Termite Digestomes as a Potential Source of Symbiotic Microbiota for Lignocelluloses Degradation: A Review

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Abstract: Termites thrive in great abundance in terrestrial ecosystems and the symbiotic gut microbiota play important roles in digestion of lignocelluloses and nitrogen metabolism. Termites are excellent models of biocatalysts as they inhabit dense microbes in their guts that produce digestive enzymes to decompose lignocelluloses and convert it to end products such as sugars, hydrogen, and acetate. Different of digestive system between lower and higher termites which lower termites dependent on their dual decomposing system, consisting of termite’s own cellulases and gut’s protists. Higher termites decompose cellulose using their own enzymes, because of the absence of symbiotic protists. Termite gut protaryotes efficiently support lignocelluloses degradation. In this review, a brief overview of recent experimental works, development and commercialization is discussed. Significant progress has been made to isolate cellulolytic strains from termites and optimise the digestion efficiency of cellulose. Future perspective should emphasize the isolation of cellulolytic strains from termites, genetically modifying or immobilization of the microbes which produce the desired enzyme and thus benefits on the microbiology and biotechnology.

Key words: Termite, lignocelluloses, polysaccharides, microbes, bacteria, flagellate

INTRODUCTION

Termites are one of the most successful wood-degraders on Earth, tunnelling and chewing on wood for millions of years (Radek, 1999). These insects made their first imprint as early as the Cretaceous period with the unearthing of oldest termite fossils of approximately 100 million years old. Termites are representing one of the social insects. They are grouped in ancient insect order Isoptera and they are closely related to cockroach and mantises (Radek, 1999; Kricher, 2011). Throughout the world, termites are traceable along the equators, in the tropical forests and Mediterranean shrublands, where dense biomass and the greatest biodiversity take place (Abensperg-Traun and Milewski, 1995). As mankind are fearful for their structures and crops damaging capabilities, the nature graciously welcome these wood-grazers. Termites also account for severe damage to wood in use. In order to enhance the resistance ability of wood towards termite’s invasion, application of termite repellent and post heat treatment on wood are now widely used (H’ng et al., 2012). Nevertheless, ecologically speaking, termites are beneficial insects that play an essential role in recycling nutrients, forming habitats, aerating and improving soils, and as food for countless predators (Radek, 1999; Kricher, 2011).

Termites survive on any wood and lignocellulose materials (H’ng and Chin, 2008; Lee et al., 2013). They convert the cellulose of wood and lignocellulose materials into carbohydrates before translated into energy. Cellulose is part of plant structures and the basic structural components of cell wall. Cellulose is a polysaccharide, a tough linear chain of glucose joined by β-1,4-glycosidic and hydrogen bonds. To disintegrate this polysaccharide into simple glucose, termites are loaded with as much as 250 different species of microorganism in their relatively tiny guts (Nadin, 2007). Nonetheless, not all the microorganisms in the gut of termites function in cellulose degradation. Each of the microorganisms is responsible in breaking down specific components of the plant structures to different end products. The termites provide the needed settlement for the microbes and feed on wood, while the microbes digest the food for their hosts in return. Such exchange reflects a mutual symbiotic relationship that benefits both the host and symbiont.

The microbial organisms inside the termite’s gut, however, do not develop by themselves. Before termites
could start munching on wood, they need to engage in trophallaxis (Wilson, 1971; Machida et al., 2001). In other words, they are exchange food and digestive fluid through mouth-to-mouth (stomodeal) or feeding on each other’s faeces (proctodeal) (Quarrieo, 2009). Such fluid contains the essential nutrients and endosymbionts that are passed on to the younger instars by the mature termites (James, 2008). Termites are lost most of their symbionts at every molt, and termites have to feed themselves with the recycled symbionts via proctodeal trophallaxis (James, 2008). They rely on symbiotic bacteria embedded on their surfaces to produce some of the digestive enzymes to degrade cellulose for their termite host (Radek, 1999). The resulting glucose and acetate are then absorbed by the termites as a primary source of energy (Radek, 1999; Okuma, 2003).

Termites’ successful survival on cellulose-rich diet suggests that significant decomposition of wood components is taking place in the gut. Almost 90% of the cellulose in wood is turned into acetate (Nadin, 2007). Many scientists are now convinced that termite’s gut resembles an efficient living bioreactor. Different species of the microbes in termite gut have different needs and release different end-products but they share a common goal-to degrade lignocelluloses into different applicable products. By turning the energy-rich cellulose into acetate and glucose, the symbiotic microbial community in termite’s gut could be the key to generate customised enzymatic-cocktails to apply in the biomass processing industry. Termites are therefore a potentially powerful catalyst for feasible bioethanol production.

**DISTRIBUTION OF TERMITES**

According to the theoretical and empirical data from Models of Population Dynamics and Models of Population Oscillations, the maximum of termite population would be gotten during 2020s years and world population may be until $10^7$-$10^8$ billion (Sapunov, 2008). In the high population of termites, there are 2,650 species of termites, and the majority of them occur only within tropical and sub-tropical latitudes (Kriecher, 2011). Termites are playing significant roles in food webs and influence the provision of ecosystem such as decomposition. They occur in vast numbers in tropical regions, which exceed 100 g m$^{-2}$ and 10,000 individuals m$^{-2}$ in the tropical forests (Eggleton et al., 1996). But their area prolongs to increase included Italy, New Zealand, and Australia.

In addition, the distribution of Drywood termites, Subterranean termites, Formosan termites, and Dampwood termites is varying by region. Drywood termites are live in the countries that do not reach freezing temperatures during winter and they are found along East Coast from the Mid-Atlantic States to South Florida, along the Gulf Coast, through the Southwest into California, and in Hawaii. The Subterranean termites which live in the soil underground; are able to survive in wide range of temperatures. In US, the subterranean termites are found in every state, except Alaska. As a pest of forest tree, Dampwood termites are rarely damage wood in buildings. They do not nest in the soil but mainly nest in decaying stumps, logs and eucalypt trees.

In Malaysia alone, it is estimated there could be 180 species of termites representing 48 genera that live in different habitats in the country (Tho, 1992). A termite can correspond to up biomass of invertebrates in decomposing trunks (Bandeira and Torres, 1985). At least ten identified species are known to invade wooden structures, paper products, cotton cloths or ornamental trees. *Coptotermes gestroi* (Asian subterranean termite) is the most common and aggressive wood-feeding termite species found in Malaysia and was reported to cause major damages (60-70%) specifically to interior wooden structures, followed by *C. curvignatus* contributing to about 20% of the total structural damage and attacking living trees as well as rubber, oil palm and coconut plantations (Yeoh and Lee, 2007). Other essentially threatening species in Malaysia include *Odonotermes* sp., *Schedorhino termes* sp., *Macro termes gigas*, *Nasuter mes* sp., *Microcerotermes crassus*, *Globitermes sulphurosus*, *Macrotermes carbonarius* and *Microtermes* spp.

**LOWER AND HIGHER TERMITES**

The order Isoptera of termites is phylogenetically classified into seven families and fifteen subfamilies (Lee and Wood, 1971). The families are: (1) Mastotermitidae, (2) Kalotermitidae, (3) Termopsidae, (4) Hodotermitidae, (5) Rhinotermitidae, (6) Seritermitidae, and (7) Termitidae. The Mastotermitidae, Kalotermitidae, Termopsidae, Hodotermitidae and Rhinotermitidae families are identified as the lower termites, whilst the Seritermitidae, and Termitidae families are acknowledged as the higher termites.

The taxonomy of lower and higher termites is based on the termites’ stage of evolution, in terms of their behaviour and anatomically. The main difference between higher and lower termites is the gut of lower termites comprises with protozoa, while the gut of higher termites is lack of protozoa (Varma et al., 1994). In the digestive tracts of lower termites, degrading of cellulose is depend on flagellates, yeasts and bacteria (Breznak and Brune, 1991).
TERMITE AND ITS DEGRADATION ON LIGNOCELLULOSIC MATERIAL

Lignocellulose can serve as a biomass material for a number of industrial biorefinery process, namely pyrolysis, hydrolysis, gasification to value-added products such as glucose, xylose, starch, ethanol (Kim and Dale, 2004; Scharf and Tartar, 2008; Chun et al., 2010, 2011; H'ng et al., 2011; Tay et al., 2013; Chin and H'ng, 2013). The main challenge facing lignocellulosic materials utilization is the energy, costs input involved in treatment and production processes. Therefore, researchers have expanded on the potential of the termite-based biological pretreatment strategy for use in lignocelluloses degradation.

Termites efficiently digest lignocellulose using their endogenous and digestive enzymes in the termite gut (Breznak and Brune, 1994; Watanabe et al., 1998; Ohkuma 2003; Scharf and Tartar, 2008; Tartar et al., 2009). The symbiotic digestion of polysaccharides by termites is involving a complex of host and its gut microbiota, which comprises bacteria, fungi, protozoa to degrade cellulose and hemicelluloses (Radek, 1999; Brune, 2009). The microbial community in the gut of termites has been attracting many scientists due to their symbiotic digestion mechanisms in the hindgut are largely controlled by the symbionts (Brune, 2009). According to previous reports suggested that termites could efficiently decompose lignocelluloses within a day by degrading 74-99% of the cellulose, 65-87% of the hemicellulose as well as 5-83% of the lignin which are able to removes most neutral polysaccharides and more than half of the acidic sugars (Breznak and Brune, 1994; Konig et al., 2006, Sun, 2008). Nevertheless, cows decompose only 30-40% of polysaccharides in their forage (Brune and Ohkuma, 2011).

Sound wood is most difficult to digest because the polysaccharides of the secondary plant cell wall are embedded in an amorphous resin of phenolic polymers which causing the barrier to enzymatic attack of the polysaccharides (Brune, 2009). Therefore, an efficient of symbiont-derived digestive enzyme in cellulolytic system is required to the polysaccharides degradation (Scharf and Boucias, 2010). Therefore, the incredible metabolic capability of the termite gut is potential biocatalyst in aerobic fermentative degradation of carbohydrates, and in metabolism of lignin-derived aromatic compounds (Brune, 1998).

ROLE OF TERMITE GUT MICROBIOAATA

Let us take a look at how is the role of termite gut microbiota in lignocelluloses digestion and may bring potentially beneficial in industrials application. For instance, the lignocelluloses digestion is highly achieved in the termite gut as the termite's digestome is apparent as a pool of host and symbiont genes (Scharf and Tartar, 2008; Tartar et al., 2009).

The cellulose activity in the hindgut of termites is attributed to cellulose-degradation bacteria. (Schwarz, 2001). Termite gut contains a lot of microbes which can digest cellulose such as the Gram-positive bacteria:
Bacillus, Paenibacillus, Streptomyces, Actinobacteria group and Gram-negative bacteria; Pseudomonas, Acinetobacter, Faculative microbe; Serratia marcescens, Enterobacter aerogenes, citrobacter farmer. Gram-positive strains related to Cellulomonas, Bacillus and Paenibacillus showed highest CMC-degrading potential. Wenzel et al. (2002) argued that the cellulolytic bacteria are taking over the role of flagellates in higher termites.

Most of the gut bacteria are necessary for the survival of their hosts even though they are indirectly involved in cellulose degradation in termites gut (Slaytor, 1992; Radek, 1999). In a termites’s gut, cellulose is broken down into simple sugar by certain cellulolytic species, subsequently metabolized to form pyruvate. Other microbial species collaborate in turn to transform the pyruvate to different end-products, such as CO₂, acetate, methane or ethanol, depending on availability of oxygen supply (Nadin, 2007). While concentrations in the midgut are aerobic, oxygen concentrations are low in the hindgut (Radek, 1999). Eventually the transformation cycle repeats again on another type of substrates. As much as 250 microbial species are adapted to live in a termite’s gut together, but each is individually involved in different transformation of varying substrates.

Termites are mostly feed on the dead grass, wood, and other plant material to obtain essential energy from the digestion of cellulose (Andersen and Jacklyn, 1999; Pearce, 1997). Therefore, it is an opportunity of termites biomass used as food sources for the aquaculture, pig, and poultry industries. At present, termite microbes have been proven useful in poultry feed additive. Purwadaria et al. (2003) detected cellulolytic activities in the fresh extract of termites (Glyptotermes montanus) that increases the digestion of poultry feedstuffs containing high lignocelluloses such as rice bran, wheat pollard, Palm Oil Mill Effluent (POME), Palm Kernel Cake (PKC), corn and soybean meals. Rich of protein in termite gut replace 50% fishmeal in formulations and it is a useful supplement for family poultry. Nutrients left behind in termite wastes may also be useful for horticultural purposes, particularly compost which potential be a novel resource for organic biofertilizers (Chai et al., 2013; Peng et al., 2013).

Current studies showed that termite symbionts have involved as cellulolytic or lignin-derived component and degradation of aromatic hydrocarbons compounds. Hence, that would be useful for industrial application such as biomass consumption, environmental remediation and fine-chemicals production. Advances in the conversion technology will add value to existing biochemicals production and boost exciting economic opportunities of bio-based applications as well as fuels, chemicals and pharmaceuticals. Despite slower reaction time and careful control of microbial growth conditions, biological system involving termite symbionts appears to be more appealing (Sun and Cheng, 2002; Zheng et al., 2009) for lignocelluloses degradation.

CURRENT RESEARCH AND FUTURE PERSPECTIVE ON TERMITE’S GUT MICROBIOA

As discussed, termite lignocellulose digestion has been considered as a gut-symbiont-mediated process. The termite gut is explored as a source of novel microorganisms and may bring many benefits to large scale industrial applications (Tokuda et al., 2004). In fact, the symbiotic association of termites with their diversity intestinal macrobiotic is receiving interests from various aspects such as microbiology, biochemistry, protozoology, insect physiology and ecology, socio-biology, evolutionary biology, and even in atmospheric chemistry (Sanderson, 1996; Higashi and Abe, 1997; Sugimoto et al., 2000). Hence, researches have been further expanded on the anaerobic food web and nitrogen metabolism in the termite gut.

In addition, in microbial gut of termites, also include nitrogen fixing bacteria (Benemann, 1973; Breznak et al., 1973; French et al., 1976; Potrikus and Breznak, 1977; Prestwich and Bentley, 1981). Nitrogen fixation by termite gut microbes has been known for years ago (Breznak, 2000) and nitrogen fixation contributes as much as 60% of N in some termite colonies (Tayasu et al., 1994). Since the nitrogen compound are insufficient in wood and soil, the nitrogen fixing bacteria (e.g., Enterobacter, Rhizobium, Desulfovibrio) is play a vital role in symbiotic community (Lovelock et al., 1985; Radek, 1999). However, the wood-feeding termites are strongly nitrogen limited (Brune and Okhuma, 2011). Researchers showed that the hindgut microflora of termites includes a morphologically diverse population of N-fixing Spirochetae bacteria which have reached 50% of all prokaryotes (Paster et al., 1996; Breznak, 2002). The spirochetes are involved in acetogenesis and N₂ fixation process to provide the carbon, nitrogen and energy needs of their termite host. The N-fixing bacteria produce amino acids that are partly liberated and may be used by termites and flagellates. Nonetheless, the metabolic role of Spirochetae is entirely unknown (Radek, 1999). Hence, more researches are needed to study the metabolic properties of Spirochetae especially the spirochetes contribution to H₂/CO₂-acetogenesis and N₂ fixation. Next, the study on the properties of spirochetes (or of the termite gut itself) enables them to become such a prominent component of the microbiota is needed in the field of researches also.
In addition, there are fermenting bacteria also found in the termite gut from the anaerobic food web. The low concentrations of soluble sugars and the accumulation of their metabolites in the hindgut fluid of termites indicate that polysaccharides depolymerization is coupled to the fermentative degradation of its hydrolysis products (Brune and Okhuma, 2011). The fermenting bacteria (e.g., of the genera Streptococcus, Bacteroides, Fusobacterium, and Lactobacillus), profit by the low amount of mono-, di-, and oligosaccharides, liberated by the flagellates (Breznak, 1984; Radek, 1999). The fermentation of cellulose is following the equation as shown below (Odelson and Breznak, 1985; Brune and Okhuma, 2011):

$$[\text{C}_{6}\text{H}_{10}\text{O}_{5}] + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COO}^- + 2\text{H}^+ + 2\text{CO}_2 + 4\text{H}_2$$

The metabolic end product of the anaerobic fermentation food web is acetate (termite’s fuel), and other organic acid, which can be transported across the gut wall for reabsorbed by the host and form the basis for its energy metabolism (Radek, 1999; Brune and Okhuma, 2011). According to the few researches, two species of fermenting bacteria were identified which include Acetobacteriger longum by Kane and Breznak (1991) and Enterococcus sp. by Tholen et al. (1997).

In the new study, researchers have suggested the termite’s enzyme could be boon to cellulosic ethanol by fermentation process. Researcher claim that a type of bacteria that helps termite digests wood could be a key to making ethanol economically from non-food crops such as wood and grass (REF). Meanwhile, wood decay in the guts of termites generates hydrogen gas from lignin as a key intermediate product. This explosive, energy-rich hydrogen gas can be combined with ethyl acetate to make ethanol or provide energy for gasification. As a result, research is currently applied to understanding the interactions of lignocelluloses degradation and symbiotic microbes in termites gut to provide innovations in technology to address this challenge for producing ethanol (Nadin, 2007; Brune, 1998; Scharf and Bouciuas, 2010; Li et al., 2012). Bioethanol production with emphasis on cellulosic ethanol brings a scientific challenge of achieving cost effective degradation of complex cellulosic biomass (pre-treatment and hydrolysis stages) (McMillan, 1994). Thus, the microbes living in termites gut provide a fast and efficient hydrolysis of biomass if harnessed and applied appropriately to produce cellulosic ethanol at an industrial scale.

For increased efficiency and reduced production costs, future findings should highlight the isolation of cellulolytic strains or microbial species from termites, genetically modifying or immobilizing of microbes which produce the desired enzymes. As such, termite gut digestomics is a relatively new area of research. In the future, termite guts can potentially advance the bioconversion of lignocellulosic materials to valuable product such as fuel as it is an effective, economic, and sustainable ways.

**CONCLUSION**

Termites are regarded as harmful insects because of their ability in destroying various materials including lignocellulosic biomass. The termites’ digestion process on the cellulose is fast and efficient which typically achieving 95% conversion in 24 h or less. However, the microbiology mechanism is different between the two classes of termites which are ‘lower termites’ and ‘higher termites’. In which, the differences of microbiology of lower termites and higher termites may also differ in their role in degrading cellulose. In recent years, termites have captured the interests of researchers from various disciplines to investigate their gut microbial symbionts and their incredible ecological importance to the global carbon cycle. The ability of termites to hydrolyse a broad assortment of chemical bonds and break down the lignocelluloses into monomer sugars quickly has astonished researchers. Apparently, the development of low-cost enzymatic approach with termites is promising and ecological to accomplish the bioconversion of lignocelluloses into useful products such as glucose and ethanol.

**REFERENCES**


