International Journal of Academic Research and Development
ISSN: 2455-4197
Impact Factor: RJIF 5.22
www.academicsjournal.com
Volume 3; Issue 2; March 2018; Page No. 1730-1735

Termites: A classical system of insect evolution displaying mutualistic cooperation with multiple Symbionts

Shewane Bishnoi
Haryana Agricultural University, Hisar, Haryana, India

Abstract

Termites (*Dictyoptera, Isoptera*) comprise of approx. 2800 species, roughly divided into socalled lower and higher termites with maximum diversity found in tropical and sub-tropical regions, close to the equator, while fewer species live at higher altitudes. Lower termites harbor a dense and diverse population of prokaryotes and flagellated protists (single-cell eukaryotes) in their gut. Higher termites comprise only one apical family (Termitidae) but more than three-quarters of all termite species. While they also harbor a dense and diverse array of prokaryotes, higher termites typically lack flagellated protists. Cellulose digestion in termites depends upon mutualastic interaction with a variety of symbionts present in gut of termites. These microorganisms play a crucial role in the nutritional physiology of termites. Diverse microorganisms inhabit the intestinal tracts of all termite groups at a high density of 106–107 cells per µl of gut volume. The ability of Isopteras to digest complex molecules is mostly attributed to the action of these intestinal microbes. Comprehensive study of bacterial diversity in termites showed that microbes from all known domains, i.e., *Bacteria*, *Archaea*, and *Eucarya* were represented in gut symbionts of termites which include many cultured and uncultured species. The midgut contains a purely prokaryotic microbiota, whereas the hindgut is colonized by prokaryotes and special single-celled eukaryotes. As a result of the microbial activities, high concentrations of fermentation products accumulate in this system The enlarged hindgut, also known as paunch, is the "hotspot" of the microbial activity. In the hindgut, the symbiotic microbiota polymerizes cellulose and hemicellulose, which are further fermented to short-chain fatty acids; to be used as the main energy source by the host termite. Apart from their importance in the nutrition of the termites, the microorganisms in the termite may also include novel species with potential for exploitation in biotechnology. They may have large industrial applications as they are potentially good resource of functional genes which can be exploited using classical enrichment culture technique and recent metagenomic approach. Recent researches have shown that termites and its symbionts have not only cellulolytic or lignin decomposition activity but also aromatic hydrocarbons degradation. These functions would be useful for biomass utilization, environmental remediation, and fine-chemicals production.

Keywords: *Dictyoptera, lsoptera*, symbiont

Introduction

Insecta is the most diversified and populated group of organisms. There are more insects in one square mile of rural land than there are human beings in the world. Termites (Commonly known as White Ants) are terrestrial, eusocial insects, with differentiated castes, complex, coordinated group behaviours of nest construction and foraging, the evolution of which are poorly known compared with other eusocial groups. They constitute a large part of total insect population. It is estimated that around 250 trillion termites are alive at any given time with numbers exceeding 6000 individuals per m^2, whose weight is around 4 billion tons (Lee and Wood, 1971) [29]. It is said that about 700 kg of termites exist for every human on the planet. As some of the most abundant and efficient lignocellulose decomposers on the planet, termites tremendously impact lignocellulose biorecycling, and rank as one of the most important "ecosystem engineers" (Shaomei *et al.*, 2013) [53]. Termites thrive on recalcitrant materials like wood and soil and thus play important roles in global carbon recycling and also in damaging wooden structures. Termites are important arthropods involved in the biodegradation of complex substances like cellulose and hemicellulose found in plant materials (Wood and Sands, 1978; Brune, 1998) [69, 11]. Termites owe their success to their ability to extract nutrients from lignocellulose (a major component of wood) with the help of gut-dwelling symbionts (Raychoudhury *et al.*, 2013) [49]. Therefore, these insects are important not only for their roles in carbon turnover in the environment, but also as potential sources of biochemical catalysts for converting wood into biofuels (Warnecke *et al.*, 2007) [67]. Recently, termites have attracted interest through the architecture of the mounds of *Macrotermes michaelseni* (Turner, 2000) [65], which are being viewed as a possible model for cheap and energy efficient buildings (Mackenzie *et al*., 2007) [33].

Due to pest nature, they are very important commercially too. Only one species *Coptotermes formosanus* is responsible for more than one billion US$ loss per year in the USA for preventive and remedial treatment (Lax and Osbrink, 2003) [28]. Worldwide, approximately 2700 species of termites are present (Kambhanpati and Eggleton, 2000) [27]. Their total ecological and commercial value can thus be predicted.

Phylogeny of Termites

Termites (order: Isoptera) are phylogenetically closely related to cockroaches (Deitz *et al*., 2003; Inward *et al*., 2007) [17, 26]. Depending on the presence/absence of cellulolytic flagellate protozoa in the hindgut, termites are distinguished into phylogenetically lower and higher termites, respectively. While higher termites can be fungus-cultivating, wood-feeding or soil or humus-feeding (Noirot, 1992; Abe *et al*., 2000) [38, 1], lower termites are strictly wood-feeding (Cleveland, 1926; Waller and La Fage, 1987) [15, 66]. Termites are divided among seven families, six of which belong to lower termites (Mastotermitidae, Kalotermitidae, Hodotermitidae, Termopsidae, Rhinotermitidae, Serritermitidae) and one belonging to higher termites (Termitidae). Termitidae alone contain approximately 85% of all known genera of termites (Kambhanpati and Eggleton, 2000) [27].

Termites with cockroaches and mantids form a well established lineage, the Dictyoptera. Termites are generally known as order Isoptera. However, in recent times most phylogenetic studies have shown their molecular relatedness to woodroaches and hence they are now considered as social cockroaches (Inward *et al*., 2007) [26]. Currently, a taxonomic rank of "Isoptera" is controversial, since Inward *et al*. (2007) [26] proposed to downgrade the order "Isoptera" to the family "Termitidae" within the order Blattaria on the basis of their molecular phylogenetic research, which is already in use to describe an apical lineage of termites (higher termites). However, Eggleton *et al*. (2007) [26] suggested the name Termitoidea as an epifamily to be replaced with Isoptera within Blattaria but Lo *et al*. (2007) suggested to keep Isoptera as unranked taxon in Blattaria. One other recent study (Legendre *et al.,* 2008) [30] also supports the monophyly of Mantodea and the paraphyly of Blattaria with respect to termites, with Cryptocercus as sister taxon of termites. More comprehensive phylogenetic analysis is hence needed to conclude an actual taxonomic position of termites.

Termites: Insects with Multiple symbionts

Despite their small size, termites harbor an abundant and astonishingly diverse intestinal microbiota. The relationship between the termites and their gut fauna is a textbook example of symbiosis. Termites and their gut microbes engage in fascinating dietary mutualisms. Less is known about how these complex symbioses have evolved after first emerging in an insect ancestor over 120 million years ago (Zhang and Leadbetter, 2012) [75]. Most of these microbes are thought to depend on, and to have co-speciated with, their host and each other for millions of years (Rosenthal *et al.,* 2011) [50]. The symbionts converts a substantial portion of dietary components to microbial components to microbial fermentation products, which are eventually reabsorbed by the intestinal components (Brune, 2013) [10]. Most of the termites contain a vast variety of fauna in their gut including bacteria, protists which include a variety of not-yet-cultured intestinal microorganisms, details of which are given later. While it is well established that the eukaryotic flagellates play a major role in the degradation of lignocellulose, much less is known about the identity and function of the prokaryotic symbionts associated with the flagellates (Strassert *et al.,* 2010) [60]. Termite symbionts has lead to ecological niche expansion of termites, which allows termites to utilize a food source and conveys resistance to environmental stresses, predators, or parasites (Hussa and Goodrich-Blair, 2013) [24]. In these contexts, we expect that future explorations of termite genomes will contribute not only to our knowledge on fundamental biology but also to applications such as pest control, bioengineering of lignocellulolytic enzymes, and developing culturing methodology for difficult-to-culture microbes (Matsui *et al*, 2009) [34].

Termite hindgut: a structured environment

The gut of termite consists of foregut, midgut and hindgut. The enlarged hindgut, also known as paunch, is considered as the "hotspot" of the microbial activity (Breznak, 2000; Brune, 2005). The gut contains large variety of fauna as it is divided into four fundamentally different microhabitats: (i) the midgut (contains host enzymes), (ii) the wall of the hindgut paunch (continuous influx of oxygen), (iii) the hindgut fluid (unattached bacteria and protozoa), (iv) the symbiotic protozoa. However, also the relatively simple guts of wood-feeding termites, which possess only a single hindgut dilatation, harbor an enormous number of different microbial morphotypes (Breznak and Pankratz, 1977; To *et al*., 1980; Czolij *et al*., 1985) and phylotypes (e.g., Ohkuma and Kudo, 1996; Lilburn *et al*., 1999; Hongoh *et al*., 2003). Very diverse and uneven bacterial population is found in different gut parts (>200 ribotypes) and comprise representatives of several phyla, including *Firmicutes* (mainly clostridia, streptococci, and *Mycoplasmatales*-related clones), *Bacteroidetes*, *Spirochaetes*, and a number of *Proteobacteria* (Yang *et al*., 2004).

Microhabitats in gut and the microbial interactions within and between the habitat boundaries create a variety of niches for the intestinal microbiota (Brune and Friedrich, 2000; Tholen and Brune, 2000) [32].

Bacteria create a microoxic periphery (50-200 μm) around the anoxic centre in the paunch by utilizing oxygen in respiration. Since termite guts have a high surface area per unit volume, it seems that more than 40% of the paunch is oxic. Oxygen and Hydrogen (produced by the anaerobic parabasalid flagellates) are important metabolites in the paunch (Brune, 1998). Microelectrode measurements showed steep radial gradients O_2 and H_2 partial pressure (whose concentration varies among different termite species; Pester and Brune, 2007) [47] which create fundamentally different microhabitats and necessitate the differentiation between the microoxic gut wall and the anoxic lumen (Brune *et al*., 1995; Ebert and Brune, 1997; Brune, 1998) [14, 18]. Some studies show that the intestinal carbon fluxes are also controlled by the spatial organization of the different microbial guilds (Tholen and Brune, 2000) [32].

Faunal symbionts: diversity and functions

Termites thrive on plant biomass in which the major constituents are cellulose, hemicellulose (*i.e.* noncellulosic carbohydrates), and lignin. A termite's gut hosts a fairly efficient commune, almost 90 percent of the cellulose in the wood it eats, is turned into its ultimate fuel, acetate (Nadin, 2007) [35].

Termite gut is a rich reservoir of novel and diverse microorganisms which has been passed down from termite to termite for over 100 million years (Nadin, 2007) [35]. A variety of aerobic, microaerobic, and anaerobic microbes have been isolated from termite guts (Tholen and Brune, 2000) [32]. For understanding of entire structure of digestive symbiosis of termites, molecular-based comprehensive surveys of microbial diversity in termite guts have been applied to some termite species (Ohkuma and Kudo, 1996; Shinzato *et al*., 2005, 2007; Hongoh *et al*., 2003; Hongoh *et al*., 2005).

The diversity of microbes in the guts of diverse termites includes bacteria, fungi and protozoa (Brune, 1998), which have been characterized and shown to include many uncultured species (Ohkuma and Kudo, 1996; Ohkuma and Kudo, 1998; Schmitt- Wagner *et al*., 2003; Hongoh *et al*., 2005; Shinzato *et al*., 2005). This includes about 250 different species of microbes. The hindgut of lower termites is filled with numerous species of oxygen-sensitive flagellates (Hungate, 1955). The fungus-cultivating termites are also reported to harbor dense populations of bacteria and archaea (Anklin-Mühlemann *et al*., 1995; Brauman *et al*., 2001) [3]. Termites and gut bacteria, together with *Termitomyces* fungi, form a symbiotic complex (Bignell, 2000); however, the relationship within the complex and the mechanism of cellulose digestion remain unclear. However, Firmicutes form the most part of the bacterial community structure of the fungus comb (77.5% of phylotypes). Bacteroidetes (26.7%) are the second major constituent in the gut library, and third is Proteobacteria (15%).

Flagellates

A single termite hindgut can contain up to 600,000 flagellates. 430 described species of flagellates unique to lower termites and wood-feeding cockroach *Cryptocercus* were listed by Yamin (1979) which belong to Parabasalia (Cristamonadida, Spirotrichonymphida, Trichomonadida, and Trichonymphida) and Preaxostyla (Oxymonadida) (Adl *et al*., 2005). They are host-specific and very important for celloulose degradation to fermentatively decompose cellulose to carbon dioxide, hydrogen and acetate, the latter is absorbed by termite as their energy source (Breznak and Brune, 1994; Radek, 1999). Degradation of lignocellulose by termites demands a dual cellulase system, comprising cellulases of both termite and flagellate origin (Nakashima *et al*., 2002; Tokuda *et al*., 2007). Parabasalids (phylum Parabasalia) are anaerobic flagellated protists which contain at least one parabasal apparatus consisting of a parabasal body (Golgi complex) and a parabasal filament. Physiological studies on termite gut flagellates are hindered, as none of the flagellates are in permanent culture. Termite gut flagellates *Trichomitopsis termopsidis* and *Trichonympha sphaerica* were temporarily cultured (Yamin and Trager, 1979; Yamin, 1980; Yamin, 1981; Odelson and Breznak, 1985a, 1985b). The closest cultivated representative of the (termite gut) parabasalid flagellates is the human pathogen *Trichomonas vaginalis* (Steinbüchel and Müller, 1986).

Bacterial symbionts of flagellates

Cellulolytic bacteria have been frequently isolated both from lower and higher termites (Hungate, 1946; Thayer, 1976; Pasti and Belli, 1985; Paul *et al*., 1986; Saxena *et al*., 1993; Wenzel *et al*., 2002) and play a significant role both in lignocelluloses digestion and termite nutrition. 90% of the bacteria present in the hindgut are symbionts of flagellates (Berchtold *et al*., 1999). These include *Cytophaga-Flexibacter-Bacteroides*, *Proteobacteria*, *Firmicutes*, a group distantly related to *Planctomycetes*, *Anaerobaculum-Thermoanaerovibrio* and spirochetes (Mackenzie *et al*. 2007) [33].

It was revealed that distinct bacteria inhabited each of the distinct gut compartments, and most of the bacteria are considered novel species (Schmitt-Wagner *et al*., 2003). The bacterial contributions on cellulose degradation in termite guts are thought to be significant particularly in higher termites lacking protozoan symbionts from the guts, in which lignocellulose is decomposed by the enzymes provided by both bacteria and the termite.

The bacterial microbiota has remained a black box due to difficulties in cultivation of most of these bacteria. Phylogenetic positions of bacterial symbionts can be determined using culture-independent techniques (Stingl *et al*., 2005; Ohkuma *et al*., 2007). The fullcycle- rRNA approach has proven to be a useful to localize bacterial symbionts (Noda *et al*., 2003; Stingl *et al*., 2004; Stingl *et al*., 2005). Bacteria in termite belong to TG-1 phylum (Endomicrobia), Firmicutes, Bacteroidetes, Spirochaetes, Proteobacteria and many other phyla. Termite Group 1 is the best studied symbiont candidate phylum having diverse cellulolytic protozoa in termite guts (Stingl *et al*., 2005; Ohkuma *et al*., 2007; Ikeda-Ohtsubo *et al.*, 2007). The second-largest group of bacteria found in termite guts (27%) are affiliated with the *Firmicutes.* These are low G+C gram-positive bacteria (Mackenzie *et al*., 2007) [33]. *Bacteroidetes* (18%) are the third most abundant bacteria in termite gut and are quite diverse. Spirochetes are also have great populations in the gut community, which account for as many as 50% of all prokaryotes present in some termite guts (Stingl, 2004; Paster *et al*., 1996). However, due to the difficulty of cultivation, their physiological role in the hindgut ecosystem and the contribution to host termite have long been unidentified. They are capable of nitrogen fixation. 6% of gut bacteria of termite are *Proteobacteria* (Yang *et al*., 2004). Less than 2% of the bacteria belong to other phyla with the closest relatives from bacteria from the oral cavity of humans.

Conclusion

Termites or their derived symbionts posses a very high diversity and can be viewed as source of enzymes including cellulolytic (cellobiose dehydrogenase, carboxymethyl cellulase, and xylanase), lignin and lignin-derived compounds degrader, aromatic compounds degrader, petroleum-derived organic compounds degrader and most importantly plastics degrader (Matsui *et al*., 2009) [34]. Nitrogen fixing bacteria could be used for the fertilizing soil for agriculture. Termites also produce some antibiotics. Termite lysozyme (acting as a proteinaceous pheromone for egg recognition) can be used be used as a tool for termite control (Matsui *et al*., 2009) [34]. Higher termites, as feed upon diverse food resources are thus expected to provide attractive gene sources to utilize diverse natural substances. Indeed, the first metagenomic analysis of the hindgut microflora of a higher termite brought a new insight into the microbial metabolisms and relevant functional genes (Warnecke *et al*., 2007) [67] which can be utilized further. Much research can be done on *Zootermopsis*, an especially handy termite, as it can be maintained in lab its internal microbes are easily cultivated (Nadin, 2007) [35]. As, termites cellulose and antifungal gene are patented, more patents can be expected in future (Matsui *et al*., 2009) [34]. In these contexts, we expect that future explorations of termite genomes will contribute not only to our knowledge on fundamental biology but also to applications such as pest control, bioengineering of lignocellulolytic enzymes, and developing culturing methodology for difficult-to-culture microbes.

References

1. Abe T, Bignell, DE, Higashi M. (eds). Termites: Evolution, Sociality, Symbiosis, Ecology. Kluwer Academic Publishers, Dordrecht, 2000.
2. Adl SM, Simpson AG, Farmer MA. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. J Eukaryot Microbiol. 2005; 52:399-451.
3. Anklin-Mühlemann R, Bignell DE, Veivers PC, Leuthold RH, Slaytor M. Morphological, microbiological and biochemical studies of the gut flora in the fungus-growing termite *Macrotermes subhyalinus*. J. Insect. Physiol. 1995; 41:929-940.
4. Berchtold M, Chatzinotas A, Schönhuber W, Brune A, Amann R, Hahn D, *et al.* Differential enumeration and in situ localization of micro-organisms in the hindgut of the lower termite *Mastotermes darwiniensis* by hybridization with rRNA-targeted probes. *Arch. Microbiol.* 1999; 172:407-416.
5. Bignell DE. Symbiosis with fungi. *Termites: Evolution Sociality, Symbiosis, Ecology* (eds. T. Abe, D.E. Bignell & M. Higashi). Kluwer Academic Publishers, Holland. 2000; pp. 289-306.
6. Brauman A, Dore J, Eggleton P, Bignell D, Breznak JA, Kane MD. Molecular phylogenetic profiling of prokaryotic communities in guts of termites with different feeding habits. *FEMS Microbiol. Ecol*. 2001; 35:27-36.
7. Breznak JA. Ecology of prokaryotic microbes in the guts of wood- and litterfeeding termites. In Abe, T. et al. (eds.), Termites: Evolution, Sociality, Symbiosis, Ecology. Kluwer Academic Publishers, Dordrecht. 2000; pp. 209-231.
8. Breznak JA, Brune A. Role of microorganisms in the digestion of lignocellulose by termites. *Annu. Rev. Entomol*. 1994; 39:453-487.
9. Breznakj A, Pankraatzh S. *In situ* morphology of the gut microbiota of wood-eating termites *(Reticulitermes Jlavipes* Kollar and *Coptotermes formusanus* Shiraki). *Applied and Environmental Microbiology*. 1977; 33:406-426.
10. Brune A. Symbiotic Associations between Termites and Prokaryotes. *The Prokaryotes*. 2013; 545-577.
11. Brune A. Termite guts: the world's smallest bioreactors. *Trends. Biotechnol.* 1998; 16:16-21.
12. Brune A. Symbiotic associations between termites and prokaryotes. In: Dworkin, M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E. (eds), The prokaryotes: an online electronic resource for the microbiological community, 3rd edn, release 3.20. Springer, Berlin Heidelberg New York, 2005.
13. Brune A, Friedrich M. Microecology of the termite gut: structure and function on a microscale. *Curr Opin Microbiol.* 2000; 3(3):263-269.
14. Brune A, Emerson D, Breznak JA. The termite gut microflora as an oxygen sink: microelectrode determination of oxygen and pH gradients in guts of lower and higher termites. *Appl Environ Microbiol*. 1995; 61:2681-2687.
15. Cleveland LR. Symbiosis among animals with special reference to termites and their intestinal flagellates. *Quart. Rev. Biol.* 1926; 1:51-64.
16. Czolij R, Slaytor M, O'Brien RW. Bacterial flora of the mixed segment and the hindgut of the higher termite *Nasutitermes exitiosus* Hill (Termitidae, Nasutitermitinae). *Appl Environ Microbiol*. 1985; 49:1226-1236.
17. Deitz LL, Nalepa C, Klass KD. Phylogeny of the Dictyoptera re-examined (Insecta). *Entomol. Abhandl.* 2003; 61:69-91.
18. Ebert A, Brune A. Hydrogen concentration profiles at the oxic-anoxic interface: a microsensor study of the hindgut of the wood-feeding lower termite *Reticulitermes flavipes* (Kollar). *Appl. Environ. Microbiol.* 1997; 63:4039-4046.
19. Eggleton P, Beccaloni G, Inward D. Response to Lo *et al. Biol Lett.* 2007; 3:564-565.
20. Hongoh Y, Deevong P, Inoue T. Intra- and interspecific comparisons of bacterial diversity and community structure support coevolution of gut microbiota and termite host. *Appl Environ Microbiol.* 2005; 71:6590-6599.
21. Hongoh Y, Deevong P, Inoue T, Moriya S, Trakulnaleamsai S, Ohkuma M, *et al*. Intra- and Interspecific Comparisons of Bacterial Diversity and Community Structure Support Coevolution of Gut Microbiota and Termite Host. Applied and Environmental Microbiology. 2003; 71:6590-6599.
22. Hungate RE. Studies on cellulose fermentation: II. An anaerobic cellulose-decomposing actinomycete, *Micromonospora propionici*, n. sp. J Bacteriol. 1946; 51:51-56.
23. Hungate RE. Mutualistic intestinal protozoa. In Hutner, S.H.; Lwoff, A. (eds.), Biochemistry and Physiology of Protozoa, Academic Press, New York. 1955; 2:159-199.
24. Hussa E, Goodrich-Blair H. It Takes a Village: Ecological and Fitness Impacts of Multipartite Mutualism. *Annu. Rev. Microbiol*. 2013; 67:161-178.
25. Ikeda-Ohtsubo W, Desai M, Stingl U, Brune A. Phylogenetic diversity of 'Endomicrobia' and their specific affiliation with termite gut flagellates. Microbiology. 2007; 153:3458-3465.
26. Inward D, Beccaloni G, Eggleton P. Death of an order: a comprehensive molecular phylogenetic study confirms that termites are eusocial cockroaches. *Biol. Lett*. 2007; 3(3):331-335.
27. Kambhanpati S, Eggleton P. Taxonomy and phylogeny of termites. In T. Abe, D.E. Bignell, and M. Higashi, Eds. Termites: Evolution, sociality, symbioses, ecology. Kluwer Academic Publisher. 2000; pp. 1-23.
28. Lax AR, Osbrink WL. United States Department of Agriculture- Agriculture Research Service research on targeted management of the Formosam subterranean

termite *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae). Pest Management Science. 2003; 59:788-800.

29. Lee KE, Wood TG. Termites and Soils. Academic Press, London, 1971.
30. Legendre F, Whiting MF, Bordereau C, Cancello EM, Evans TA, Grandcolas P. The phylogeny of termites (Dictyoptera: Isoptera) based on mitochondrial and nuclear markers: Implications for the evolution of the worker and pseudergate castes, and foraging behaviours. Molecular Phylogenetics and Evolution. 2008; 48:615-627.
31. Lilburn TG, Schmidt TM. Breznak JA. Phylogenetic diversity of termite gut spirochaetes. *Environ Microbiol*. 1999; 1:331-345.
32. Lo N, Engel MS, Cameron S, Nalepa CA, Tokuda G, Grimaldi D, *et al.* Save Isoptera: A comment on Inward *et al. Biol Lett*. 2007; 3:562-563.
33. Mackenzie LM, Muigai AT, Osir EO, Lwande W, Keller M, Toledo G, *et al.* Bacterial diversity in the intestinal tract of the fungus cultivating termite *Macrotermes michaelseni* (Sjöstedt). African Journal of Biotechnology. 2007; 6:658-667.
34. Matsui T, Tokuda G, Shinzato N. Termites as Functional Gene Resources. Recent Patents on Biotechnology. 2009; 3:10-18.
35. Nadin E. For the love of Termites. Engineering and Science. 2007; 2:24-31.
36. Nakashima K, Watanabe H, Saitoh H, Tokuda G, Azuma JI. Dual cellulose digesting system of the wood-feeding termite, *Coptotermes formosanus* Shiraki. *Insect Biochem. Mol. Biol.* 2002; 32:777-784.
37. Noda S, Ohkuma M, Yamada A, Hongoh Y, Kudo T. Phylogenetic position and in situ identification of ectosymbiotic spirochetes on protists in the termite gut. *Appl. Environ. Microbiol.* 2003; 69:625-633.
38. Noirot C. From wood to humus feeding: an important trend in termite evolution. In Billen J. (ed.), Biology and evolution of social Insects, Leuven University Press, Leaven. 1992; pp. 107-119.
39. Odelson DA, Breznak JA. Nutrition and growth characteristics of *Trichomitopsis termopsidis*, a cellulolytic protozoan from termites. *Appl. Environ. Microbiol.* 1985a; 49:614-621.
40. Odelson D, Breznak JA. Cellulase and other polymer-hydrolyzing activities of *Trichomitopsis termopsidis*, a symbiotic protozoan from termites. *Appl. Environ. Microbiol.* 1985b; 49:622-626.
41. Ohkuma M, Kudo T. Phylogenetic diversity of the intestinal bacterial community in the termite *Reticulitermes speratus*. Appl Environ Microbiol. 1996; 62:461-468.
42. Ohkuma M Kudo T. Phylogenetic analysis of the symbiotic intestinal microflora of the termite *Cryptotermes domesticus*. FEMS Microbiol. Lett. 1998; 164:389-395.
43. Ohkuma M, Sato T, Noda S, Ui S, Kudo T, Hongoh Y. The candidate phylum 'Termite Group 1' of bacteria: phylogenetic diversity, distribution, and endosymbiont members of various gut flagellated protists. *FEMS Microbiol Ecol*. 2007; 60:467-476.
44. Paster BJ, Dewhirst FE, Cooke SM, Fussing V, Poulsen LK, Breznak JA. Phylogeny of not-yet-cultured spirochetes from termite guts. Appl Environ Microbiol. 1996; 62:347-352.
45. Pasti MB, Belli ML. Cellulolytic activity of actinomycetes isolated from termites (Termitidae) gut. *FEMS Microbiol Lett*. 1985; 26:107-112.
46. Paul J, Sarkar A, Varma A. *In vitro* studies of cellulose digesting properties of *Staphylococcus saprophyticus* isolated from termite gut. *Curr Sci.* 1986 55:710-714.
47. Pester M, Brune A. Hydrogen is the central free intermediate during lignocelluloses degradation by termite gut symbionts. *ISME J*. 2007; 1:551-565.
48. Radek R. Flagellates, bacteria, and fungi associated with termites: diversity and function in nutrition – a review. *Ecotropica*. 1999; 5:183-196.
49. Raychoudhury R, Sen R, Cai Y, Sun Y, Lietze UV, Boucias DG, *et al*. Comparative metatranscriptomic signatures of wood and paper feeding in the gut of the termite *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). *Insect Molecular Biology*. 2013; 22(2):155-171.
50. Rosenthal AZ, Matson EG, Eldar A and Leadbetter JR. RNA-seq reveals cooperative metabolic interactions between two termite-gut spirochete species in co-culture. The ISME Journal. 2011; 5:1133-1142.
51. Saxena S, Bahadur J, Varma A. Cellulose and hemicelluloses degrading bacteria from termite gut and mound soils of India. *Indian J Microbiol.* 1993; 33:55-60.
52. Schmitt-Wagner D, Friedrich MW, Wagner B, and Brune A. Phylogenetic diversity, abundance, and axial distribution of bacteria in the intestinal tract of two soil-feeding termites (*Cubitermes* spp.). Appl. Environ. Microbiol. 2003; 69:6007-6017.
53. Shaomei H, Ivanova N, Kirton E, Allgaier M, Bergin C, Scheffrahn RF, *et al.* Comparative Metagenomic and Metatranscriptomic Analysis of Hindgut Paunch Microbiota in Wood- and Dung-Feeding Higher Termites. Plos One. 2013; 8(4):1-14.
54. Shinzato N, Muramatsu M, Matsui T, Watanabe Y. Molecular phylogenetic diversity of the bacterial community in the gut of the termite *Coptotermes formosanus*. *Biosci Biotechnol Biochem*. 2005; 69:1145-1155.
55. Shinzato N, Muramatsu M, Matsui T, Watanabe Y. Phylogenetic analysis of the gut bacterial microflora of the fungus-growing termite *Odontotermes formosanus*. *Biosci Biotechnol Biochem.* 2007; 71:906-915.
56. Steinbüchel A, Müller M. Anaerobic pyruvate metabolism of *Tritrichomonas foetus* and *Trichomonas vaginalis* hydrogenosomes. *Mol. Biochem. Parasitol.* 1986; 20:57-65.
57. Stingl U, Radek R, Yang H, Brune A. Endomicrobia: Cytoplasmic symbionts of termite gut protozoa form a separate phylum of prokaryotes. *Appl Environ Microbiol*. 2005; 71:1473- 1479.
58. Stingl U. Termite gut flagellates and their bacterial symbionts: Phylogenetic analysis and localization *in situ* Ph. D. Dissertation. 2004; pp. 1-188.

59. Stingl U, Radek R, Yang H, Brune A. *Endomicrobia*: cytoplasmic Symbionts of Termite Gut Protozoa Form a Separate Phylum of Prokaryotes. Applied and environmental Microbiology. 2004; 71:1473-1479.
60. Strassert JFH, Desai MS, Radek R, Brune A. Identification and localization of the multiple bacterial symbionts of the termite gut flagellate *Joenia annectens. Microbiology*. 2010; 156:2068-2079.
61. Thayer DW. Facultative wood-digesting bacteria from the hind-gut of the termite *Reticulitermes hesperus*. *J Gen Microbiol.* 1976; 96:287-296.
62. Tholen A, Brune A. Impact of oxygen on metabolic fluxes and in situ rates of reductive acetogenesis in the hindgut of the woodfeeding termite *Reticulitermes flavipes*. *Environ Microbiol.* 2000; 2:436-449.
63. To LP, Margulis L, Chase D, Nutting WL. The symbiotic microbial community of the Sonoran desert termite: *Pterotermes occidentis*. *Biosystems*. 1980; 13:109-137.
64. Tokuda G, Watanabe H, Lo N. Does correlation of cellulase gene expression and cellulolytic activity in the gut of termite suggest synergistic collaboration of cellulases?. *Gene.* 2007; 401:131-134.
65. Turner JS. Architecture and morphogenesis in the mound of *Macrotermes michaelseni* (Sjöstedt) (Isoptera: Termitidae, Macrotermitinae) in northern Namibia. *Cimbebasia*. 2000; 16:175.
66. Waller DA, La Fage JP. Nutritional ecology of Termites. In J. F. Slansky and J. G. Rodriguez, Eds. Nutritional ecology of insects, mites, spiders and related invertebrates. Wiley-interscience. 1987; pp 487-532.
67. Warnecke F, Luginbuhl P, Ivanova N. Metagenomic and functional analysis of hindgut microbiota of a wood-feeding higher termite. *Nature*. 2007; 450:560-565.
68. Wenzel M, Schonig I, Berchtold M, Kampfer P, König H. Aerobic and facultatively anaerobic cellulolytic bacteria from the gut of the termite *Zootermopsis angusticollis*. J Appl Microbiol. 2002; 92:32-40.
69. Wood TG, Sands WA. The role of termites in ecosystems. In: Brian MV, editor. *Production Ecology of Ants and Termites*. Cambridge University Press. 1978; pp. 245-292.
70. Yamin MA. Termite flagellates. *Sociobiology*. 1979; 4:1-119.
71. Yamin MA. Cellulose metabolism by the termite flagellate, *Trichomitopsis termopsidis*. *Appl. Environ. Microbiol.* 1980; 39:859-863.
72. Yamin MA. Cellulose metabolism by the flagellate *Trichonympha* from a termite is independent of endosymbiotic bacteria. *Science.* 1981; 211:58-59.
73. Yamin MA, Trager W. Cellulolytic activity of an axenically-cultivated termite flagellate, *Trichomitopsis termopsidis*. J. Gen. Microbiol. 1979; 113:417-420.
74. Yang H, Schmitt-Wagner D, Ulrich S, Brune A. Termite gut flagellates and their bacterial symbionts: Phylogenetic analysis and localization *in situ,* Dissertation. 2004; pp. 1-212.
75. Zhang X, Leadbetter JR. Evidence for Cascades of Perturbation and Adaptation in the Metabolic Genes of Higher Termite Gut Symbionts. *mBio*. 2012; 3(4):223-232.