 1. Termite phylogeny simplified after Krishna [12].

*totermes darwiniensis*. Another primitive, but still wide-spread family is Kalotermitidae (dry wood termites) which excavate logs and maintain colonies strictly in wood without necessary soil connections. The family Hodotermitidae, which has fossils dating back to the mid cretaceous in Labrador, has retained some primitive characters such as mandible structures. Among the four subfamilies, the Termopsinae (damp wood termites) live in rotting wet wood in warm temperate regions and the Hodotermitinae (harvester termites) thrive in deserts and steppes and store pieces of grass in their nests. The Rhinotermitidae are so named since they have evolved small soldiers with snouts for the ejection of repellants in the subfamily Rhinotermitinae. Most species of this family occur in the oriental region. The Serritermitidae possess mandibles with serrations along the entire inner margin. Only one species belongs to this family which is found in Brazil. The most highly evolved family, the Termitidae, includes 75% of the more than 1800 known termite species [13] and possesses many termites with pigmented sclerotized neuter classes. This family of termites has some remarkable soldiers with snap-closing mandibles. Within Termitidae, Amitermitinae is the simplest subfamily. The

Macrotermitinae culture a fungus (*Termitomyces* sp.) or fungal combs and the Nasutitermitinae have soldiers that shoot jets of toxic and sticky material [9,10]. *Odontotermes obesus* (Rambur) is one of the most widely distributed species of termites and has been reported for most of the parts of India [10,11,14,15].

*Ecological distribution of termites*

Termites are distributed all over the world from the 47° Northern latitude to the 47° Southern latitude, but they are heavily concentrated in the tropics. Only species of the genera *Archotermopsis*, *Hodotermopsis*, *Zootermopsis* and *Reticulitermes* thrive also in temperate regions [16]. A few genera occur in dry habitats, but the majority of the species live under relatively moist conditions. Their main food is cellulose which is taken up as wood or other plant material. About 85% of the species recorded until 1955 were known to be present in three principal tropical geological regions, viz. Oriental, Ethiopian, and Neotropical, while only 13–17% of species are reported for Palearctic and Nearctic regions. Africa, south of Sahara, was reported to harbour 66 genera.

*Topography of the termite hill and nest*

The nests of termite societies exhibit large variations. Some species form only simple galleries in wood or the ground, while others construct most complex edifices. They are the wood and the ground dwellers. The wood dwellers are confined throughout their lives to wood in which they make tunnels and also nest. The termite-infested wood may be dead or alive. The wood dwellers, based on their requirement of the wood's content are further divided into two categories, damp wood termites (e.g. Termopsinae) and dry wood termites (e.g. Kalotermitidae).

The ground dwellers (e.g. Rhinotermitidae) live exclusively, or at least partly, in soil. The ground connection is necessary for their normal life and breeding. From the ground, the workers come out for foraging either in the open or into wood, having a direct ground connection or earthern runways and shelter tubes made earlier by the workers on the ground, wall surface or tree trunks. Termites that excavate hard wood or soft wet

wood cut out tunnels and then plaster them with faeces which is heavily fibrous with plastic texture. They often make partitions by placing a grain of sand and cementing it with faeces to create a lenticular fabric up to 2 mm thick [2]. Many live underground and make chambers connected by interwoven tunnels. The chambers are either for reproductives or for fungus combs or nurseries. Chambers with fungal combs are generally little bigger and are plastered with clay but have a concave floor which gives access to the soil beneath and also allows air to circulate. The most spectacular mounds are made by the Macrotermitinae. They may be either round, 20–30 m in diameter at the base and over 6 m high or 2–3 m

in diameter and reaching a height of 8–9 m. Fig. 2 shows a schematic representation of two mound types of *Macrotermes bellicosus*. The termites start under ground where a new couple have dug a shaft and chamber about 20 cm deep and rear a few small workers. These workers take down wood and build a miniature fungus comb seeded with fungi from adult faeces. This is enclosed in an envelope of 1 mm thick clay with a hole at the top. It takes only a night to make; in fact, the whole microcosm can be established within 100 days [18]. The nascent mounds break the bare surface soil after rainfall and grow at a linear rate into the final form which, according to Collins [19], may take either of two designs on the same
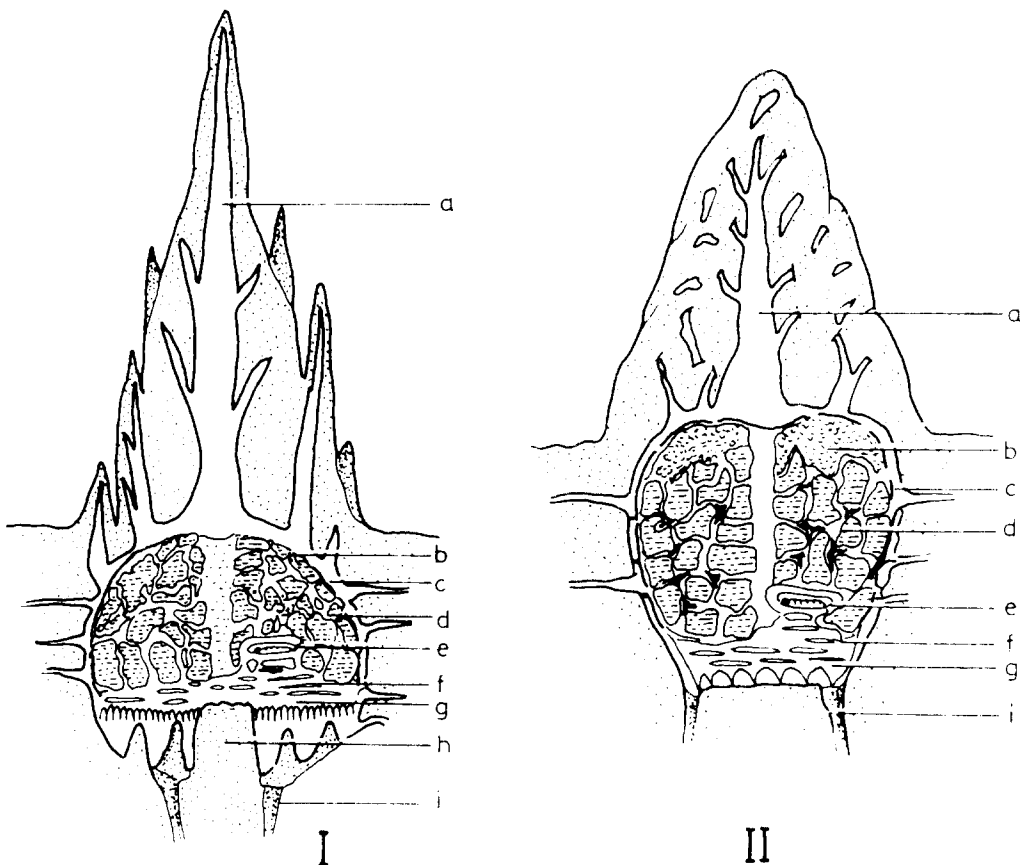


Fig. 2. Schematic representation of a mound of *Macrotermes bellicosus*. (I) spiral plate; (II) normal type. Signs indicate the different architecture of a typical termite hill. a, main shaft; b, food stores; c, envelope; d, fungus combs; e, reproductive tissues; f, galeries with larvae; g, base plate; h, caves; i, pillar.

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soil with the same drainage. This difference in design might be due to different species [20]. In one type, the brood nest with the reproductive cell, the fungus comb and galleries for the young, rests on conical pillars standing in a cavity, called the cellar, which is enlarged out of the original soil excavation, from which open chimneys arise. The other type is also called the 'cathedral' because of its pointed hollow spires which can be 6 m high and 3–4 m in diameter at the base. In this type, broad galleries together with the reproductive cell and fungus comb form a spherical assemblage, at about ground level, resting on a plate of 3.5 m in diameter. This plate is supported by a single pillar, though not actually attached to it. From the cellar, very large shafts up to 20 cm diameter descend into the soil as deep as 3 m and are probably used for mining clay. Valves or flat spirals which are up to 2.5 cm thick and covered with salt crystals hang from the plate into the cellar. The mound wall is of clay and carton, perhaps faecal in origin, and the inside is mostly clay mixed with saliva but incorporating some lime [18]. Even in sandy deserts the termites collect these materials selectively [21].

Apart from the great pyramidal mounds, they also make spheroidal nests (e.g. *Apicotermes*) in underground cavities. These are regularly and intimately designed with floors connected by ramps and a cortex of galleries under a perforated skin, the pores of which are characteristic of the species [22].

*Anatomy and chemistry of the termite gut*

The digestive system of termites consists of foregut, which includes crop and gizzard, midgut and hindgut. The hindgut may be divided into five successive segments: the proctodeal segment, the enteric valve controlling the entrance of food, the paunch which is major site of absorption and harbours a number of symbiotic microorganisms [23], and lastly there are colon and the rectum [24]. An important function of the enteric valve is that it prevents the return of paunch content to the midgut or the foregut [24]. The Malpighian tubules enter the gut at the junction of the midgut and the first proctodeal segment. In many species of higher termites, the midgut is an elongated
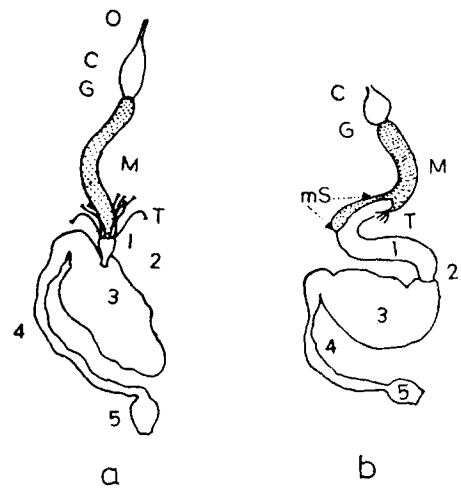


Fig. 3. Segments of a typical termite gut. (a) Lower termite. (b) Higher termites. C, crop; G, gizzard; M, mid gut; T, malpighian tubules; mS, mixed segment; O, esophagus; 1, proctodeal segment; 2, enteric valve; 3, paunch; 4, colon; 5, rectum (adopted from [24]).

part of the intestine. Fig. 3 represents different segments of a typical gut of termites.

The histology, cytology and ontogenetic variation were described in detail by Noirot [25] and Noirot and Noirot-Timothee [24]. The epithelium forms distinct sites for the attachment of symbiotic microorganisms [26]. The termite hindgut was assumed to be anaerobic [27–34], while Eutick et al. [35] suggested it to be microaerophilic. Kuhnigk et al. [34] isolated also aerobic bacteria from the hindgut and found that aromatic compounds are only degraded with oxygen by the hindgut flora, which indicates a constant diffusion of oxygen through the paunch epithelium. The oxygen is readily used by aerobic and facultatively anaerobic microorganisms and, therefore, the gut contents remain anaerobic. Using redox dye feeding technique, several authors have measured the redox state of the gut of termites [31,32,34]. The fore- and midgut of all species were aerobic with an $E_0'$ in excess of $+100$ mV. Paunch and colon were anaerobic with an $E_0'$ of up to $-270$ mV. Midgut pH was found to be neutral, whereas the hindgut had a range from 6 to 7.5, except for soil feeders (*Cubitermes severus* and *Procubitermes*

*aburiensis*), where conditions were more alkaline. In *Odontotermes obesus*, the hindgut pH was observed to be alkaline (up to pH 10.4; [32,36]), which is usually tolerant for the growth of many organisms.

The passage of the food through the alimentary canal takes 24 h. Endogenous cellulose digestion takes place in the foregut and midgut, but the hindgut is the primary site of microbial cellulose digestion. Cellulose undergoes substantial degradation in termites as does the hemicellulose. In lower termites, cellulose digestion is performed by mutualistic protozoa. In higher termites, bacteria may perform this function. Endogeneous cellulases have been found in termites and cockroaches. According to Slayter [37], there is no convincing evidence for a major involvement of bacteria in cellulose digestion. Cellulose metabolism by the termite flora leads to the liberation of acetate and hydrogen as the end products [29,38–40]. The problem of lignin decomposition is, however, still unsettled. Most probably the degradation occurs with the aid of oxygen [34]. Where lignin appears to be degraded, determination of the extent of degradation has been confounded by the coprophageal habitat of termites and the recycling of faecal material which subjects the compounds to repeated digestion [41].

## Microbes from the termite environment

In most semi-arid ecosystems, the polysaccharide cellulose consitutes the major component of the flow of carbon and thus most studies on microbial polysaccharide decomposition were focussed on cellulolytic microorganisms. Termites play a key role in the carbon cycle of native soil ecosystems. The diet of termites is rich in cellulose and hemicellulose. The microflora inhabiting termite soil and their gut play a significant role in the dissimilatory activity. Microbial population estimates in termite-infested soil mounds in Kenya (Africa) showed that bacteria and actinomycetes are the most abundant during the wet season [42]. The highest density of bacteria recorded was $10^6$ and that of actinomycetes $10^5$
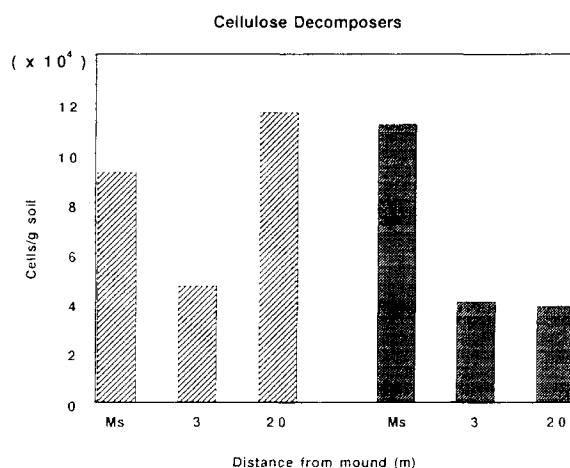


Fig. 4. Cellulose-degrading bacteria from termite *Odontotermes obesus* mound soils. hatched, 'dead' mound; crosshatched, 'live' mound; Ms, mound soil (adopted from [42]).

per gram of dry soil. In contrast, fungi, which dominate only during dry periods, numbered $10^4$ and declined to $10^2$ cells per gram of dry soil during the wet period. Fungi, actinomycetes, bacteria and protozoa occurred in higher numbers in 'dead' than in 'live' mounds. However, during the wet period, bacteria were dominant in 'dead' mounds. Cellulose decomposers were more numerous than all other groups of bacteria examined and were more abundant in mound soils than in adjacent soils (Fig. 4).

Indirect evidence of the significant role of microorganisms inhabiting termite mound soil was obtained from the observation of Coe [43]. The action of termites (*Odontotermes* and *Microtermes*) on elephant dung, a source of partially digested plant litter, increases during the dry season. A survey in the National Park in Kenya revealed that the rate of removal of dung may be up to $8.7 \times 10^3$ kg of faeces per $km^2$ from the ground surface. The termites therefore play an important role in immobilizing nutrients in their nests and mounds.

The seasonal variation obtained in the population of microorganisms from termite mounds revealed the dominance of cellulose degraders as well as other bacterial populations to be the highest during the monsoon and minimum in
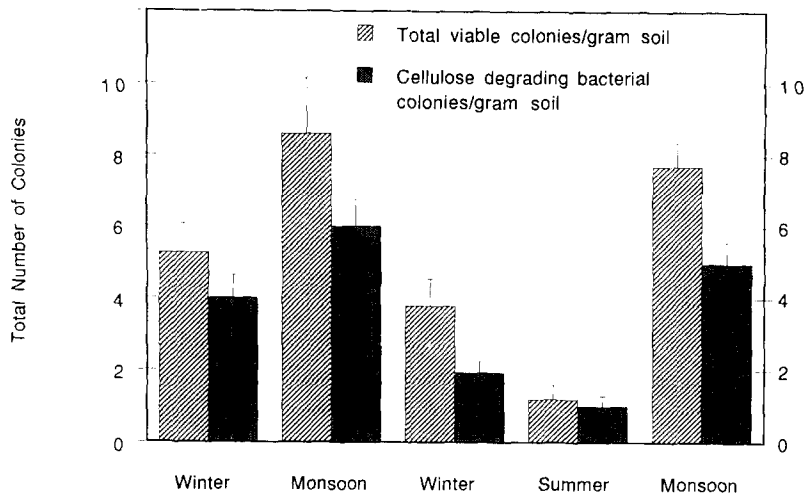
Fig. 5. Seasonal variations in cellulolytic bacterial population from live termite mound soils located in semi-arid areas. Data are the average of seven replicates.

summer (45–50°C). In winter months (5–15°C), the population was moderate. However, no significant decline was observed in comparison to the total population during summer (Fig. 5).

Polysaccharide-depolymerizing microorganisms isolated from termite habitats are compiled in Table 1. Some bacterial cellulolytic isolates such as *Cellulomonas* and *Bacillus* species from termite-infested soil have been characterized in more detail [44–48]. Among the cellulose de-

Table 1

Cellulose- and xylan-decomposing microorganisms isolated from termite hills and nests

| Organisms | Associated termite | Habitat | Reference |
|---|---|---|---|
| **A. Cellulose-decomposing microorganisms** | | | |
| I. Fungi | | | |
| *Formes lividus* (white rot) | *Nasutitermes exitiosus* | Infested wood | [6] |
| *Acremonium* sp. | *Reticulitermes flavipes* | Termite surface | [55] |
| *Aspergillus* sp. | | | |
| *Alternaria* sp. | | | |
| *Cladosporium* sp. | | | |
| *Fusarium* sp. | | | |
| *Stachybotrys* sp. | | | |
| *Penicillium* sp. | | | |
| *Trichoderma* sp. | | | |
| II. Bacteria | | | |
| *Bacillus* sp. | *Odontotermes obesus* | Mound soil | [48,163] |
| *Bacillus 'thermoalkalophilus'* | *Odontotermes obesus* | Mound soil | [5] |
| *Cellulomonas galba 'Cellvibrio flavifaciens'* | *Odontotermes obesus* | Mound soil | [5] |
| *Cellulomonas* sp. | *Odontotermes obesus* | Infested soil | [47] |
| *Cellulomonas* sp. | *Odontotermes obesus* | Mound soil | [7] |
| **B. Xylan-decomposing microorganisms** | | | |
| *Bacillus* sp. | *Odontotermes obesus* | Mound soil | [48,163] |
| *'Bacillus thermoalkalophilus'* | *Odontotermes obesus* | Mound soil | [6] |
| *Cellulomonas* sp. | *Odontotermes obesus* | Mound soil | [7] |

graders, a mesophilic *Bacillus* sp. [48] was found to contain a complete set of cellulolytic as well as hemicellulolytic enzymes. Similarly, Saxena et al. [49] also isolated a *Micrococcus* sp. which is able to produce all the three enzymes. Another cellulose degrader, *Bacillus thermoalkalophilus*, a unique bacterium which grew and metabolized in a wide range of pH 7–9 and temperature (37–60°C), was isolated from termite soils [5]. Another unique feature of these isolates was that cellulose degradation was invariably accompanied by change in pH of incubation medium from neutrality to alkalinity [11].

In the termite environment, associated fungi could be found [50–60]. A well-known example is the symbiotic relationship of the Macrotermitinae and species of *Termitomyces*. Recently, Zoberi and Grace [55] isolated 40 fungal species from living termites of the species *Reticulitermes flavipes* and infested soil and wood. A number of the isolated fungi also produce cellulases.

## Microbes from termite gut

### Colonization of the gut

In recent years there has been an increase in the number of studies on the gut microbiota of xylophagous insects like termites, with the object of determining their role in the metabolic and digestive processes of these insects.

In the phylogenetically 'lower' termites (Mastotermitidae, Kalotermitidae, Hodotermitidae, Rhinotermitidae) the intestinal microbiota include bacteria, as well as unique genera and species of oxymonad, trichomonad, and hypermastigote protozoa which are not found anywhere else in nature [23,61,62] except in members of the genus *Cryptocercus*. From the 205 examined species of the lower termites and the two species of *Cryptocercus*, 434 species of protozoa have been described until 1979 [62]. Many of these protozoa are capable of ingesting wood particles and are cellulolytic [23,61]. In the phylogenetically 'higher' termites (Termitidae), which constitute roughly 75% of all termite species, the gut microbiota essentially consist of bacteria alone. The bulbous 'paunch' region of the hindgut

is particularly important, because it is almost invariably colonized by a dense microbiota.

The role of the symbiotic flora has been described in several articles [3,4]. The anaerobic flagellates produce acetate, $CO_2$ and $H_2$ from cellulose. Acetate is also formed from $CO_2$ and $H_2$ by acetogenic bacteria [60–65] such as *Sporomusa termitida* from *Nasutitermes nigriceps* [66,67], *Acetonema longum* from *Pterotermes occidentis* [68] and *Clostridium mayombei* from *Cubitermes speciosus* [69]. Acetate is absorbed by the gut epithelium and functions as a major carbon source of the termites. Spirochetes are of constant and abundant occurrence in the hindgut of all termites and they are part of the autochthonous paunch flora [70,71]. They occur free in the gut fluid as well as attached to the protozoa in the hindgut of lower termites. Since the attempts for in vitro cultivation of these organisms failed, their biochemical role in gut system is still obscured. The heterotrophic bacteria fulfil several functions in the hindgut. They fix dinitrogen and recycle the nitrogen of uric acid, maintain a low redox potential, synthesize amino acids, produce acetate and other lower fatty acids and protect the hindgut from foreign bacteria [3,4].

Breznak and co-workers [41,72,73] found the hindgut of *Reticulitermes flavipes* and *Coptotermes formosanus* to be colonized by a morphologically heterogeneous assemblage of bacteria and protozoa. Most of the bacteria were situated close to the epithelium. Lesser numbers of bacteria were observed free in the lumen of the paunch, which is mainly occupied by protozoa. The midgut was more sparsely colonized, but did possess distinctive cuboidal-shaped, endospore-forming bacteria situated in between the microvilli of the epithelium. Twenty-seven bacterial morphotypes were distinguished in the higher termite *Nasutitermes exitiosus* [74]. Similar observations were made with the other lower and higher termites [3,4,75,76,77]. Prominent epithelial spines as specific attachment sites of symbiotic bacteria are present in the gut of *Procubitermes aburiensis* [26]. Sulfate-reducing bacteria [78,79] are another group of prokaryotes present in the termite. In addition, the flagellates (e.g. *Mixotricha paradoxa*) themselves harbour extracellular and intra-

cellular prokaryotes of unknown taxonomic position except for extracellular spirochetes [75,80]. Therefore, one may regard the termite gut as a complex ecosystem with free-living and attached microorganisms.

In the soil-feeding higher termites *Procubitermes aburiensis* and *Cubitermes severus*, the proctodeum was heavily colonized by bacteria. The crop, mesenteron and mixed segment also harboured bacteria [26]. As determined by direct microscopic count, population levels of bacteria ranged from $10^6-10^7$ bacteria per gut [3,71], whereas levels of protozoa were about $3-4 \times 10^4$ per gut for *Reticulitermes flavipes* [81]. Odelson and Breznak [82] determined that 61% of the hindgut contents of *Reticulitermes flavipes* consisted of microbial cells, the remainder being extracellular hindgut fluid. Attempts at enumeration and identification of termite gut bacteria through microbiological culture techniques was reviewed by Breznak [3] and O'Brien and Slaytor [4]. Results indicated that $0.3-1.3 \times 10^6$ colony-forming units per gut can be isolated from workers of *Reticulitermes flavipes*. Most of the bacteria isolated from lower and higher termites proved to be facultative or strict anaerobes. These included strains of *Streptococcus*, *Bacteroides*, various members of Enterobacteriaceae, *Staphylococcus* and *Bacillus*. The presence of *Staphylococcus saprophyticus*, *Micrococcus roseus*, *Bacillus circulans* in the gut fluid of *Odontotermes obesus* [5,36] confirmed the above-mentioned facts. In vitro cultivation of the above strains revealed that these were potent cellulose degraders.

*Degradation of cellulose*

Of all the intriguing activities and properties of termites, none seems as widely recognised or as often quoted as their ability to utilize wood as a food source. Indeed, many species of termites thrive on healthy, decay-free wood which contains as little as 0.03–0.1% (dry weight basis) nitrogen [83]. Many species prefer wood that is partially decayed by fungi, whereas some termites actually cultivate fungi in elaborate gardens for use as a nutrient source. Still others, depending on the species, feed on leaves and roots of grasses, dung of herbivorous animals, humus or soil [2].

Clearly, the diet of termites as a group is quiet diverse, but is basically rich in cellulose, hemicellulose and lignin or lignin derivatives, the main polymers of wood. Celluloses and hemicelluloses undergo substantial dissimilation on passage through the termite gut and the assimilation efficiency of wood feeders is quite high. Nevertheless, a key question has been, and continues to be, the contribution of specific gut organisms versus the termite's own enzymes, to the digestion process. Bulk of wood polysaccharides digestion occurs in the hindgut of termites. The physicochemical characteristics of the gut are similar in both higher and lower termites [31,32,34,36]. By contrast, midgut regions appear to be aerobic. Anaerobicity of the hindgut contents is consistent with the oxygen sensitivity of the hindgut protozoa of lower termites, with the demonstration of strict anaerobic bacteria in termite hindgut contents and in situ methanogenesis [29,30,41,63,67–69,72].

Feeding experiments with wood clearly showed that the two main constituents cellulose and hemicellulose are subject to degradation in termites. Cellulose digestion is also found in other insect groups such as the Thysanura, Orthoptera, Coleoptera and Hymenoptera. The mechanisms involved in cellulose degradation of termites are discussed controversially [37]. The extent of cellulose digestion in wood was found to be between 59% and 99% [84–86], resulting in the production of lower fatty acids, mainly acetic acid, and $CO_2$ and $H_2$ [39,82]. Already since the 1920s several authors demonstrated the breakdown of cellulose by termites (e.g. [3,4,23,87–92]).

Wood particles ground down by termites are passed to the hindgut where they are endocytosed by protozoa, as often found in higher termites [23]. Cellulose is fermented anaerobically within the protozoa to acetate, $CO_2$ and $H_2$ which are liberated from the cells. Acetate is subsequently absorbed by the termites and used as their major oxidizable energy source. This model (Fig. 6) is conceptually appealing, because it permits acquisition of energy (ATP) by both protozoa (via anaerobic fermentation of cellulose) and the termites, via aerobic oxidation of acetate.
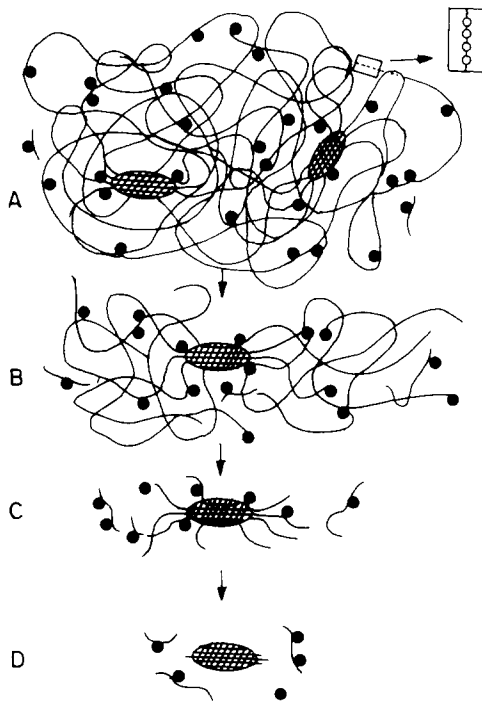
Fig. 6. Proposed working model for wood polysaccharide degradation in the hind gut of lower termites. ●, cellobiase; ▰, crystalline cellulose.

Considering the extent to which wood polysaccharides are dissimilated in termites, it appears that the protozoa are quite efficient at stripping these polymers from their complex with lignin. In 1924, it was already realized that protozoa may possess the potential to degrade cellulose [87]. The protozoa ingest the wood particles at the posterior end of the cell [23,93]. Three protozoa have been isolated, two of which are cellulolytic, *Trichomitopsis termopsidis* and *Trichonympha sphaerica* from *Zootermopsis* require cellulose for growth and protozoa, also free of bacteria, exhibit cellulase activity [23,40,61,62,90,94–100]. Protozoal cellullases could be detected in the hindgut of *Mastotermes darwiniensis* and *Coptotermes lacteus* [101,102]. By feeding sawdust amended with ¹⁴C-labelled polymers to *Reciculitermes flavepus*, Odelson and Breznak [100] found that 80% of the acetate derived was from cellulose, whereas about 20% was from hemicellulose. Little work has been done on the role of gut organisms in digestive processes of higher termites.

Many attempts to isolate cellulolytic bacteria from the hindgut of termites failed [72,87,89,91, 103] or the methods for isolation and the role of

Table 2

Cellulose- and xylose-decomposing microorganisms isolated from the termite gut

| Organisms | Termite | Reference |
|---|---|---|
| **A. Cellulose-decomposing microorganisms** | | |
| I. Fungi | | |
| *Alternaria alternater* | *Odontotermes obesus* | [52,122] |
| *Aspergillus awamuri* | | |
| *Aspergillus clavatus* | | |
| *Aspergillus flavus* | | |
| *Aspergillus nidulans* | | |
| *Cladosporium* sp. | | |
| *Paecilomyces fusiporus* | | |
| *Rhizopus stolonifer* | | |
| II. Protozoa | | |
| *Trichomitopsis termopsidis* | *Zootermopsis* | [95,97, 100] |
| *Trichonympha sphaerica* | *Zootermopsis* | [96] |
| III. Bacteria | | |
| *Alcaligenes* sp. | *Reticulitermes hesperus* | [110] |
| *Arthrobacter* sp. | *Reticulitermes hesperus* | [110] |
| *Bacillus cereus* | *Reticulitermes hesperus* | [100,114] |
| *Bacteroides* sp. | *Zootermopsis* | [100,164] |
| *Cellulomonas fimi* | *Odontotermes obesus* | [5] |
| *Clostridium* sp. | *Amitermes minimus* | [117] |
| *Clostridium termitidis* | *Nasutitermes lujae* | [115] |
| *Micrococcus roseus* | *Reticulitermes hesperus* | [5,7] |
| *Micrococcus luteus* | *Reticulitermes hesperus* | [5,7] |
| *Micrococcus* sp. | *Odontotermes obesus* | [5] |
| '*Micromonospora acetiformici*' | *Reticulitermes lucifugus* var. *santonensis* | [108] |
| *Micromonospora propionici* | *Amitermes minimus* | [117] |
| *Micromonospora* sp. | *Odontotermes microtermes* | [118] |
| *Serratia marcescens* | *Reticulitermes hesperus* | [110,114] |
| *Staphylococcus saprophyticus* | *Odontotermes obesus* | [5,36] |
| *Streptomyces* sp. | *Macrotermes amitermes* | [118] |
| IV. Hindgut | | |
| Hindgut homogenate | *Mastotermes darwiniensis* | [112] |
| **B. Xylan-decomposing bacteria** | | |
| *Micrococcus luteuns* | *Reticulitermes hesperus* | [7] |
| *Micrococcus resens* | *Reticulitermes hesperus* | [7,164] |
| *Micrococcus* sp. | *Odontotermes obesus* | [7] |
| *Micrococcus* sp. | *Odontotermes obesus* | [7] |
| *Pseudomonas aeruginosa* | *Reticulitermes santonensis* | [150] |
| *Bacillus cereus* | *Nasutitermes nigriceps* | [150] |

bacteria in the hindgut were not well documented [88,104–111]. Eutick and co-workers [103] were not able to isolate cellulose-degrading bacteria from nine species of Australian termites, and Schultz and Breznak [72] showed that cellulolytic bacteria were absent in *Reticulitermes flavipes*, while streptococci using cellobiose were abundant. The removal of gut bacteria in *Nasutitermes exitiosus* and *Coptotermes lacteus* had little effect on the cellulase activity [112]. Therefore, bacteria may play little role in the breakdown of cellulose in the termite hindgut and there is no direct evidence that they are of quantitative importance in cellulose digestion [2–4,37,41].

Nevertheless, several bacterial, fungal and protozoan isolates which hydrolyze cellulose [105,108,109,111,113–120] or starch [121] have been obtained in pure culture from the termite gut (Table 2). Among the actinomycetes, Hungate [117] isolated for the first time a cellulose-decomposing actinomycete, *Micromonospora propionici*, from the alimentary canal of the worker termite of the wood-eating species *Amitermes minimus*. Thayer [110] isolated *Bacillus*, *Serratia* and *Arthrobacter* strains with β-cellulase activity, and carboxymethylcellulase activity was detected in *Bacillus* and *Serratia* strains. Mannesmann [105] and Wood [120] also reported that some hindgut bacteria were cellulolytic or at least took part in cellulose digestion. With the help of an electron microscope, Bignell et al. [26] studied the gut wall of the soil-feeding termite species *Procubitermes aburiensis* and concluded that actinomycetes formed novel associations with the host in the mesenteron, mixed segment and colon. Pasti and Belli [118] reported different species of *Streptomyces* and *Micromonospora* from the hindgut of four termites belonging to the genera *Macrotermes*, *Odontotermes*, *Amitermes*, and *Microcerotermes*. All strains were shown to degrade soluble and insoluble cellulose; optimum pH for growth was 6.2–6.7 at 28°C and three strains could grow at 48°C.

Fungi are found in the gut contents [122], but seemed not to play a major role in the digestion of cellulose [37]. Interactions with wood-rotting fungi are common [57,58] and fungus-growing termites are found in the subfamily Macrotermitinae [50]. They are associated with *Termitomyces* sp., which they grow in their nests. The fungus nodules, which are consumed by the termites possess $C_1$- and $C_x$-cellulases. The consumed fungal enzymes led to the hypothesis that fungus-growing termites such a *Macrotermes natalensis* and *Microtermes subhyalinus* [92,123–126] acquire cellulases for the digestion of cellulose and feeding on cellulose depends on the ingestion of enzymes. *Macrotermes natalensis* contains $C_1$-cellulases, $C_x$-cellulases and β-glucosidases, which accomplish the cellulose breakdown. According to Martin and Martin [124], the $C_1$-cellulases found in lower and higher termites originate from protozoa, bacteria or fungi, while the other glycolytic enzymes are produced by the termites themselves. On the other hand, Veivers et al. [127] and Slaytor [37] found no evidence for a higher activity of acquired enzymes.

While Cleveland [87] and Trager [90] postulated that the gut symbionts are the source of cellulase, Yokoe [128] demonstrated for the first time that cellulases were produced by the termites themselves. Meanwhile many authors confirmed this observation [102,124–126,129–146]. The endogenous cellulases are mainly produced in the midgut and in minor portions in the foregut and the salivary glands. Termites produce $C_x$-cellulases and cellobiases, but no $C_1$-cellulases. Species where microbial or endogenous cellulases have been demonstrated are compiled in Table 3. After a critical evaluation of the obtained data on cellulose digestion in termites and cockroaches, Slaytor [37] came to the conclusion that cellulose is mainly hydrolyzed in these insects by endogenous cellulases. This view is supported by the hypothesis that also defaunated termites incorporate radioactivity in lipids when they were fed on $^{14}$C-labeled cellulose [33,147].

*Degradation of hemicellulose and other saccharides*

Hemicelluloses are digested to a high degree by termites [38,86]. Mishra [86] determined values between 49% and 78%. Xylan constitutes the main portion of hemicelluloses and in *Neotermes bosei* xylanase activity was observed [146]. In addition, termites can possess different kinds of

Table 3

Detection of cellulose degradation by microbial or endogenous cellulases in different termites

| Family | Species | Reference |
|---|---|---|
| Mastotermitidae | *Mastotermes darwiniensis* | [102] |
| Kalotermitidae | *Kalotermes flavicollis* | [84] |
| | *Neotermes bosei* | [146] |
| Hodotermitidae | *Hodotermes mossambicus* | [135,136] |
| | *Zootermopsis angusticollis* | [94,95] |
| Rhinotermitidae | *Coptotermes formosanus* | [33] |
| | *Coptotermes lacteus* | [130] |
| | *Coptotermes lacteus* | [132] |
| | *Heterotermes indicola* | [84] |
| | *Reticulitermes flavipes* | [85,147] |
| | *Reticulitermes hesperus* | [114] |
| | *Reticulitermes lucifugus* | [84] |
| | *Reticulitermes speratus* | [128,144] |
| Termitidae | *Amitermes evuncifer* | [145] |
| | *Macrotermes michaelseni* | [127] |
| | *Macrotermes mülleri* | [137,138] |
| | *Macrotermes natalensis* | [124] |
| | *Macrotermes subhyalinus* | [126,127] |
| | *Microcerotermes edentatus* | [125] |
| | *Nasutitermes ephratae* | [84] |
| | *Nasutitermes exitiosus* | [129,132] |
| | *Nasutitermes walkeri* | [129,140] |
| | *Odontotermes obesus* | [141] |
| | *Termes obesus* | [124] |
| | *Trinervitermes trinervoides* | [123,134] |

carbohydrases, such as sucrase, maltase, trehalase and raffinase [145,146]. Krishnamoorthy [148] found, among peptolytic and lipolytic enzyme activities, carbohydrases such as amylase, invertase, sucrase, maltase and laccase in *Heterotermes indicola*. β-Amylase occurs mainly in the salivary glands of *Mastotermes darwiniensis* [102]. Feeding of *Mastotermes darwiniensis* on starch resulted in the loss of the four large protozoa and also in the loss of cellulase activity in the hindgut. *Mastotermes darwiniensis* survived the starch diet for over a year. In *Neotermes bosei* chitinase activity could be found, which may be produced by the microbial symbionts. Chitin digestion plays a role during cannibalism at times of food shortage [143,149].

Only a few xylan-decomposing bacteria have been obtained from the termite gut [7,150]. A list of the isolates belonging to different genera is given in Table 2.

*Degradation of lignin*

The question of lignin degradation by termites is intriguing, since much of the termite gut is anaerobic and natural anaerobic mechanisms for lignin degradation are unknown. Conclusions based on analyses of termite faeces were conflicting as some authors reported as much as 83% lignin degradation [2,84,151–155], while others reported virtually none [85]. The controversy concerning lignin degradation was discussed by Breznak [3] and O'Brien and Slaytor [4], because it is believed that lignin is not degraded under anaerobic conditions [156]. Butler and Buckerfield [151] found that the higher termite *Nasutitermes exitiousus* readily respired $^{14}$C-labelled lignin. 14–32% and 15–16% of the $^{14}$C-label was evolved from synthetic and maize lignins, respectively, which were labelled in various positions in the polymer (methoxy; $C_2$; ring). A control was their demonstration that maximum $^{14}CO_2$ emission required the presence of live termites in the incubation vessels. Little or no $^{14}CO_2$ was evolved when the termites were removed. The specific site of degradation in the gut was not determined, nor was the involvement of gut microbes in the process. It could not be excluded wether the lignin was already inoculated by bacteria from the body surface of termites before ingestion. Data on the polymerization degree of the labelled lignin preparations are also required, because low-molecular mass lignins are degraded anaerobically without the involvement of ligninases. Butler and Buckerfield [151], however, speculated that the polymer might be degraded in the gut to smaller-molecular mass derivatives which might be taken up through the gut epithelium and oxidized aerobically by termite tissues. The assumption that oxygen is required for lignin breakdown is supported by the finding that in the oxygenated paunch the lignin degradation increases [157]. In order to approach the problem on lignin degradation, Kuhnigk et al. [34] studied recently the degradation of lignin monomers by the intestinal flora of the lower termites *Mastotermes darwiniensis* and *Reticulitermes santonensis* and of the higher termite *Nasutitermes nigriceps*. From the three termites, 53 anaerobic, facultative anaerobic and aerobic bacterial strains were iso-

lated in media containing lignin monomers and other aromatic compounds as carbon source. The isolates belonged to 20 genera (Table 4). Most of the aromatic compounds were degraded aerobically by mixed and pure cultures. Under anaerobic conditions 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 was obtained under anaerobic conditions. The results suggested that in the anaerobic hindgut the breakdown of aromatic ring systems requires oxygen, which is most probably supplied via the aerated paunch epithelium. The termite gut seems to be an anaerobic gradient system, which is constantly supplied with oxygen via the paunch epithelium. Beside stomodeal and proctodeal food exchange termites feed also on faeces. During passage of wood particles through the digestive tract they are inoculated with microorganisms. The microorganisms may start lignin breakdown aerobically near the paunch epithelium and then continue the digestion outside the termite gut. Therefore, repeated recycling of faecal material may increase the efficiency of the digestion of wood particles by a termite colony. Recently, a lignin-solublizing actinomycete was isolated from a termite gut [158], but no microorganism breaking down intact lignin has been obtained from the termite gut yet. Wood contains a large number of extractable low-molecular mass phenolic compounds, which may be used by aromatics-degrading bacteria (Table 4).

*Characteristics of microbial cellulases and xylanases*

Hydrolysis of cellulose is a complex process and has been well reviewed by Ljungdahl and Eriksson [159]. This requires the participation of at least three enzymes, viz, (a) endo-(1,4)-$\beta$-D-glucanase (E.C. 3.2.1.4) sometimes referred to as endoglucanase, carboxymethyl cellulase (CMC-ase), $C_x$-cellulase or avicelase which initiates random attack on crystalline cellulose producing cellodextrins, cellobiose and glucose (b) Exo-(1,4)-$\beta$-D-glucanase. Exoglucanases are also known as $C_1$-cellulases of which there are at least two types: (i) 1,4-$\beta$-D-glucan cellobiohydrolase (E.C. 3.2.1.91) which removes cellobiose units one by one from the non-reducing ends of cellulose chains; and (ii)

Table 4

Bacterial isolates from termites with the capability to degrade or modify aromatic compounds [34]

|  | Number | Isolate | Termite [a] |
|---|---|---|---|
| Aerobic | 1 | *Acinetobacter calcoaceticus – baumannii* group | *M. darwiniensis N. nigriceps* |
|  | 2 | *Arthrobacter*-like bacteria [b] | *N. nigriceps R. santonensis* |
|  | 3 | *Aureobacterium liquefaciens* [b] | *N. nigriceps R. santonensis* |
|  | 4 | *Bacillus firmus* [b] | *R. santonensis* |
|  | 5 | *Comamonas acidovorans* | *N. nigriceps* |
|  | 6 | *Ochrobactrum anthropi* | *M. darwiniensis N. nigriceps R. santonensis* |
|  | 7 | *Ochrobactrum*-like bacteria [a] | *N. nigriceps* |
|  | 8 | *Pseudomonas aeruginosa* | *M. darwiniensis R. santonensis* |
|  | 9 | *Pseudomonas putida* | *N. nigriceps* |
| Facultative anaerobic | 10 | *Bacillus cereus* | *R. santonensis* |
|  | 11 | *Bacillus licheniformis* | *R. santonensis* |
|  | 12 | *Citrobacter amalonaticus* [a] | *R. santonensis* |
|  | 13 | *Enterobacter aerogenes* | *M. darwiniensis* |
|  | 14 | *Enterobacter cloacae* | *R. santonensis* |
|  | 15 | *Enterobacter* sp. [b] | *R. santonensis* |
|  | 16 | *Klebsiella pneumoniae* [b] | *M. darwiniensis* |
|  | 17 | *Listeria innocua* | *R. santonensis* |
|  | 18 | *Serratia marcescens* [b] | *M. darwiniensis* |
|  | 19 | *Serratia ficaria* [b] | *R. santonensis* |
| Strictly anaerobic | 20 | *Clostridium sporogenes* [b] | *M. darwiniensis* |

[a] M, *Mastotermes*; N, *Nasutitermes*; R, *Reticulitermes*.

[b] Preliminary identification.

1,4-$\beta$-glucan glucohydrolase (E.C. 3.2.1.74) which removes glucose molecules one by one from non-reducing ends of the chain; and (iii) $\beta$-(1,4)-glucoside glucohydrolase also known as $\beta$-glucosidase or cellobiase which hydrolyses cellobiose to glucose. $\beta$-Glucosidases share common substrates with glucohydrolases, but differ from them in four respects, viz:

(a) they have greater activity on dimers than on higher cello-oligosaccharides and polymeric substrates,

(b) they retain anomeric configuration during hydrolysis,

(c) they are more sensitive to inhibition by gluconolactone, and

(d) they hydrolyse $\beta$-1,1; $\beta$-1,2; $\beta$-1,3; $\beta$-1,4 and $\beta$-1,6 linkages whereas exoglucanases are highly specific for $\beta$-1,4 linkages. The $\beta$-glucohydrolases were reported to be specific for hydroxyl group at $C_4$ [160].

Hemicellulose, whose major constitutent is xylan, is analogous to that of cellulose, but composed of D-xylose instead of D-glucose. Complete breakdown of xylan requires the action of endo-1,4-$\beta$-xylanase, which attacks randomly the polysaccharide back, $\beta$-xylosidase, which hydrolyses xylobiose to D-xylose, and arabinosidase. Microorganisms often produce more than one type of xylanase. These enzymes differ in substrate binding site and consequently in the number of unsubstituted xylose units in series required in the polysaccharide backbone. Several xylanases occur in multiple forms as a result of differential mRNA processing partial proteolysis or differences in the degree of amidation and glycosylation [161].

Exoxylanases have not been reported in bacteria until today. Though reported from few fungi, claims were doubtful as the enzyme was not purified to homogeneity. Cellulolytic and hemicellulolytic activities of some strains isolated from termite mound soil and gut are summarized in Tables 1 and 2.

Cellulases or hemicellulases [150] were extracellularly produced in many microbes living together with termites. These enzymes were generally inducible [5–7]. However, *Bacillus cereus* and *Serratia marcescens* isolated from the hindgut of the termite *Reticulitermes herperus* produced carboxymethylcellulase in the absence of any cellulosic materials which indicates that the enzyme must be constitutive [114]. Furthermore, addition of glucose, the end product of cellulose hydrolysis, to cultures of *Bacillus cereus* increased production of CMCase without increasing the bacterial protein. This observation led us to conclude that synthesis and activity of CMCase from this isolate was not subject to end product regulation. Similarly, in another species of *Bacillus* isolated from mound soil of the termite *Odontotermes obesus*, glucose enhanced endoglucanase synthesis and activity [48], while xylose, one of the end products of hemicellulose hydrolysis, repressed endoxylanase synthesis in two specis of *Bacillus* [6,48].

Generally, bacterial cellulases and xylanases are active in neutral pH while enzymes of fungal and actinomycetes origin are active in acidic range. The bacterial enzymes are active at higher temperatures ranging from 40 to 60°C (Tables and 2). Reports regarding the purification and properties of these enzymes from microbes living in association with termites are scarce except for those from our laboratory as is evident from the Tables 1 and 2. Endoxylanase from *Bacillus thermoalkalophilus* was purified to homogenity and the molecular mass of the enzyme was found to be 19.5 kDa by SDS-PAGE [6]. The enzyme was thermostable ($t_{1/2}$ at 60°C was 2.5 h). This strain could find potential use in biotechnological applications due to its unique combination of depolymerizing properties. The strain was a good producer of xylanase and grew optimally at 60°C $\pm$ 3°C and pH 9.0. This combination has wide use in industry for

(a) minimizing contamination,

(b) avoiding expensive cooling systems in fermentors,

(c) as a source of thermostable enzymes, and

(d) increasing overall recovery of products [6].

## Perspectives of exploitation of cellulases and hemicellulases

Industrial application of cellulase and hemicellulases is plagued by such problems as catalytic

constant towards insoluble substrates, low thermostability, high degree of product inhibition, and low adsorption coefficient towards the substrates. Klyosov [162] in his thought-provoking paper on these aspects presented data and argued in favour of cellulases with predetermined molecular characteristics which he called 'third-generation cellulases'. To find a source of such third-generation cellulase a new strategy for screening is required with emphasis not only on 'overall' activity, but on the above characteristics which have a direct implication in biotechnological application. Information on these characteristics of xylanases is very scarce, but they may also behave similarly as cellulases. Enhanced cellulase and xylanase synthesis can be achieved by three means: (i) optimization of cultural parameters; (ii) strain improvement; and (iii) genetic engineering.

There has been a tremendous awareness of the value of organic wastes as inexpensive sources of energy and plant nutrients. These must be fully exploited and used carefully and effectively. Processing of agricultural and forestal wastes is attractive since they hold the potential of producing indefinitely renewable clean energy sources and at the same time solving a waste management problem. Therefore, it is imperative to understand the physiology and biochemistry of carbohydrate-degrading processes and the intricate interspecific actions which characterize this microbial-induced process in nature. Enzymatic hydrolysis of lignocellulosic materials is preferred to acid hydrolysis as this catalyst is non-corrosive, environmentally non-hazardous, potentially reusable and offers an efficiency of more than 90%. Work on enzyme catalyzed hydrolysis should be accelarated as the product is a potential source of fuel and economically important chemicals. Cellulases and xylanases are of commercial importance and problems associated with them are comparable. Some of the major aspects which need urgent attention are:

1. Development of microbial strains having high rates of enzyme production.
2. Thermal stabilization of hydrolytic enzymes.
3. Development of methods to recover and reuse cellulase and xylanase from the fermenters.
4. Immobilization of bacterial cells and the hydrolytic enzymes to promote continuous and repeated use in large scale industrial applications. This will ensure cost efficient and higher yield of more pure products. Thus, not only resources could be conserved, but pollution could be minimized.
5. Screening of potential and efficient lignocellulolytic bacteria.

Both termite soil and termite gut bacteria play an important role in polymer depolymerization. Gut bacteria have the capacity to degrade cellulolytic and hemicelulolytic materials more efficiently. Most of the isolates have all the three enzymes which can be used as a tool for bioconversion of cellulolytic waste materials which can be used for biogas production. From the eyes of a microbiologist there is a great scope for improving the depolymerization processes and the termite microniches are potential source for boosting the research.

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