



## Research Article

# Identification of Termites's Hindgut Bacterial Symbionts (*Coptotermes curvignathus* Holmgren) and the Feasibility in Rice Straw Decomposting

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## ABSTRACT

Cellulotic bacteria can be used as breakers of cellulose bonds in rice straw, one of which is cellulotic symbiont bacteria found in the hindgut part of termite *Coptotermes curvignathus* Holmgren. The study aimed to characterize and identify bacterial isolates from the back intestine of *C. curvignathus* termites and test the ability of bacterial isolates to decompose rice straw. The results of isolation on worker caste termites found one bacterial species that was successfully cultured in *Carboxymethyl Cellulose* (CMC) media. Biochemical test results for isolates, showed that, bacteria found in rod form (rod shape), aerobic, gram negative, motile and produced catalase enzyme. Sequencing of 16S rRNA genes in bacterial isolates showed similarities with *Bacillus cereus*. A dose of 10 ml symbiont starter, 50% in concentration, applied to 2 kg rice straw can shorten the decomposition time from 12 weeks to 4 weeks, reducing the required decomposition time which is about 75% of the normal time.

Keywords: decomposition of rice straw, symbiotic bacteria, symbiotic starter bacteria termite hindgut

## INTRODUCTION

Lignocellulose biomass, such as rice straw, has the potential as a base for making organic fertilizers which can increase soil nitrogen and soil uplift (Singhania, 2009). In one millimeter of cubic rice, straw contained 46.13% C-organic, 0.52% N-total, 32% cellulose and 13.3% lignin (Nandi *et al.*, 2000). On the other hand, cellulosic bacteria found in soil and cow dung have the potential to break down cellulose bonds in rice straw. Cellulolytic bacteria were also found in the hindgut (termite hindgut) (Papoola & Opayele, 2012). Diba *et al.* (2012) successfully identified *Staphylococcus* sp. found in the hindgut of the *Macrotermes gilvus* termite and proved to be able to decompose organic matter.

*Coptotermes curvignathus* Holmgren is a termite known as wood and building destroying insect. Apart from being a pest, termites also play an important role in recycling plant nutrients through the process of disintegration and degradation of organic material from wood and plant litter (Subekti *et al.*, 2008). Cellulose degradation by termites is

carried out by cellulase enzymes produced by microorganisms present in termite hindgut (Ni & Tokuda, 2013). The results of Aini's (2015) study concluded that bacteria from *M. gilvus*'s termite intestine produced compost with a total bacterium of  $2.75 \times 10^8$  cells/g. Compost products with a starter concentration of 50% and composting time of 5 weeks are black, textured crumbs, scented like soil, have a C/N ratio of 15.11 with a neutral pH (7.0) and humidity of 60.80%.

Based on the description above, identification of bacterial isolates originating from the hindgut of *C. curvignathus* termites was identified to be further tested for their decomposition ability against symbiotic bacterial isolates in rice straw.

## MATERIALS AND METHODS

Termites used as the object of the study were *C. curvignathus* from the worker caste. Termite samples were taken from the MIPA forest at the Universitas Sumatera Utara, then collected in a collection tube. Carboxymethyl cellulose (CMC) media was prepared for bacterial development

media to be isolated. The pure and isolated bacteria were then stained with gram and biochemical tests [Simmon's Citrat Agar (SCA) test, Triple Sugar Iron Agar (TSIA) test, Simmon Indol Motility (SIM) test, gelatin test. The study used a descriptive observation method by characterizing the species of symbiont bacteria present in *C. curvignathus* termite hindgut and observed the potential of these bacteria to decompose rice straw.

### ***Isolation and Purification of Termite Hindgut Bacteria***

The body of termites was sterilized using 70% ethanol and washed with sterile distilled water. The digestive tract that has been taken was finely crushed. The suspension was then diluted with distilled water in a serial dilution of  $10^{-1}$  to  $10^{-9}$ . The dilution results were taken as much as 100  $\mu$ l and then spread using the scatter method on CMC media. Furthermore, the isolates were incubated for 24 hours in an incubator at 37°C. The bacterial colonies that have been purified were determined by representatives of each group who had the same characteristics. Colonies that were located far apart from other colonies were chosen to be replanted on CMC media until several replications (minimum three replications) until pure bacterial isolates were obtained.

### ***Biochemical Test***

Pure bacterial isolates were then conducted in a biochemical test to see bacterial characteristics. The tests carried include gram staining, TSIA test, Indol test, Motility test, SCA test, and Catalase test (Ramin *et al.*, 2008).

### ***Identification of Bacteria through 16S rRNA Sequencing***

The method used for 16S rRNA sequencing was the method that has been carried out by IPBCC (Bogor Culture Collection Institute of Agriculture). The isolated DNA was used for amplification of the 16S rRNA gene using the Polymerase Chain Reaction (PCR) machine. The primers used for 16S rRNA gene amplification are 63F primers (5'-CAG GCC TAA CAC ATG CAA GTC-3') and 1368 R (5'-GGG CGG WGT GTA CAA GGC-3') which are universal primers for various bacterial strain. The PCR machine is programmed and carried out at the condition of 94°C predenaturation for 2 minutes 45 seconds, annealing 45°C for 45 minutes, extension 72°C for 1 minute 2 seconds rotation.

Then the next stage of denaturation is 74°C for 45 seconds, annealing for 45°C for 45 seconds, extension 72°C for 1 minute and carried out 35 cycles. The final stage of amplification was carried out at 72°C for 10 minutes.

The PCR results were visualized on 1% agarose gel and stained with ethidium bromide. The amplified DNA was purified and then commercially sequenced to determine the sequence of DNA bases. The sequence data is then compared with the data in GenBank from the NCBI database (<http://www.ncbi.nlm.nih.gov>) using the BLAST program.

### ***Testing of the Ability of Decomposition of Symbiotic Bacterial Isolates in Rice Straw***

Straw decomposition was carried out by small scale testing, rice straws were chopped into small pieces with a size of 2–5 cm. After that, the straws substrate were put into a plastic bag. The substrate was separated into two parts, one substrate bag for control treatment and another one substrate bag for treatment with a bacterial starter. The amount of substrate used was 2 kg/treatment. The starter stock solution was made by planting bacteria grown on CMC media into 9 ml Nutrient Broth (NB) media. Bacteria grown on NB media were incubated for 2 times 24 hours at 37°C. After incubation, the starter solution is stored in the freezer.

Treatment by giving bacterial starter conducted with the sterilized straw substrate using autoclave at 121°C for 30 minutes. The substrate then put in a bucket, followed with a solution of 10 ml of symbiont bacteria starter. The concentration bacterial starter was 50%, that was made by adding 5 ml of the starter stock solution dissolved in 5 ml NB. For the control treatment, the straw substrate was left in a plastic bag without given any treatment.

## **RESULTS AND DISCUSSION**

### ***Isolation of Bacteria Symbiont Originating from C. curvignathus Termites Hindgut***

Based on the results of isolation that has been carried out, it was found that 1 isolate was able to grow and colonize CMC media. The isolate grown from the hindgut isolation of *C. curvignathus* termites on CMC media were then observed based on the morphology of the colonies macroscopically by looking at the form of colonies, the surface of colonies, the edges of colonies, and the color of

colonies. Macroscopic observation of bacterial morphology on CMC showed rounded colony, convex morphological surface, intact colony edge, and whitish color characteristics (Figure 1).

#### ***Identification of Bacteria Symbiont Originating from *C. curvignathus* Termites Hindgut***

Characteristic results showed that bacterial isolates were rod-shaped and gram-positive bacteria. The biochemical tests showed different results between each test (Table 1). The gelatin test showed a negative result which was characterized by a culture that still frozen after being removed from the freezer. This indicates that the bacterial isolate does not produce gelatinase enzymes that are able to hydrolyze proteins into amino acids so that the gelatin will remain liquid even at 4°C. The TSIA test also showed a negative result, there was no change in color to blackish brown so that it can be considered that bacteria are not able to ferment glucose.

Simmon's citrate test also showed a negative result, because there was no change in the color of the medium from green to blue. The catalase test showed a positive result, which was indicated by the presence of air bubbles around the colony after H<sub>2</sub>O<sub>2</sub> was added.

Based on Bergey's Manual of Determinative Bacteriology (Buchanan & Gibson, 2003) isolates obtained from the results of the bacterial symbiont isolation from hindgut *C. curvignathus* Holmgren termites showed the same morphological and physiological characteristics as the *Bacillus* genus, thus being identified in the *Bacillus* group. Some of these bacteria produce extracellular enzymes that can hydrolyze proteins and complex polysaccharides. Thayer (1976) succeeded in isolating *Bacillus cereus*, *Serratia marcescens*, and *Arthrobacter* sp. from termites *Reticulitermes hesperus*, all bacteria can grow on cellulose agar media.



Figure 1. Morphological appearance of the colony of symbiotic bacterial isolates from the hindgut of the termite *Coptotermes curvignathus* Holmgren with 4×10 magnification

Table 1. The result of the identification of the bacterial isolate

Parameter	Result	Notes
Cell shape	rod	Rod shape bacteria
Gram's reaction	(+)	Bluish bacterial cells
TSIA test	(-)	No change in color to blackish brown
Motility	(+)	The roots of the colony spread to the media
Catalase	(+)	There is an air bubble around the colony
Sitrat test	(-)	There is no change in green to blue
Gelatin	(-)	Culture media do not melt and remain frozen

### Identification of Bacterial Symbiont Species by 16S rRNA Sequencing

The identification results based on 16S rRNA sequencing from bacterial symbiont isolate from the hindgut of *C. curvignathus* termites using 63 F primers and 1368 R produced one amplicon measuring around 1200 pm (Figure 2). Based on the results of identification of the BLAST program, the results showed that the bacterial symbiont isolates originating from *C. curvignathus* termite hindgut had a close relationship with *Bacillus cereus* strain MCCC 1A00359 with 99% similarity. Ramin *et al.* (2009) found that the hindgut of *C. curvignathus* termites had also been isolated from the same bacterial species, namely *Bacillus cereus*. The first bacterial isolate was identified as *B. cereus* strain Razmin from Order Bacillales and Genus Basil (Ramin *et al.*, 2008). Several *B. cereus* strains have also been isolated and identified by Kuhnigk *et al.* (1994) from the type of termite *Mastotermes darwiniensis*.

Analysis of the 16S rRNA gene with a sequence of 18 oligonucleotides is an effective way to find out the prokaryotic taxonomy including the genus *Bacillus* and can be directly linked to data from phylogenetic trees (Fox *et al.*, 1977). Figure 3 showed that there

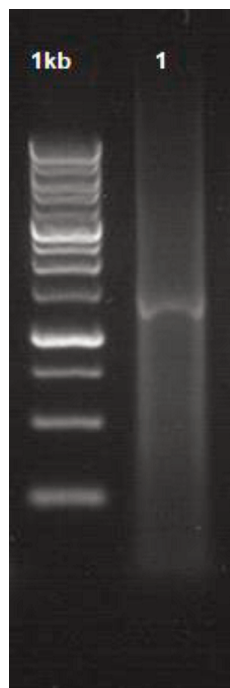


Figure 2. DNA band pattern from PCR *Bacillus cereus* analysis using 63 F and 1368 R primers, producing one amplicon of 1200 pb

are two groups of bacterial kinship division in the phylogeny tree, where *B. cereus* strain MCCC 1A00359, *B. cereus* MCCC 1A04098, *B. toyonensis*, *B. thuringiensis*, and *B. cytotoxicus* are in one group. Whilst *B. cereus* strain MCCC 1A00359 with *B. marisflavi*, *B. anthracis*, and *B. solani* are in a different group.

### Test on the Ability of Bacterial Symbiont Isolate in Rice Straw Decomposing

The test showed difference result between the decomposition process of rice straw using a starter of bacteria symbiont (*Bacillus cereus*) originating from the hindgut of *C. curvignathus* termites and without the use of a bacterial starter (control). In the 4th week, the rice straw which was given a bacteria starter had shown a change in shape (Figure 4a), whereas rice straw without the use of a bacterial symbiont (starter) had not changed in shape at all (Figure 4b). In the 12th week, the material from the control rice straw had decomposed with the appearance of the material that became brittle and destroyed (Figure 4c).

The residual weight of the substrate at the end of decomposition showed a different percentage between the addition of the bacterial starter and the control. The weight of the rest of the control rice straw material showed a percentage of 45.56% (900 g) while the residual weight of the rice straw material with the addition of symbiotic bacteria starter from hindgut *C. curvignathus* termite showed a percentage of 48.67% (971.2 g). Depreciation of weight can be caused by the change of material by microbes so that the water content of the material decreases and due to the heat generated during composting so that the evaporation occurs. Based on the "Munsel Soil Color Chart" book, the hue value and the value of the decomposition of rice straw with the symbiont starter was 7.5 YR 3/3. Murbandono (2000) suggested that giving a bacterial starter during the decomposition process can help accelerate the maturation of compost and the maturity of faster compost colors. The color of the decomposition of rice straw with bacterial symbiont starter showed dark brown color, while the color of the control treatment decomposition of rice straw was light brown which had a value of hue and values 7.5 YR 6/4. Color changes occurred due to the formation of humic compounds. Polyphenol compounds which were the result of decomposition



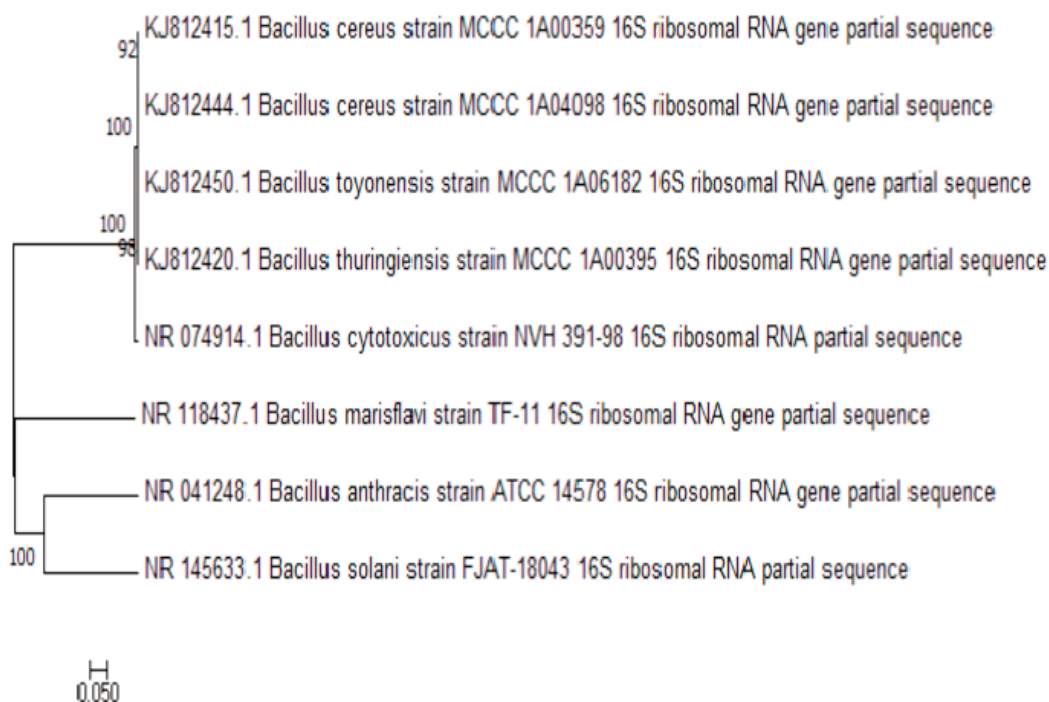


Figure 3. Tree phylogeny of termite bacterial symbiont with CLUSTAL W and 1000 replications from GenBank

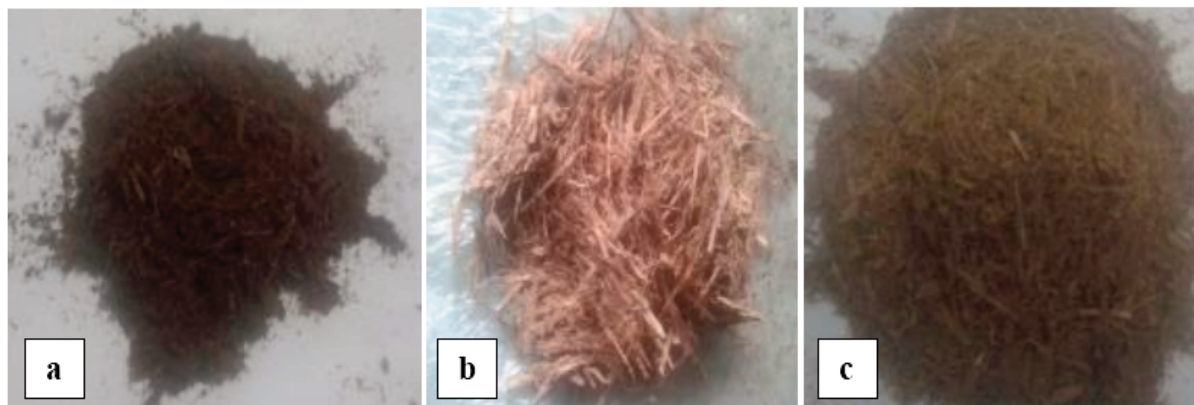


Figure 4. (a) final result of decomposition of rice straw with bacterial symbiont starter at week 4; (b) final result of decomposition of control rice straw in the week 12; (c) final result control rice straw decomposition at week 12

of lignocellulose compounds into quinones will react with amino compounds to form humic acid or fulvic acid which is dark or black.

The C-organic content on rice straw substrate with the bacterial symbiont starter was different from rice straw without bacterial symbiont (control) starter, which was 9.10 % and 11.55 %, respectively. The C/N ratio of each treatment had different value as well. The value of C/N substrate with a bacterial

starter was 13.58% while the C/N value of the control substrate was 15.4% (Table 2). Sutanto (2002) stated that microbes that play a role in the decomposition will bind nitrogen, but depend on carbon availability. If the availability carbon was limited (low C/N ratio), carbon compounds used by microbes as an energy source to bind all free nitrogen will not be enough. In this case, the amount of free nitrogen will be released in the form of

Table 2. The result of observations of rice straw decomposition

Study	Control	Starter treatment <i>Bacillus cereus</i>	SNI Straw rice compost
Color	7.5 YR 6/4 Light brown	7.5 YR 3/3 Dark brown	Dark brown
Structure	Fragile	Crumb	Crumb
Smell	Do not smell of soil	Smell of soil	Smell of soil
C-organic (%)	11.55	9.10	9.8–32
N (%)	0.75	0.67	>0.1
C/N	15.4%	13.58%	10–20%
pH	6.78	7.05	6.0–7.49
Temperature	27°C	25°C	27°C
Time	12 weeks	4 Weeks	6–7 Weeks

NH<sub>3</sub><sup>+</sup>gasses. In the decomposition process, the total carbon content will decrease, while the nitrogen content increases and the temperature stabilizes. The value of C/N at the end of composting will be stable with a relatively low value and indicates that the compost has matured (Simanungkalit *et al.*, 2012).

The final temperature of decomposition of straw with the addition of starter was at a range of 25°C, while the decomposition temperature of the control rice straw was at a range of 27°C. The ideal temperature range for composting is 55°C–65°C. At a temperature of 60°C the activity of microorganisms decreases, but the thermophilic microorganisms was at the optimum. Composting temperature below 20°C will result in low microbial decomposition activity, even the decomposition process stops. Decomposition that occurs affects the activity of microorganisms, so that increasing temperatures indicate that microorganisms are increasingly active in carrying out the degradation process.

The *Bacillus* genus has the ability to decompose organic compounds such as proteins, starch, cellulose and hydrocarbons (Clous & Barkeley, 1986). Various sources of cellulose waste such as rice straw, oil palm empty bunches, and samples of forest soil that are rich in plant litter are utilized by cellulolytic bacteria in decomposing organic matter (Ashwani *et al.*, 2014). This is in line with the research of Ardani (2016) who has managed to isolate cellulolytic bacteria from bat feces. These bacteria were identified as *Bacillus cereus* species. The lytic lignocellulose microbes are used as a starter for compost inoculants because they have the ability to produce cellulase enzymes, as well as xylanase which is able to hydrolyze lignin, cellulose and

hemicellulose compounds which are commonly contained in rice straw (Howard *et al.*, 2003).

## CONCLUSION

Based on the results of biochemical tests and sequencing of 16S rRNA bacterial isolate obtained from the hindgut of termite *C. curvignathus* were identified in the *Bacillus cereus* strain MCCC1A 00359 species and had a similarity rate of 99%. This bacteria can be used as a starter to help in rice straw decomposition process. By adding starter solution of *B. cereus* isolate with a dose of 10 ml/2 kg of straw (50% concentration) can shorten the decomposition time of rice straw which was about 4 weeks, produce crumbs, black, and have a C/N value of 13.38 % which was in accordance with SNI.

## LITERATURE CITED

- Aini, A.N. 2015. *Peranan Bakteri dalam Usus Rayap Tanah Macrotermes gilvus Hagen sebagai Agen Biologis Degradasi Bahan Organik*. Skripsi. Biologi. Fakultas MIPA. Universitas Negeri Semarang, Semarang. 62 p.
- Ardani, F. 2016. *Isolasi Bakteri Selulolitik dan Karakterisasi Enzim Selulase dari Feses Kelelawar*. Tesis. Sekolah Pascasarjana. Institut Pertanian Bogor, Bogor. 31 p.
- Ashwani, K., L. Saida, & K.V. Reddy. 2014. Isolation, Screening, and Characterization of Cellulolytic Bacteria from Forest Soil Sample. *International Journal of Current Microbiology and Applied Sciences* 3: 679–685.
- Buchanan, R.E. & N.E. Gibbons. 2003. *Bergey's Manual of Determinative Bacteriology*. The William & Wilkins Company, Baltimore, USA. 787 p.

- Clous, D. & R.C.W. Berkeley. 1986. *Genus Bacillus*, p. 1105–1139. In P.H.A. Sneath (ed.). *Bergeys Manual of Systematic Bacteriology*. Vol 2. Williams and Wilkins, Baltimore.
- Diba, F., S. Khotimah, & U. Febriyana. 2012. Isolation and Identification Cellulolytic Bacteria from the Termite *Coptotermes curvignatus* Holmgren and *Macrotermes gilvus* Hagen from Secondary Forest in West Kalimantan Indonesia, p. 105–111. In P.R. Termite Research Group (eds), *Proceedings of the 9th Pacific Rim Termite Research Group Conference*. Hanoi. February 27–28, 2012.
- Fox, G.E., K.R. Pechan, & C.R. Woese. 1977. Comparative Cataloging of 16S Ribosomal Ribonucleic Acid: Molecular Approach to Prokaryotic Systematic. *Journal of Systemic Bacteriology* 27: 44–57.
- Howard, R., L.E. Abotsi., J.V. Rensburg, & S. Howard. 2003. Lignocellulose Biotechnology: Issue of Bioconversion and Enzyme Production. *Journal African of Biotechnology* 2: 602–619.
- Kuhnigk, T., E. Borst., A. Ritter, P. Kampfer, A. Graf, H. Hertel, & H. Konig. 1994. Degradation of Lignin Monomers by the Hindgut Flora of *Xylophagous Termites*. *Journal Systemic and Applied Microbiology* 17: 76–85.
- Murbandono, L. 2000. *Membuat Kompos*. Edisi Revisi. Penebar Swadaya. Jakarta. 60 p.
- Nandi, N., F.H. Rahman., N.B. Sinha, & J.N. Hajra. 2000. Compatibility of Lignin-Degrading and Cellulose Decomposing Fungi during Decomposition of Rice Straw. *Journal of the Indian Society of Soil Science* 48: 387–389.
- Ni, J. & G. Tokuda. 2013. Lignocellulose-degrading Enzymes from Termites and their Symbiotic Microbiota. *Journal Biotechnology Advances* 31: 838–850.
- Papoola, K.O.K. & A.V. Opayele. 2012. Morphometrics of *Macrotermes bellicosus* (African mound termite) (Blattodea: Termitidae) and the Impact of its Saliva Amylase on the Strength of Termitarium Soil. *New York Science Journal* 5: 207–216.
- Ramin, M., A. Ralimon., N. Abdullah., J.M. Panandam, & K. Sijam. 2008. Isolation and Identification of Three Species of Bacteria from the Termite *Coptotermes curvignathus* (Holmgren). Present in the Vicinity of University Putra Malaysia. *Journal Microbiology* 3: 288–292.
- Ramin, M., A.R. Alimon, & N. Abdullah. 2009. Identification of Cellulolytic Bacteria Isolated from the Termite *Coptotermes curvignathus* (Holmgren). *Journal of Rapid Methods & Automation in Microbiology* 17: 103–116.
- Sutanto, R. 2002. *Pertanian Organik Menuju Pertanian Alternatif dan Berkelanjutan*. Penerbit Kanisius, Yogyakarta. 50 p.
- Simanungkalit, R.D., M.D.A Suriadikarta., R Sarswati., D. Setyorini, & W. Hartatik. 2012. *Pupuk Organik dan Pupuk Hayati*. Balai Besar Penelitian dan Pengembangan Sumber Daya Lahan Pertanian, Bogor. 283 p.
- Singhania. 2009. Cellulolytic Enzymes, p. 371–381. In P. Singh., Nigam., A Pandey (eds.), *Biotechnology for Agro-Industrial Residues Utilization*. Springer, Dordrecht.
- Subekti, N., D.Nandika., D. Duryadi, S. Suryokusumo, & S. Anwar. 2008. Sebaran dan Karakter Morfologi Rayap Tanah *Macrotermes gilvus* Hagen di Habitat Hutan Alam. *Jurnal Ilmu dan Teknologi Hasil Hutan* 1: 27–33.
- Thayer, D.W. 1976. Facultative Wood-digesting Bacteria from the Hindgut of the Termite *Reticulitermes hesperus*. *Journal of General Microbiology* 95: 287–296.