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MICROORGANISM SYMBIONTS OF SUBTERRANEAN TERMITE *MACROTERMES GILVUS* (TERMITIDAE) ISOLATED FROM THEIR GASTROINTESTINAL AND NEST

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A B S T R A C T

Study aimed to determine the diversity and the role of symbiont bacteria found in the digestive tract of termites and symbiont fungus grown in the termite nest. Location of research is a rubber plantation which is located about 60 km from the city of Palembang, and 20 km from the district capital Ogan Ilir regency, Inderalaya. Area of the garden is about 2 hectares. In the land of rubber plantation was discovered many termite nests. Symbionts bacteria were identified with the test of characteristics and biochemical activities of bacteria, by using the key books of Bergey's Manual of Determinative Bacteriology. Identification of fungus was done by matching the characteristics of the fungus obtained from observations with identification book Compendium of Soil Fungi. *M. gilvus* was symbiosized with 3 species of bacteria that live in their digestive tracts, two species were found in the midgut, identified as; *E. agglomerans* and *S. hominis*. One species of bacteria was found in the hindgut, identified as *P. paucimobilis*. On the "fungus comb" inside nest of *M. gilvus* discovered two species of fungi which were identified as *Trichoderma* sp. and *Mucor* sp.

Keywords: *Macrotermes gilvus*, Symbiont, *E. agglomerans*, *S. hominis*, *P. paucimobilis*, *Trichoderma* sp., *Mucor* sp.

INTRODUCTION

Subterranean termites is able to decompose the material of died plants, mainly cellulose and restore the nutrients to the environment. The ability of termites digest cellulose was collaborated with mutualistic symbiont of various microorganisms in the digestive tract (Ohkuma, 2003). Subterranean termites are important decomposer in the dry soil, especially in nutrients recycling, soil forming and maintaining soil moisture (Mommer, 2003; Ackerman *et al*., 2009). The ability of termites, especially the caste of workers, in degrading cellulose is supported by the presence of cellulolytic bacteria and other enzymes in the digestive tract of termites. Bacterial symbionts in the termite gut are specific, for different termite species, its bacteria simbions are different (Eutick *et al*., 1978). Subterranean termites can also grow fungus inside the nest. However, the exact function of the fungal symbionts are unclear and the information is still different between the genus and species of termites (Bignell, 2000; Rouland-Lefèvre and Bignell, 2001; Hyodo *et al*., 2003; Ohkuma, 2003). The level of diversification and the role of mutualistic fungal symbionts termites seem to be different. These differences are associated with plant materials that are edibled and used to build fungus combs inside the termite nest (Rouland-Lefèvre *et al*., 2006).

Study about the role of termites in the soil litter decomposition processes had been widely published. Most studies are conducted in the field of forest land, grassland, desert, and non-tropical areas. Subterranean termites have an important role in the decomposition processes, especially in the arid areas (Jones and Eggleton, 2000; Ackerman, 2009; Hemachandra *et al*., 2010). Termites have the ability to adapt to the dry environment well in the decomposition process while other decomposers such as fungi and bacteria cannot do

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(Mommer, 2003). The rubber plantation land managed naturally use organic fertilizers and without the use of pesticides, there was found many termite mound nests spread relatively uniform and the termite identified as a species *Macrotermes gilvus* (Arifin *et al*., 2014). Therefore, these subterranean termites which were found in the area of the rubber plantation needs to be examined and studied more. The research aimed to discover about the diversity and the role of symbiont bacteria found in the termite digestive tracts and symbiont fungus which grown inside the termite nest of *Macrotermes gilvus* in the rubber plantation land.

MATERIALS AND METHODS

Study site: The study was conducted in October 2013 to May 2014 on public productive rubber plantation, in the village of Tanjung Batu, Ogan Ilir regency. Research activities were carried out in the form of observations and direct measurements in the field (insitu), Observation, measurement and testing in the laboratory. Location of the gardens is about 60 km from the city of Palembang, south sumatera, Indonesia, and 20 km from the district capital Ogan Ilir regency, Inderalaya. The width of field of rubber plantations studied is about 2 hectares. Rubber plantation was specified intentionally or purposively that a garden is managed using organic fertilizers and without the use of pesticides. This management is expected to describe the condition of the rubber plantation as a natural ecosystem that supports the existence of subterranean termites to live.

Inventory symbionts in the digestive tract and the termite nest

- **Test of characteristics and biochemical activity of intestinal bacteria:** Bacteria were identified by using the key books of Bergey's Manual of Determinative Bacteriology (John *et al*., 1999). Tests were carried out; gram staining test, motility test, carbohydrate fermentation test, indole test, test TSIA, urea test, test-Proskauer voges, methyl red test, Simmon citrate, test dihydrolase Arginine, Lysine and Ornithine decarboxylase and oxidase test.
- **Identification of the symbiont fungus:** Identification of the fungus was done by matching the characteristics of the fungus with identification book, Compendium of Soil Fungi Domsch *et al*., (1980); Gandjar and Sjamsuridzal (2006). Fungus was cultured on dextrose agar medium.

RESULTS

Symbionts bacteria In Gastrointestinal of Termites: The results showed that in the digestive tract of subterranean termites *M. gilvus*, caste workers, discovered three species of bacteria, two species of bacteria found in the midgut, and one species found in the hindgut. In the front part of the digestive tract or foregut is not found any bacteria. The existence of different bacterial species in each part of the digestive tracts is suspected to relate with the physico-chemical conditions in each part of the digestive tract. Each bacterium tends to have a different tolerance for physico-chemical conditions in that microhabitat. Physico-chemical conditions in each part of the digestive tract of termites *M. gilvus* are different. Different species of bacteria could be found in any part of the termite digestive tract. The results of identification for bacteria were found in the digestive tract of worker caste of termites *M. gilvus*. Three species bacteria are known to exist. The three species are *Staphylococcus hominis*, *Enterobacter agglomerans* and *Pseudomonas paucimobilis*. The species *S. hominis* and *E. agglomerans* were found in the midgut. *P. paucimobilis* species was found in the hindgut. Each colonies of bacteria was grown in the medium can be seen in figure 1 below.

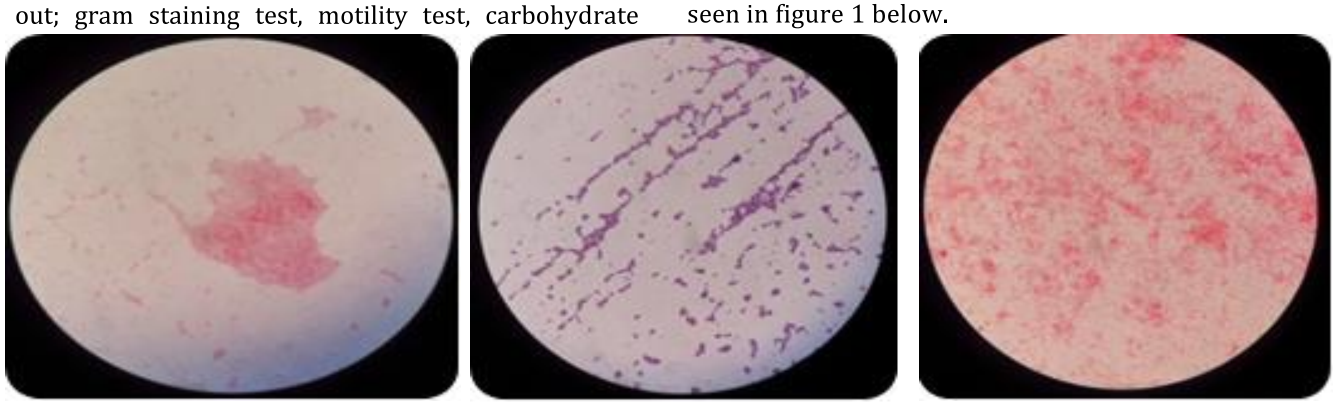

Figure 1. The form of colonies of bacteria found in the digestive tract of termites *M. gilvus* grown in the medium. Colonies of bacteria *Enterobacter agglomerans* (a), *Staphylococcus hominis* (b), and *Pseudomonas paucimobilis* (c).

In the foregut of digestive tract of termites *M. gilvus*, the bacteria were not found. Allegedly it was linked to the front of the digestive tract that serves as a container and performs mechanical digestion of food. In the front part of gastrointestinal tract does not have glands of the enzyme, while the midgut and the hindgut have glandular enzymes. Cellulolytic bacteria in the digestive tract of termites produce cellulase enzymes that help the digestive process of cellulose in the digestive tract of termites. Inside the digestive tract of termites *M. gilvus*, the bacteria *E. agglomerans* was found in the midgut, a bacillus shaped bacterium, oxidase; respond positively to lactose, glucose, sucrose and maltose. The bacterium reacts positively to amino acids such as lysine, ornithine and arginine. Biochemical test results show that *E. agglomerans* has a role in the fermentation of sugars, such as glucose, sucrose, lactose, maltose and mannitol. It shows that the bacterium *E. agglomerans* role is in degrading cellulose. The positive respond of the *E. agglomerans* to amino indicates that *E. agglomerans* also function in nitrogen fixation. Degrading of cellulose in the digestive tract of termites, aims to produce organic matter as a source of energy for the termites *M. gilvus*. The energy produced in the form of Adenosine three phosphate (ATP) molecules and inorganic phosphate. The abilities of *M. gilvus* termites decompose and degrade cellulose were supported by the existance of the bacteria *E. agglomerans* which is producing endogenous enzymes. General overview of the digestive tract of the termites with section parts can be seen in figure 2 below.

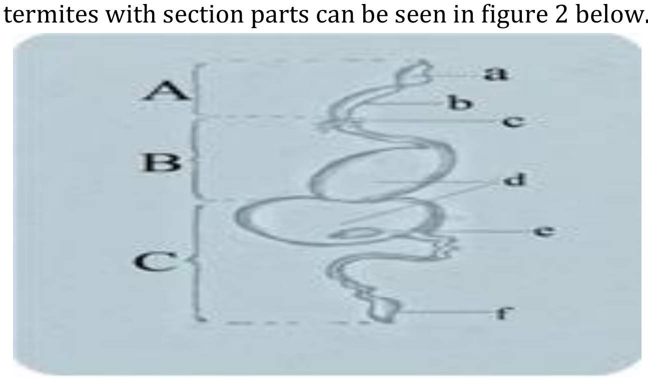

Figure 2. The termite digestive tract of *M. gilvus*. (a. Cache; b. Mesenteron: MS. Mixed segment: MT. Malphigian tubules: d. Proctodeal segment: e. Diverticulum. f. Rectum. A. Frontgut; B. Midgut; C. Hindgut).

Symbiont fungus in termites nest: The results showed that in termite mounds, “fungus comb” was found. A comb was constructed from dead plants that are not digested in the form of secondary termite feces. A fungus comb was taken directly from the ground where *M. gilvus* termite nests were found in a rubber plantation. A fungus comb is a building that has a cavity or tunnel, yell taking fungus comb, and is found large number of termite larvae inside. The fungus comb taken from the termite nests can be seen in figure 3 below.

Figure 3. Fungus comb of termite *M. gilvus* (a) and white nodules of fungus (b).

The results of identification show that there are two species of fungus that grow on the fungus comb which was identified as *Trichoderma* sp. and Mucor sp. Species of *Trichoderma* sp. which is found in the "fungus comb" in the soil termite nests of *M. gilvus* have morphological features as follows: septate hyphae (a), septate and conidiophores hyaline (b). Phialides inherent in conidiophores perpendicular. Phialides solitary or cluster. Conidia unicellular round or elliptical (c) branched conidiophores (d). walled conidia smooth or rough and attached at the end phialides. Conidia are smooth-walled, hyaline colored initially, the becoming greenish-white, dark green and then, white especially in the part where there are many conidia. Conidiophores increasingly resembles a pyramid can be branched to the shorter end of the ramifications, figure 4 below.

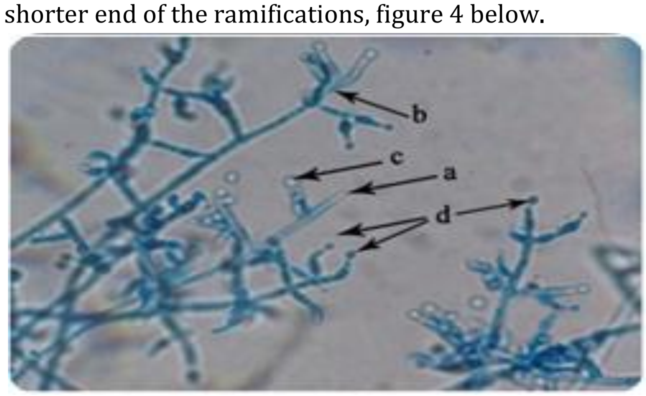

Figure 4. The microscopic view of colony of fungus *Trichoderma* sp. Cultured on Dextrose agar medium.

The microscopic observation of *Mucor* sp. colonies can be seen that the fungus mycelium is shaped like cotton fibers with surface shaped colonies. Color of *Mucor* sp., first colony mycelium white initially and then turned into a brownish gray. White microscopically mycelium *Mucor* sp. has stolons but do not have rhizoid, conidiophores short, smooth and hyaline colored. Septate not hyphae, conidia are round or oval, smooth surface, and colored hyaline. Sporangiophore is rounded in shape and length, sporangia is round or oval in shape and inside it looks like a collection of spores. Arthrospore (oidia) is located right on the tip of the hyphae. Sporangia spherical shape, colored gray to black and it contains spores in it. Zygosfor is grown in the mycelium. Overview of mycelium, sporangiofor, sporangium and spores of fungi Mucor sp. can be seen in figure 5 below.

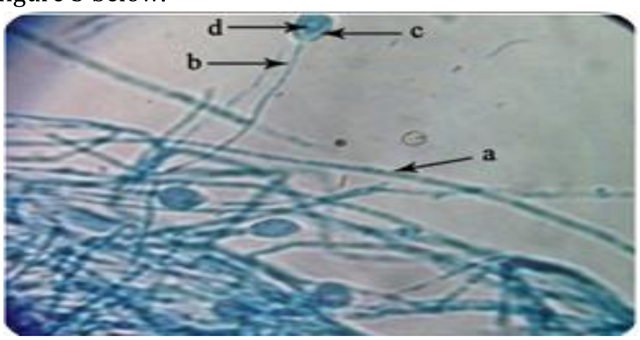

Figure 5. Microscopic view of *Mucor* sp. mycelium (a) sporangiofor (b), sporangium (c) and spores (d).

DISCUSSION

BACTERIA SYMBIONT IN GASTROINTESTINAL OF TERMITES

Enterobacter agglomerans: The digestive tract of termites, between foregut and midgut are separated by a valve. The valve controls the entry of food from the front to the next section. Termite cellulase in a termitidae family secreted in the intestine or in front bowel of the midgut. Cellulase is an important part of the process of cellulose digestion that occurs in the gut of termites (Tokuda *et al*., 2004). According to Tokuda *et al*., (2005) hindgut is also an important place in the process of digestion of cellulose. The bacterium inside the digestive tract of subterranean termites is critical symbionts in termites symbiotic systems, for subterranean termites with the diet has low nitrogen content. Bacteria *E. agglomerans* is a termite symbiont which acts an important role as a nitrogen binder (Breznak, 2002). Termites remove residual nitrogen in the form of uric acid and excreted it into the digestive tract. Uric acid will be degraded by microorganism symbionts that live in the digestive tract of termites (Ohkuma, 2003). *E. agglomerans* is a facultative anaerobic bacterium and can bind nitrogen. Bacteria *E. agglomerans* synonymous with *Erwinia herbicola*, *Erwinia uredovora* and *Erwinia stewartii*. The bacterial species are heterogeneous and associated with the plant material as a saprophyte or pathogen (Beji *et al*., 1988). Gastrointestinal tract of Termitidae, midgut of termite produces more enzymes than in the hindgut and the foregut does not produce the enzyme. Therefore, in the digestive tract of termites, bacterial symbionts produce enzymes to degrade lignocellulose. The enzymes work together to decipher the material cellulose and lignin into simple sugars monomer (Hongoh, 2011). Termite digestive tract is an anaerobic gradient system, a constant supply of oxygen which is introduced through the epithelium. Therefore, in any part of the digestive tract of termites occurs spatially differentiated of pH (Noirot, 1970; Bignell, and Eggleton, P., 1995). Front part of termite gut in the form of cache and gizzard muscle, midgut is a core part of the secretion of digestive enzymes and absorption of dissolved food ingredients. Hindgut is part of the digestive tract that most large in size, where digestion can also occurs here. The size of the termite hindgut reaches 1/3 of body weight of termites (Brune, 2000). On the hindgut, bacteria are anaerobic and microaerophilic (Konig *et al*., 2006). Cellulolytic bacteria in the digestive tract of termites are facultative anaerobic or microaerophilic. *M. gilvus* termite digestive tract consists of three parts: foregut, midgut and hindgut (Noirot, 1970). Lignocellulose degradation in the digestive tract of termites occurs in three stages. The first stage is the hydrolytic degradation of cellulose substrats. In this stage, the cellulase enzyme that acts as derived from termites. The second stage is the oxidative degradation of cellulose which is carried out by the enzyme β-glucosidase. In this stage the role of microorganisms residing in the digestive systems of termites are bacteria of the genus *Enterobacter* and *Pseudomonas*. In the third stage act acetogenik bacteria, and bacterial decomposition of methanogenik sulfate. At this third stage, the bacteria *Enterobacter* degrade cellulose and hemicellulose substrats (Konig et al., 2006). Hydrolysis of hemicellulose produces three kinds of monosaccharides that are xylan, arabinose and glucose. Arabinose hydrolysis of hemicellulose produces glucose. Hydrolysis of hemicellulose through fermentation by microorganisms that can break down some of the substrate in the form of pentose sugars. According to Tokuda *et al*., (2012) the percentage of total endogenous enzyme activity (EG) in the digestive tract of highest termites (Termitidae) are in the mouth and getting back into the digestive tract which content a few endogenus enzymes. Endogenous enzyme activity in the mouth termites reached 69.1%, in the midgut 28.3%, in hindgut 2.3% and 0.3% in foregut. Endogenous cellulase enzymes present in the digestive tract of termites are not able to decipher the entire cellulose. The inability indicates that the symbiont bacteria inside the digestive tract of termites play an important role as an energy supply for termites. Tokuda *et al*. (2012) stated that in the middle of the termite gut is still unexplained mechanism of cellulase activity increase in the crystalline cellulose.

***Staphylococcus hominis*:** Bacteria *S. hominis* together with the bacteria *E. agglomerans* found in the middle part digestive tract of termites. Bacteria *S. hominis* is a bacteria that works on oxidative stage in substrats hemicellulose. Allegedly hemicellulose is a product of the decomposition of the termite diet produced or decomposed by bacteria *E. agglomerans*. Oxidative phase in the digestive tract of termites aided by the enzyme β-glucosidase. At this stage of digestion termites were performed by Enterobacter bacteria, followed by Pseudomonas bacteria inside the rear digestive tract of termites. In the final stages of food digestion in the digestive tract of termites, the role of acetogenik bacteria, and bacterial decomposition methanogenik sulfate. S*. Hominis* are able to produce aerobic acid from glucose, fructose, sucrose, trehalose, and glycerol (Konig *et al*., 2006).

***Pseudomonas paucimobilis*:** In the hindgut, bacterium *P. paucimobilis* was found. The bacteria are known to be actively involved in the fermentation of glucose. *P. paucimobilis* bacteria are gram-negative, slightly motile, aerobic and do not grow in anaerobic condition. *P. Paucimobilis* bacteria grow optimally at temperatures of 30 ^{0} C. *P. Paucimobilis* bacteria do not produce urease, but capable of decomposing and synthesizing macromolecules such as cellulose. Pseudomonas is a group of bacteria that are active through hydrolytic process that perform decomposition with the aid of water (Konig *et al*., 2005). The hind digestive tract can accommodate the rest of the digestion of organic materials in large quantities. Part of hindgut content is a termite fecal that aggregate formation. *M. gilvus* termites that digest organic material had eaten and returned it to the ground in the form of fecal. The termite fecal is an organic material derived from food of termites that physico-chemical form of stable soil aggregates. Aggregate faecal is a final outcome residual termite digestion which empties into the anus which is end part of hindgut of termites. Organic materials which are removed from the anus of termites are known as primary termite feces.

Primary faecal issued termites can still be used by the termites as a food source for termites inside the nest. It shows that the process of digestion occurs in the gut of termites at first rudimentary. Termites can also utilize the fungus growth in the nest as a food source. Fungi are known to have lignoselulase enzymes, which contribute to digest organic material contained in the feces frimer. Primary termite feces still contain organic material that is not digested as lignocellulose. Furthermore, from next termite digestion will produce secondary feces. Soil eating termite, along with the digestive tract, the degree of acidity (pH) is extremely changed. Kappler (1999) suggested that the digestive tract of soil eating termite can be very alkaline. However, it is not known with certainty how the process of fermentation in the hind digestive tract of termites done (He *et al*., 2013).

Sigit *et al*., (2006) stated that the termites were instrumentally in changing the dead wood and other organic materials that contain cellulose to be litter. Based on the place and the material degraded, cellulolytic microorganisms in the digestive tract of termites have different characteristics. Microorganisms contribute cellulase enzymes to help digestion of crude fiber for termites. Previous study which uses termites *M. gilvus*, showed that microorganisms were symbionized with termites digest food diet in the form of fiber, but the results are still low, compared with rumen microorganisms. The average dry matter digestibility obtained approximately 13.88%, and digestibility by rumen microorganisms can reach 17.27% (Setianegoro, 2004).

FUNGUS SYMBIONTS IN TERMITES NEST

***Trichoderma* sp:** Fungus *Trichoderma* sp. belonging to the phylum Ascomycota, class Euascomycetes, the Order Hypocreales, Family Hypocreaceae, genus Trichoderma. *Trichoderma* is composed of several species including *Trichoderma harzianum*, *Trichoderma koningii*, *Trichoderma longibrachiatum*, *Trichoderma* *pseudokoningii*, and *Trichoderma viride* (Harman, 2007). The existence of *Trichoderma* in agricultural land allegedly due to the way of land preparation is managed organically, without the use of chemical fertilizers, synthetic pesticides, which can reduce the pollution of soil and water (Purwantisari and Hastuti, 1999). Organic soil management can affect soil fertility and trigger the growth of fungi such as *Trichoderma*, an antagonist fungus. *Trichoderma* sp. is the most common saprofitic fungus found in soil. Termites grow fungus (Termitidae; Macrotermitinae) symbionized mutualitically with fungi grown in the nest. The fungus is a food source for termites (Kirk *et al*., 2001). In the savana ecosystem, approximately 20% of the carbon mineralization events conducted by termites grower fungus (Wood and Sand, 1978). Termites grower fungi that have a symbiotic relationship are specific to certain fungi grown in the nest, (Aanen *et al*., 2002). Growth of fungus inside termite nests through spores or conidia are carried in the digestive tract reproductive termites. Spores were inoculated by the reproductive termites to the newly constructed “fungus comb”. These events are known vertical transmission. Inoculation spore to fungus comb is also done by termites caste workers. Worker caste termites out of the nest to look for food to be brought spore into the nest. During searches of the food, even the workers carry a certain fungus spores were grown in "fungus comb". The growth of the fungus known as horizontal transmission (Korb and Aanen, 2003). Conidia, spore obtained from the fungus comb, rich in nitrogen. Termites utilize fungus by eating parts of the fungus that has been mature in the form of nodules. Primary fecal or stool out of the body worker caste termites will continue to be added or placed on the surface of the fungus comb. In the newly laid surface of the substrate that it will grow and develop mycelium fungus quickly. Fungus inside the nest will degrade lignin which can then be broken down by cellulase owned termites (Hyodo *et al*., 2000). Termites, belonging to the family Termitidae, are able to produce endogenous cellulase enzymes to digest cellulose (Ohkuma, 2003). Symbiotic fungi inside termite nests of Macrotermes spp. are important in the degradation of lignin (Hyodo, 2003).

Mucor sp.: According to Michelli (2007) Mucor sp. belong to the phylum Zygomycota, Order Mucorales, Mucoraceae Family, Genus Mucor. Mucor is a mold that forms filaments (filamentous). Some species *Mucor* include Mucor amphibiorum, Mucor circinelloides, Mucor hiemalis, Mucor indicus, Mucor racemosus, and Mucor ramosissimus. *Mucor* sp. capable of producing heat. The resulting heat is released into the microenvironment inside the nest. The existence of fungus in termite mounds is able to retain the microclimate conditions inside the nest. The temperature inside the termite nest is relatively stable despite fluctuating temperatures outside the nest. Fungus is also a food source to the termite inside nest. Termites *M. gilvus* mutualized with fungi of the genus Ascomycetes, is known as Termitomyces, fungus of termite nests. The symbiont fungus grows specifically in medium or comb inside the nest which is managed by termites. Fungus comb is made of plant litter matters that are not digested as it passes through the gut of termites. Generally cellulosic material containing lignin or lignocellulose that cannot be digested by termites and accommodated in the hind of the digestive tract and subsequently issued in the form of fecal feces. Fecal feces resulting compacted to create a matrix such as a comb inside nest building. Fungus comb will overgrown which are known to utilize lignocellulosic as the growth substrate. Mature fungus comb, mycelium will grow and produce nodules in the form of conidia, which are then used as food for the termite inside nest. Older fungus comb will be consumed by termites caste workers who are inside the nest (Sieber and Leuthold, 1981). Some fungi symbionts role as providers of heat and humidity in the nests of termites (Noirot and Darlington, JPEC., 2000). Matsumoto (1976) stating that the conidia formed can act as providers of nitrogen and enrichment source of nitrogen in food ingredients derived from the metabolism termite fungus (Collins, 1983).

Rohrmann (1978) indicates that old comb of the termites *M. ukuzii* and *M. natalensis*, content of lignin was decreased. In higher plants, cellulose is protected by lignin and prevents the digestion of cellulose, because of the accessibility of the enzyme to decompose cellulose is protected by the presence of lignin (Kirk and Chang, 1981). Higashi and Abe (1997) also state that the lignin is a barrier for the termites to obtain energy and carbon sources of cellulose. Conidia produce a nitrogen rich food source for the larvae and nymphs of termites inside the nest (Collins, 1983). Therefore, old fungus comb is a provider of the most suitable food source for larvae and nymfa termites in the nest. From the standpoint of the ecosystem, lignin decomposers association with fungi allows the grower termites can utilize almost the entire lignocellulose consumed. It is reflected in the volume of lignocellulose found few in number in the dirt or termite feces (Darlington, 1994). Some degree of diversification mutualistic seems to happen with the different roles of symbiont. It has to do with differences in plant material used by termites to build fungus comb, taxonomically are different. It is still not enough information available to explain the event.

Mutualistic fungi and termites work on a reciprocal basis and depend on each other. Termites rely on fungi which become food for their survival depends on termites and fungi to establish a presence in a habitat (Korb and Aanen, 2003). *M. gilvus*, subterranean termites eat the old fungus comb. The lignin that has degraded so cellulose much easier to digest by termites. Symbiotic fungi are to address the lignin barrier and improve the digestibility of cellulose by termites. The young composer fungus comb, containing more carbohydrates than lignin. Termites do not digest cellulose and carbohydrates as a whole when the food material is in the digestive tract of termites. Darlington (1994) states the primary fresh faeces or faecal termites have not been digested properly by termite digestion. Carbohydrate content is still high in the primary termite feces and very important because fungus can not only utilize lignin as the growth substrate, but also requires more carbon energy sources, such as glucose, cellulose on the substrates or fungus comb (Kirk *et al*., 2001).

CONCLUSION

1. Termites, *M. gilvus* symbionized with three species of bacteria are inhabitants in their digestive tract. Two species of bacteria found in midgut are identified as; *E. agglomerans*, *S. hominis* .One species of bacteria is found in the hindgut is identified as *P. paucimobilis*. Otherwise, in the foregut was not found any bacteria.
2. In the "fungus comb" which is grown by the termite, two species of fungi identified as *Trichoderma* sp. and *Mucor* sp., were discovered.

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