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Isolation and Activity of Cellulolytic Bacteria Isolated from Hindgut of Odontotermes spa Subteran Termite On Wasian (Elmerrelia celebica L.) an Endemic Wood to North Sulawesi

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Abstract- The aim of this study was to determine the cellulolytic bacterial isolates originating from the gastrointestinal tract (hindgut) of Odontotermes sp subteran termites live in Wasian wood (*Elmerelia celebica*) which is an endemic wood to North Sulawesi. The isolation was consisted of the study of potentially cellulolytic bacteria include the number of colonies, population density, cellulolytic index (ratio between the diameter of the clear zone and colony diameter) while the characterization study were about the incubation time , temperature and pH of cellulolytic enzyme activity (nkat / ml). The results showed that the average number of bacterial colonies obtained ranged from 1542.23 to 2803 colony. Average number density of the bacterial population was 3.08 x 106 per ml to 5.01 x106 CFU/ml. There were four isolates BHGR1 (*Microbacterium sp*), BHGR4 (*Bacillus sp*) , BHGR5 (*Microbacterium sp*), and BHGR8 (*Eubacterium sp*) which showed cellulolytic activity on congo red test using CMC media. Optimal daily cellulolytic activity for all three isolates HGR2 , HGR5 and HGR8 was shown on day 5, day 3 and day 7, respectively. The optimum temperature of the three isolates were 30^0C , 30^0C and 40^0C while the optimum pH were 4.0, 5.5 and 6.0, respectively. Specific cellulolytic enzyme activity of the isolates had resulted the HGR5 isolate as the highest at 2.5 (nkat / mg) with optimal daily production activities on day 3 at pH of 5.5 and a temperature of 30^0C, followed by HGR2 at 1.6 (nkat / mg) on day 5 , pH 5.0 and temperature 30^0C, then HGR8 isolateat 0.9 (nkat / mg) on day 7, pH 6.0 and at 40^0C, respectively.

Keywords- *Isolation, activity,cellulolytic bacteria,wasian, endemic wood*

I. INTRODUCTION

Climatic and soil conditions, including many kinds of plant species in Indonesia are very supportive to termites's life (Nandika *et al*.,2003). Termites are known as wood destroying pests (Husseneder *et al*., 2005) because of their main food which is wood and other material containing cellulose. Wood-eating termites as *Odontotermes sp* is a subteran termites spread over Southeast Asia, and in most cases attacked plants in Indonesia (Nandika *et al* , 2003). One important role of termites are able to produce useful metabolites and enzymes to degrade complex compounds such as polysaccharides. Theimportance of this insects is due to its ability to describe the fibrous material containing cellulose, hemicellulose and lignin (Harazona et al . 2003) . Termites are able to decompose cellulose significant portion (74-99 %) and hemicellulose (65-87 %) (Ohkuma , 2003). The ability to decompose cellulose , because the digestive tract of termites contain microbial flora. The digestive tract in high level termites Odontotermes obesus contains bacterial microflora such as: spirochaeta, pseudomonas and actinomycetes . These bacteria play a role in decomposing cellulose and hemicellulose (Paul et al. 1993). Innoculated dominant bacteria of Odontotermes formosanus digestive tract is a genus of Bacillus which acts as lignocellulose degrading agent andfungi growth supressor agent (Mathew et al. 2011). Microbial population density is quite high in the gastrointestinal tract, showing approximately 1012 per ml (Bignell, 2000). Number of microbes in the termite varies among the same colony (Minkley et al. 2005), because the termites that live in the same colony have the same nutrient sources (Matsuura, 2001). Geographical differences of termite colonies and ecological conditions allow distinction of different sources of nutrients. Differences in the chemical components of food allows microbial differences (Hussender et al 2005). Therefore, the number of bacterial colonies and population density of local termiteOdontotermes digestive tract need to be investigated .

In addition, the role of cellulolytic potentially bacteria is highly depend on the termite digestive system tool that consists of multiple compartments, such as forgut, midgut and hindgut, which contains a section hindgut intestinal microbiota and is often regarded as the "fermentation chamber" (Brune and Friedrich, 2000) . Fermentation chambers in the gut of termites is analogous to the rumen ruminants sheep and oxen, which is often regarded as the smallest container compared with rumen fermentation ruminants (Brune, 1998). Ecological conditions of termite fermentation container allows the growth of microorganisms, including bacteria, protozoa and fungi (Wenzel et al. 2002) which has the main function to decompose fiber food materials (Brune, 2007).

Many studies have reported that cellulose decomposition is done by bacteria found in the digestive tract (Breznak and Brune , 1994 ; Prabowo et al 2007 , Cho et al . , 2010 , Lee et al . , 2010) Cellulolytic activitiesfrom several termites had been reported by Hetheder et al . , 1991 ; Purwadaria et al (2003) ; Zhou & Smith (2007) and Lee et al . , (2010) , but there were still a few research on its characteristics and activities. To the best of the author's knowledge, the research on the cellulolytic potential of bacterial isolate from local termites Odontotermes, which lived in wasian, a type of wood endemic to Sulawesi have not been reported. Geographical differences in termite colonies had resulted in ecological differences as well as nutrition sources differences . Differences in the chemical components of the food might possibly caused in microbial differences (Hussender et al 2005). In addition, many symbiotic bacteria in the gut are difficult to cultivate still many important aspects of their role in the metabolim system are not yet known (Radek , 1999) . Therefore, searchingmicrobes of local termite digestive tract that live in natural habitats is so crucial, especially termites that live in wasian an endemic wood to Sulawesi.

II. Materials And Methods

2.1 Termite Samples

The source of the isolates comes from the digestive tract (hindgut) of worker Odontotermes sp supplied by the neighborhood people of rural wasian wood forest at Rumoong village, South Minahasa regency.

2.2 Isolation of Bacteria from Termite Digestive Tract

The isolation technique of bacteria from the gut of termites was performed under Huseneder et al (2009) method. Ten head of worker termites anesthetized using ice, mouth and anus termites smeared with paraffin and surface sterilized using 80% ethanol to avoid contamination. Intestines were separated using sterile surgical instruments. Separated from the whole midgut and hindgut homogenized in 10 ml of sterile water in a sterile glass container and then subjected to vortex for 4 minutes.

Isolation of bacteria was carried out using the modified spread plate method by Lindquist (2005). A total of one ml suspension of each bacterial colony was suspended into 9 ml of SDW in a test tube, then diluted up to 10,000 times. Further dilution of each suspension were taken using a 0.5 ml micropipette and was plated by the spread plate method in NA media. The vials after wrapped with parafilm, were incubated in light banks in the inverted position for 48 hours.

Bacterial colonies on NA were calculated to determine the density of the bacterial population. Bacterial colonies which were different in margin, elevation, color, shape and surface structure were then purified slant media.

2.3 Isolation and Selection of cellulolytic bacteria

Pure isolateswere cultivated and spotted on enrichment media to see the cellulolytic activity. Induction used medium was a medium containing 5 g / L carboxymethyl-celulose (CMC). The isolates were incubated at 30^0C for 3 days. One milliliter of culture was transferred to nutrient agar petri dishes and incubated for 24 hours at 30^0C. Colony isolates were made in a series of culture. Another used technique as was done by Wensel et al (2002) to isolate bacteria in workerstermite. The conditions at preparation stage was the same, except for the medium. After the termite gut separated, mixed with 15 mL of medium containing 5g / L CMC, 0.1 g / L malt extract, 0.04 g / L yeast extract and 2 g / L CaCO3 at 6.7 pH. The cultures were incubated for 4 weeks at a temperature of 30^0C. After incubation, 1 mL was transferred on agar (medium with supplements 12 g / L agar) and incubated for 24 h at 30^0C.

Cellulolytic isolates that have potential in induction media will form a clear zone around the colony. Observation of clear zone was punctuated by the addition of 1% congo red. Clear zone was measured to determine the cellulolytic index (IM), which was the ratio between the diameter of the clear zone and colony diameter (Aurora *et al*., 2003).

2.4 Identification of Bacterial Isolates

Bacterial isolates were identified by biochemical analysis through several stages. The first step was gram staining using a staining test by Lay (1994), followed by motility testing by Cappuccino and Sherman (1992), carbohydrate fermentation test, indole by Lay (1994), methyl red test by Cappuccino and Sherman (1992), Voges Proskauer test (Lay, 1994), citrate and oxidase test by Ijong (2003) and catalase test by Lay (1994), respectively.

2.5 Cellulolytic activity test

Selected bacteria isolates were rejuvenated in CMC medium and grown at room temperature for ± 48 hours. The ioculum was made by taking a 1-2 inoculum colony and grown in 10 ml of liquid CMC medium in a test tube, and then incubated at room temperature for 24 hours. A total of 1 ml of inoculum cultivated in Erlenmeyer flasks each containing 100 ml of liquid media CMC. Incubation was performed in an incubator shaker with a speed of 80 rpm at room temperature. Enzyme activity measurements performed every 12 hours for each isolate. Enzyme crude extract obtained by centrifugation of the culture at 2860 rpm for 25 min at 4^0C. Enzyme crude extract used for enzyme activity assays and protein levels. Enzyme activity was measured by the formation of reducing sugars using the DNS (Dinitrosalicylic Acid) (Miller, 1959). Cellulase enzyme activity wasmeasured by calculating µmol enzyme per minute using the formula which shown below:

Acticity (nKat/ml)= $((S-K)\ x\ 1000\ x\ Fp\ x\ 16.67)/(incubation\ time\ x\ sugar\ MW)$

Description:
S: reducing sugar concentration of samples
K: reducing sugar concentration of control
Fp: the dilution factor

Enzyme specific activity can be calculated based on the obtained enzyme activity value divided by protein content. Protein assays was performed using the method of Bradford (1976) and BSA as a standard.

III. RESULT AND DISCUSSION

3.1 Termite Specimen

The source of the isolates comes from the digestive tract (hindgut) of worker *Odontotermes sp* collected from rural wasian wood forest at Rumoong village, South Minahasa regency (Figure 1 & 2)

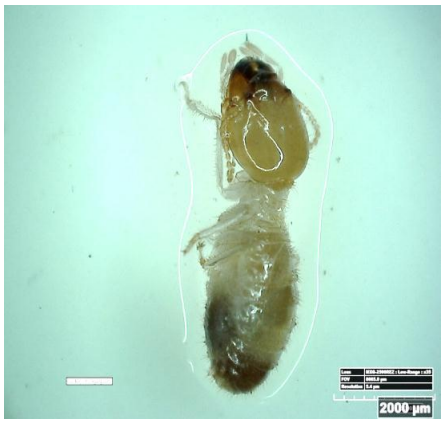


Figure 1. Worker Termite odontotermes sp.

Figure 2. Digestive tract of odontotermes sp.

3.2 Bacterial isolation from hindgut

The colonies of bacteria on NA media was shown on Figure 3.

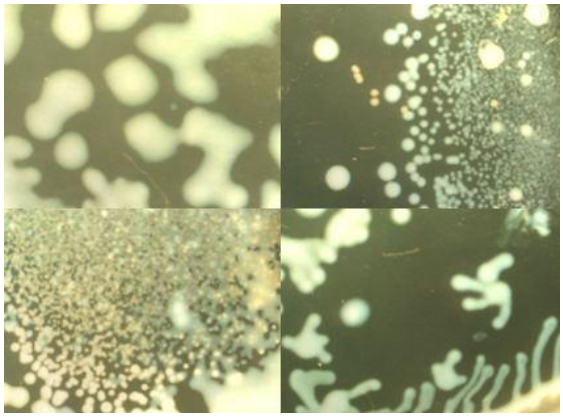
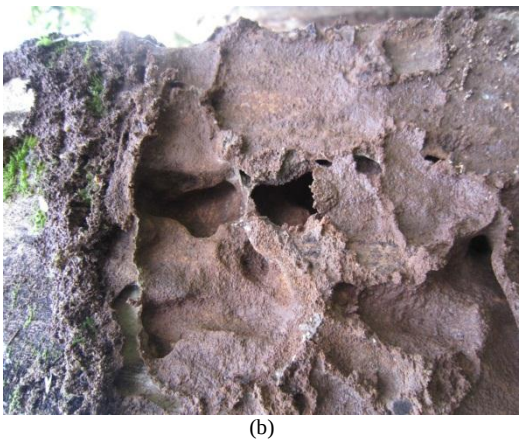


Figure 3. Bacterial colonies on NA media from the hindgut of odontotermes sp

The number of obtained colonies were counted as well asits bacterial density populations . The average number of bacterial colonies obtained ranged from 1542.23 to 2803 colony . The number of colonies were different on each sampling site. The highest number of colonies was found in the digestive tract of termites Odontotermes sp derived from Wasian dead wood compared to colonies taken from alive Wasian . Likewise, the average bacterial density . Average number density of the bacterial population is 3.08 x 106 per ml to 5.01 x106 CFU / ml . The result obtained is quite high when compared with the population density of cellulolytic bacteria extracted from Saperda vestita (Coleoptera : cerambycidae), Ips pini and Dendroctonus frontalis (Coleoptera : Curculionidae) which ranged between 2.4 x105 to 3.6 x106 CFU / gut (Delabira *et al* . , 2005) . This suggested that the termites that live in dead Wasian wood has undergone a process of weathering / decay , their digestive tracts had a higher bacterial population than the termites that live in alive Wasian wood . A research conducted by Husseneder et al (2009) showed that the difference in termites habitats (in nature and in laboratory conditions) showed differences in the composition of the number and type of bacteria obtained.

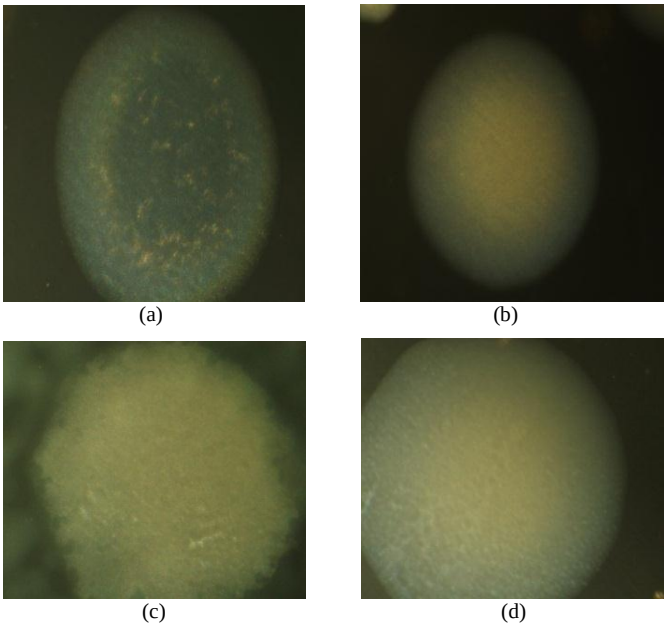
(a)



(b)

Figure 4. (a) and (b) The alive Wasian wood and the decay one as a sampling site of Odontotermes sp

Bacterial colonies that have differences in margin, elevation, color, shape and surface structure were then purified and cultured onslant media. Fourth isolates have bacterial colonies with different characteristics and colors (5). HGR2 isolate has a yellow color, round and smooth. HGR4 isolate has a transparent light blue color, the edges are uneven, irregular shape and spreaded.



(a) (b) (c) (d)

Figure 5. Characteristic of purified colonies. (a) HGR 2 isolate, (b) HGR 4, (c) HGR 5 and (d) HGR 8

HGR5 isolate has a milky white color, flat edge, slippery and slimy, while HGR8 has white, wavy edges and round shape. The fourth isolate obtained is then purified in a slanted media to test the cellulolytic potential activity.

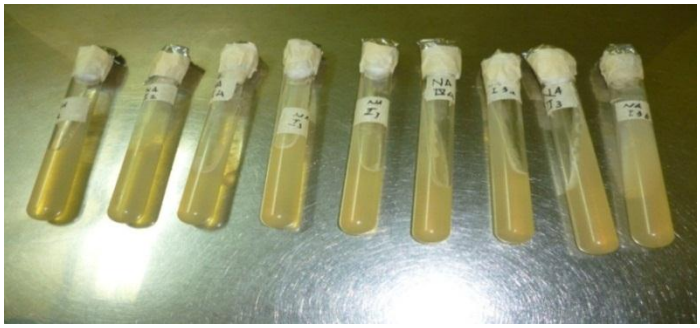


Figure 6. Purufied Bacterial isolates cultured on slant media

The use of SEM to observe the presence of bacteria in the digestive tract of *Odontotermes sp* hindgut (Figure 7).

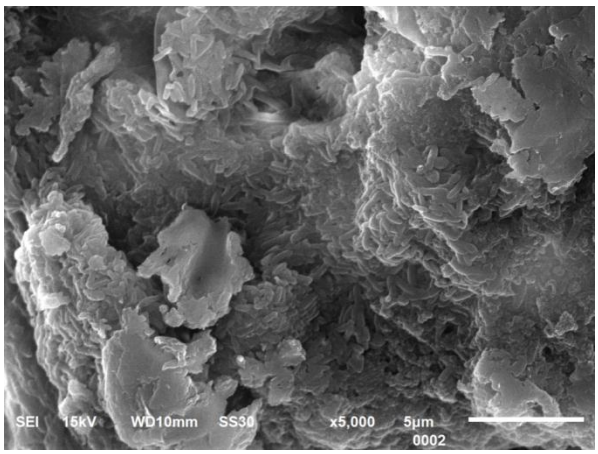


Figure 7. Cellulolytic bacteria in the digestive tract of *Odontotermes sp* hindgut captured by SEM to 5000 magnification.

Analysis of biochemical tests had resulted in the identification of bacteria species. The result wasshown in Table 1.

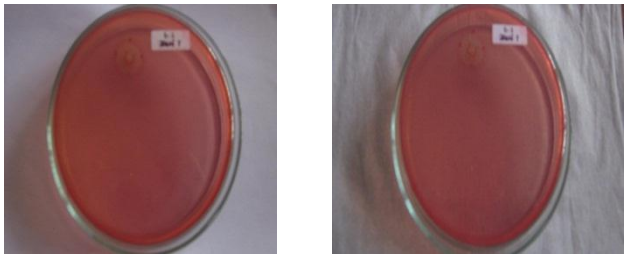
TABLE I. IDENTIFICATION OF BACTERIAL ISOLATE IN ODONTOTERMES SP HINDGUT

Code	Gram Staining	Motility	Catalase	Oxida-se	Indol	Methyl Red	Voges Proska-uer	Citrate	Carbohydrate Fermentation				Species
									G	L	S	M	
HGR2	+ Cocobasil	-	-	+	-	-	-	-	AG	AwG	AwG	AG	Microbacterium sp
HGR4	+ Cocobasil	+	-	+	+	-	-	-	AG	AG	AG	AG	Bacillus sp
HGR5	+ Basil	-	-	+	-	-	-	-	AG	AwG	AG	AwG	Microbacterium sp
HGR8	+ Cocobasil	+	-	+	-	-	-	-	AG	AG	AG	AwG	Eubacterium sp

All isolates were Gram positive, coco-basil in shaped, except for HGR5 shape which was bacillus isolate. Identification results showed that HGR5 and HGR2 belong to Microbacterium sp, HGR4 to Bacillus sp, whereas HGR8 was to Eubacterium sp.

3.3 Selection of potentially cellulolytic bacteria

Screening procedure was conducted after isolating and identifying the isolates. All bacterial cultures were isolated from the termite hindgut Odontotermes sp were grown at 50^0C on a screening media (Congo red-cellulose agar) to produce clear zone (Figure 8).



(a) (b)

Figure 8. Clear zone on a screening media (Congo red-cellulose agar) after 48 h'ours incubation.

CMC is a soluble pure cellulose or an amorphous cellulose which is more easily hydrolyzed than if taken from natural ones that is still bound to lignin and hemicellulose and still has a high crystalline structure (insoluble) (Scharf and Tartar, 2008). Carboxymethylcellulose, measuring the activity of endo-β-1,4-glucanase, which is an important artificial substrate for measuring cellulase activity due to the high solubility of these compounds in the water. CMC is used as a test medium to study the decomposition of cellulose in termites and other insects (Tokuda et al 2005). Thus, isolates that produce the biggest diameter of clear zone is considered to have the highest cellulolytic activity.

The fourth isolate has the ability to degrade CMC substrate. It can be seen from the clear zone around the colony. The formation of clear zone indicates that the CMC substrate agar is hydrolyzed by cellulase. Theability to form clear zones on CMC substrate showed the enzyme endo - β - 1 ,4 - glucanase can break the bonds of 1.4 glycosides on the cellulase fibers randomly and the number of amorphous regions on a substrate can hydrolyze CMC more efficiently (Goto et al., 1992). Several previous studies have reported the aforementioned fact as it happened in Reticulitermes Hesperus (Thayer, 1976), in bacteria actinoycetes on various termites (Watanabe et al., 2003) and in potentially cellulolytic bacteria from Nasutitermes lujae (Hethener et al., 1992) . Ramin et al. (2009) have managed to obtain and identify the bacterial isolates derived from intestinal cellulolytic local termite Coptotermes curvignathus (Holmgren), namely Bacillus cereus, Enterobacter aerogenes, Enterobacter cloacae, Cryseobacterium kwangyangense, and Acinetobacter. All bacteria were able to grow on CMC and cellobiose media at temperature of 39^0c and pH 7.1 . The addition of congo red dye can clarify the diameter of clear zone. Formation of a clear zone around the colony explains the secretion of extracellular cellulase. Four isolates HGR2, HGR4, HGR5, HGR8 (Table 2) that grown at 50^0C were then selected for further study.The measurement of isolates clear zone diameter showed that the highest clear zone was onHGR81.666 followed by HGR4 1.300, HGR51,166 and HGR2 1.083, respectively. Those cellulolytic index value were higher than the isolate cellulolytic bacteria isolates obtained from forest soil (Hatami et al . 2008). Thus each hindgut termites bacterial isolate showed cellulolytic activity on Congo - red test . As was done by Upadhyaya et al. (2012) which obtained several strains of bacteria from the gut of termites showed cellulolytic activity through the congo - red test and have identified cellulolytic bacteria such as Citrobacter, Enterobacter and Cellulomonas.

TABLE II. THE RESULT OF SELECTION OF CELLULOLYTIC BACTERIAL ISOLATES AT 50^0C FOR 48 HOURS AND THEIR CELLULOLYTIC POTENTIAL INDEX

No	Code	Species	Growth Test at 50^0C	Cellulolytic Potential Index
1	HGR 2	Microbacterium sp	+	1.083
2	HGR 4	Bacillus sp	+	1.300
3	HGR 5	Microbacterium sp	+	1.166
4	HGR 8	Eubacterium sp	+	1.666

3.4 Cellulolytic Enzyme activityof Odontotermes sp hindgut bacterial isolates

Bacteria isolated from hindgut subteran termite can grow on CMC media. Cellulolytic bacterial isolates (HGR 2) began to enter the exponential phase on day 5, and then the activity decreased afterward (Figure 9). Exponential phase is the phase in which the bacteria grow optimally or the fastest period of bacterial growth.Differences on bacterial growth show physiological diversity and responsestoward physical conditions in the environment (Pelczar and Chan, 2007).

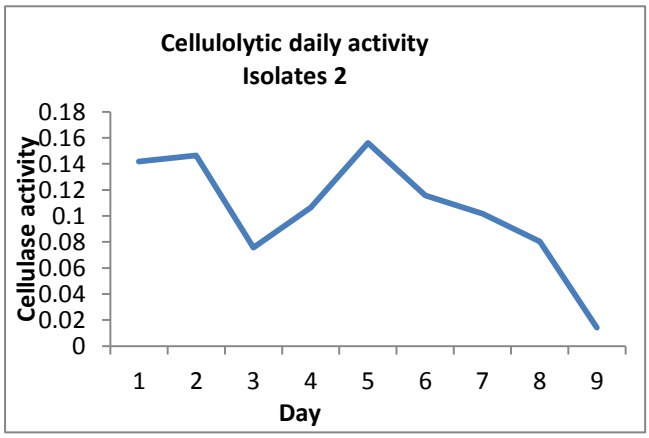


Figure 9. Cellulolytic Bacterial Daily Activity Curve of HGR2

It was not easy to determine cellulase activity of HGR4 isolate due to the unstable productivity of daily enzyme as can be seen on Figure 10.

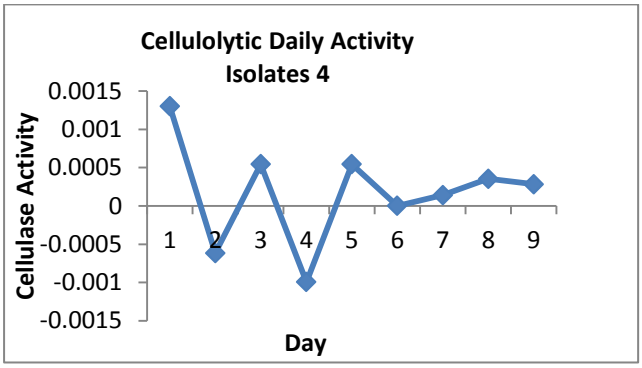


Figure 10. Cellulolytic Bacterial Daily Activity Curve of HGR4

Cellulolytic activity of HGR5 began entering maximum cellulolytic enzyme production at day 3. (Figure 11)

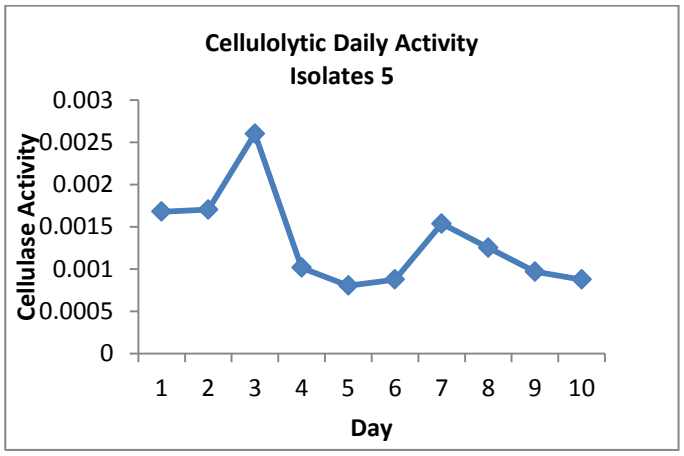


Figure 11. Cellulolytic Bacterial Daily Activity Curve of HGR5

Optimum daily cellulolytic enzyme production of HGR8 began to enter the exponential phase at day 7. (Figure 12)

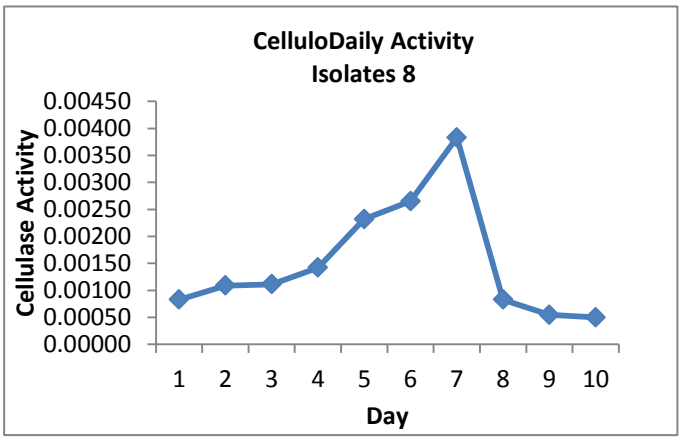
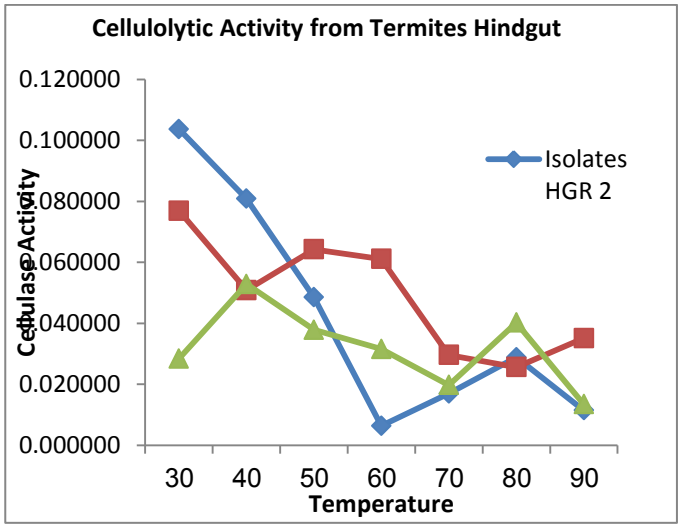


Figure 12. Cellulolytic Bacterial Daily Activity Curve of HGR8

The isolates which were grown on CMC media have different optimum time to produce the enzyme. It showed a physiological diversity between each isolates in harnessing carbon source (CMC). Cellulolytic enzyme reached the highest activity on the third day of incubation as shown by HGR5 isolate (Figure 11) followed by HGR2 (figure 9), HGR8 (Figure 12), respectively. This means that the enzyme can work optimally at the certain incubation time. When it reached the optimum time, the bacteria secrete enzymes optimally. Enzyme activity increases as its bacterial cells gorwth. But, when the cells reach stationary phase, the activity decreases. This is due to the stationary phase of cell division rate equal to the rate of cell death as well as cell lysis, at this stage there is possibility that another enzyme is produced, whereas HGR2 isolate was at the stationary phase, when enzyme activity fluctuated. This is due to the uneven degradation time ofsubstrate cellulose bacteria, besides the facts that happen during the incubation time of the complex enzyme which work at different times or appear to produce many enzymes. The optimum cell conditions in the inoculum as well as the optimum number of cells affect the enzyme activity. HGR2 isolateshowed more than one enzyme activity peaks. The activity of enzymes that having more than one peak may due to the presence of different isoenzymes which are proteins that can catalyze the same reaction also can inhibit the action of the enzyme activity (Madigan & Martinko, 2006). On these three isolates HGR2, HGR5 and HGR8, enzyme activity decreased until its death phase. This suggests that competition to find the substrate occurs in this phase so for the bacterial cells are not getting the carbon source will experience death. With the reduced number of bacterial cells, the production of enzyme is also decreased. The most decreased isolate was HGR2 followed by HGR8 and HGR5. So it can be concluded that the long period of time can affect the stability of the enzyme and can reduce enzyme activity.

Temperature and pH value can also affect the value of specific enzyme activity of each isolates. The highest specific cellulolytic activity value was HGR5 isolate 2.5 (nkat / mg) on day 3 and on pH 5.5 at a temperature of 30^0C, followed by HGR2 specific activity value 1.6 (nkat / mg) on day 5, pH 5.0 and temperature 30^0C, HGR8 0.9 (nkat / mg) on day 7, pH 6.0 and at a temperature of 40^0C. Increase in the value of specific activity in accordance with the increase in enzyme activity. Comparison of the specific activities among the three isolates can be seen in Figure 13 and 14.



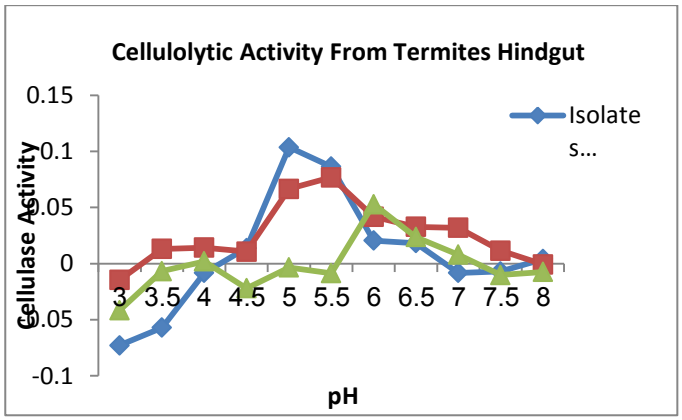


Figure 13. Comparison of the specific activities on the isolates from Odontotermes sp hindgut at different temperature

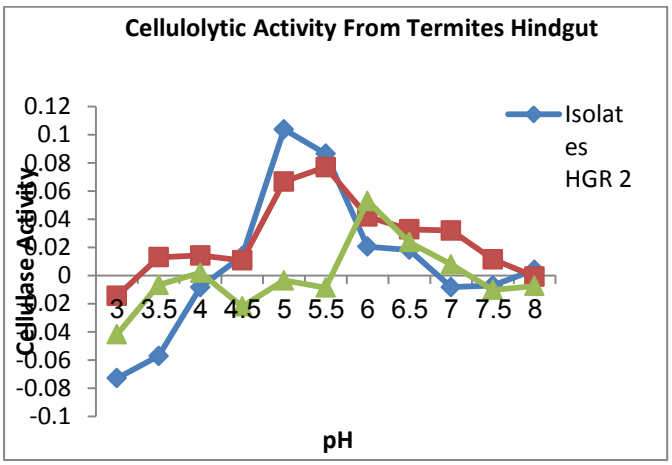


Figure 14. Comparison of the specific activities on the isolates from Odontotermes sp hindgut at different pH

The ability of microorganisms growth depends on pH, temperature, incubation time and substrate concentration(Fardiaz, 1992). In this study, the specific activity of the cellulolytic enzyme was influenced by pH and temperature. Cellulolytic activity of HGR2 isolate have optimal activity at a temperature of 30^0C and the higher the temperature the more diminished enzyme activity. On HGR5 isolate, optimum cellulolytic activity is at 30^0C, but this isolate showed higher cellulolytic activity at 50^0C compare to other isolates. HGR8 isolate exhibited highest activity at 40^0C.Thus, temperature can affect the optimum cellulolytic activity. Each of these isolates showed different activities at the same temperature. As reported by Ramin et al (2009) that the cellulolytic bacteria from the gut of local termites Coptotermes curvignathus (Holmgren), namely Bacillus cereus, Enterobacter aerogenes, Enterobacter cloacae, Cryseobacterium kwangyangense, and Acinetobacter, can grow on CMC and cellobiose at 39^0C and pH 7,1. The higher the temperature,the lower the cellulolytic activity. This may occur due to the denaturation of protein which further decreases the enzyme activity. Based on Michaelis - Menten coeficient, lower substrate concentrations can reduce reduce the enzyme decomposition capacity . Further study could be directed to termite hindgut microbial DNA especially DNA cloning to obtain maximum enzyme results.

Cellulolytic enzyme activity on three isolates showed different activity at different pH conditions. HGR2 isolate showed optimal activity at pH 5, whereas HGR5 at pH 5.5 and HGR8 at pH 6. This suggests that the range of pH from 5 to 6 is the optimal pH conditions for cellulolytic activity among the three isolates. The results are not much different from the previous research conducted by Purwadaria *et al*. (2003) on Glyptotermes Montanustermites whichexhibited a high activity ofendo-β-D-1,4-glucanase (CMCase), but showed low activity of avicelase, β-D-1,4-mannase, β-D-1, 4 - xylanase, and β-D-1,4-glucosidase. The optimum pH for CMCase enzyme, β-D-1,4-mannase, and β-D-1, 4 - glucosidase was 6.2, 5.0 and 5.8 and a optimum temperature row between 45-55^0C, 50 -55^0C, 42-45^0C respectively.

Catalytic value of the three isolates HGR2, HGR5 and HGR8 are 1.5 , 1.6 and 2.5 nkat / mg . Cellulolytic catalytic value obtained in this study was higher than the cellulase activity of the four invertebrates were reported by Gupta et al (2012). Extracellular cellulase activity offilter papercellulace (FPC) only range from 0.012 to 0.196 IU/mL whilefor endoglucanaserange between 0.162 to 0.400 IU/ml. Thus, the activity of cellulolytic bacteria isolated from local subteran termite hindgut living in North Sulawesi endemic wood become very important information for designing industrial processes especially for producing biofuels and lignocellulosic -based materials as well as for developing eco-friendly bioinsecticide technology through the use of termite hindgut cellulolytic bacteria. Comparing to the current developed methods for obtaining glucose from lignocellulosic biomass is less effective if it is done by outlining plants biomass. Insects have endogenous enzymes and symbionts enzymes which is more efficient if using lignocellulosic materials as a source of glucose metabolic (Willis *et al*., 2010)

IV. CONCLUSION

1. The average number of obtained bacterial colonies ranged from 1542.23 to 2803. The average number of bacterial population density is 3.08 x 106 per ml to 5.01 x106 CFU / ml .
2. There are four bacterial isolates namely BHGR2 (*Microbacterium sp*), BHGR4 (Bacillus sp), BHGR5 (Microbacterium sp), and BHGR8 (Eubacterium sp) which showed cellulolytic activity on congo red test using CMC media .
3. Optimal daily cellulolytic activity for all three isolates HGR2 , HGR5 and HGR 8 was on day 5, day 3 and day 7. The optimum temperature for the three isolates was 30^0C, 30^0C and 40^0C while optimum pH was 4 , 5.5 and 6, respectively.
4. Specific cellulolytic enzyme activity among the three isolates showed the highest value for HGR5 at 2.5 (nkat / mg) with the optimal daily production activities on day 3 on pH 5.5 and a temperature of 30^0C, followed by HGR2 at 1.6 (nkat / mg) on day 5 , pH 5.0 and a temperature of 30^0C , HGR8 at 0.9 (nkat / mg) on day 7, pH 6.0 and a temperature of 40^0C

V. SUGGESTION

This research should be continued to identify each isolate and expand the scope on existing local termite species in North Sulawesi. Further research may proceed to microbial diversity using culture method based on comparison of differentgeographical and ecological environment. It is also needed to exploreseveral groups of beneficial bacteria such as nitrogen fixation bacteria, cellulose degrading bacteria, acetogenic bacteria, termites nutritional function and its relationship to ingut microbial ecology. Microbial identification using DNA test is also consider as an urge.

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