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SCREENING AND IDENTIFICATION OF SUPERIOR LIGNOCELLULOSE DEGRADING BACTERIA FROM TERMITES

Mudita I M[1]*, I G. Mahardika[1], I. B. G. Partama[1], I N. Sujaya[2], N. N. Suryani[1], I W. Suarna[1]

1. Faculty of Animal Husbandry, Udayana University, Denpasar - Bali, Indonesia.
2. Faculty of Public Health, Udayana University, Denpasar - Bali, Indonesia.

ABSTRACT: A research had been carried out to screening and identification of superior lignocellulose degrading bacteria isolates from termites. Screening of lignocellulose bacteria degrader based on lignocellulase specific enzyme activities such as ligninase, cellulase (endoglucanase and exoglucanase) and xylanase. Identification of superior bacteria isolates carried out by molecular biology technologies (PCR).The result showed that from termites has isolation 36isolates of lignocellulosic degrading bacteria such as 10 lignocellulolytic bacteria, 7 lignolytic bacteria, 9 cellulolytic bacteria and 10 xylanolytic bacteria isolates. Based on lignocellulase specific enzyme activities, thesuperior lignocellulolytic bacteria is *Aneurinibacillus sp. strain BT4LS* (homology 99% with Acc. No. KR063553); superior lignolytic bacteria is "*Aneurinibacillus sp. strain BT5LG"* (homology 81% with Acc. No. KP980744); the superior cellulolytic bacteria is "*Bacillus sp. strain BT3CL"* (homology 100% with Acc. No. KX879851), and the xylanolytic bacteria "*Bacillus sp. strain BT8XY"* (homology 100%with Acc. No. KT981879).

KEYWORDS: Lignocellulolytic bacteria, Identification, characterization, termites.

Corresponding Author: I Made Mudita, SPt., MP

Faculty of Animal Husbandry, Udayana University, Denpasar - Bali, Indonesia.

Email Address: muditafapet_unud@yahoo.com

1.INTRODUCTION

The development of "Simantri" (*Sistim pertanian terintegrasi*-In Indonesia or *integrated farming system*-In English) were ones local model featured for empowerment of the public rural on economic development based on agriculture in Bali. Simantri program use oriented on development agricultural-livestock business without (zero) waste through utilization agricultural waste as feed,

livestock waste such as urine, feces and feed rest as fertilizer and biogas/energy. Eventhough, the utilization agricultural waste has various limitations mainly due to the highly lignocellulose content were contrains utilization for livestock including ruminants such as sapi bali[1, 2]. Lignocellulose is composed of three polymers compounds, including lignin, cellulose and hemicellulose which form complex compounds with strongly bonding[3,4]. Completely degradation of its three polymer will supplyall potential nutrients contents. Lignocellulosic compounds can only be degraded by certain microbes, such as lignocellulolytic bacteria. So the effort to develop lignocellulolytic bacteria is very important in optimizing utilization agricultural waste as feedstuffs [5, 6, 7, 8]. Termites is one of resources of lignocellulolytic bacteria isolates. Termites is an insect feeding wood higly lignocellulose. The body cell, saliva and gut tract of termites containing various microbes and fiber degrading enzyme [9, 10]. Purwadaria*et al.*[10] so reported that on digestive stract of termites there are bacteria, protozoa and fungi were produce cellulase and hemicellulase enzyme. Ramin *et al*. [11] has isolation cellulolytic bacteria from termite *Coptotermen curnignathus* were *Bacillus cereus Razmin, Enterobacter aerogenes Razmin* and *Chryseobacterium kwangyangense Strain Cb*. Tresnawati*et al.* [10] reported that the fresh termites extract has the enzyme activity on rice bran of 25,30 μmol.g^{-1} DM, 8,32 μmol.g^{-1} DM on wheat pollard, 0,56 μmol.g^{-1} DM on soybean meal, and 0,17 μmol.g^{-1} DM on corn meal. Based on the ability of lignocellulosic substrates degradation, bacteria isolatedfrom termites was made to explore for optimising utilization agricultural waste as feedstuff on the development Simantri Program in Bali.

2. MATERIALS AND METHODS

Isolation of source and sample preparation

The sample of termites taken from wood that is decaying that there are around the area of Research Stasiun of Faculty of Animal Husbandry Udayana University, Bukit Jimbaran, Badung Regency, Bali Provence-Indonesian Country. As many as 15 – 20 termites crushed using a mortal and than suspension with 10 ml NaCl 0,85% soluton. The solution used as a source of bacterial isolates.

Growth Media and Isolation Activities

The solution source of bacterial isolate samples were grown in selective media by Hungate method (Media No. 6 on Ogimotoand Imai) [12] were countaining 0,02g Monopotassium phosphate (KH_2PO_4); 0,03g Dipotassium phosphate (K_2HPO4); 0,01g Magnesium Sulfate ($MgSO_4$); 0,01g Calcium Tetrachloride ($CaCl_4$); 0,10g Sodium Cloride (NaCl); 0,10g Ammonium Sulfate {$(NH4)_2SO_4$}; 0,10ml Rezasurin 0,1% solution; 0,02g Cystein-HCl.H_2O; 0,40g Sodium Carbonate (Na_2CO_3); 30,00 ml rumen liquid; 1,00g substrate; 70,00ml Aquadest and 1,8% Agar. Selective substrate used were tannic acid (as lignin source), xylan (as hemicellulose source) and Carboxy Methyl Cellulose/CMC (as cellulose source) or combination tannic acid-CMC-xylan (as lignocellulose source). All ingredients were mixed in erlenmeyer, pH was determined 6.8 and heated until all ingredients dissolved. The flask then transferred aseptically with oxygen-free CO_2 gas

displacing all air, sealed and then sterilized in the autoclave at 121ºC for 15 minutes. The solution source of bacterial isolate samples was transferred aseptically to a petri disc with oxygen free CO_2 gas displacing all air. Then medium in the enlenmeyer flask was inoculated into the petri disc and closed. The culture was incubated at 39ºC for 3 -5 days. The colonies thus grown on this media were selected for the isolation

Purification of Lignocellulolytic Bacteria Isolates

The individual bacteria colonies thus grown on solid selective growth media were pricked using ose needle. The bacteria were then transferred to plate selective growth media (solid) anaerobically with gasses oxygen-free CO_2 with *streak quadrant method.*. The plate was incubated at 39ºC for 3 – 5 days. The bacteria colonies thus grown and that is invisible show single colony were transferred to tube containing selective media (aslant) for incubation and than were evaluated purification with gram staining method used *Gram Stain Kit Merk Pronadisa.*

Lignoselulase specific enzyme activity

Specific enzyme activity (unit/U) (μmol/g/minute) of lignocellulolytic bacteria based on degrading ability of lignocellulose (lignin, cellulose, hemicellulose) form of simple compound (releast of vanillin/glucose/xylose equivalent) by 1 gram protein enzyme each minute. Specific enzyme activity were evaluated such as *ligninase, cellulase (endoglucanase* and *exoglucanase)* and *xylanase* with selective substrates. Tannic acid substrates for ligninase, CMC for endoglucanase, avicel for exoglucanase and xylan for xylanase. Production of crude enzyme extract done with growing bacteria isolates in liquid selective growth media on 0,5 abs. λ 660 nm, than incubated on temperature 39ºC during 3 day on *an-aerobic* condition. Crude enzyme extract was collected from centrifuged liquid media culture in 12.000 rpm for 15 minutes at 4ºC. The protein contents of crude enzyme extract evaluated use *Bicinchoninic Acid* (BCA) method with *Pierce™ BCA Protein Assay Kit* (Production of Thermo Scientific). Analysis of protein content follow *microplate* procedure use *bovine serum albumin*/BSA standard on wavelength 562 nm. On this study, the regression equation BSA are Y=0,001X + 0,190 (R^2 = 0,987). Extracts enzyme were tested in 1% selective substrates (following specific enzyme activity were evaluated) in 50 mM acetate buffer pH 5.5. Each substrate liquid in buffer was taken (8 ml), added 1 ml enzymes source, and 1ml aquadest. The mixture then were shaken by shaking bath, enzyme activity was measured in 30 minutes, 1 hour, 3 hours, 6 hour, 12 hour and 24 hour durations. Reduction sugar (glucose from CMC/avicel and xylose from xylan), or vanillin from tannic acid (lignin) produced from the reaction were the endoglucanase/exoglucanase/xylanase or ligninase enzyme activities [13]. For sugar reduction:1 ml of sample was added to 3 ml DNS reagent and 1 ml aquadest [14], for vanillin: 1ml of sample was added to 4 ml methanol, then measured the absorbent by spectrophotometer in wavelength (λ) 508,5 nm for glucose, 509 nm for xylose and 279 nm for vanillin. Ligninase/cellulase/xylanase enzyme activities was estimated by using vanillin/glucose/xylose calibration curve were the

ligninase enzyme activity follow the regression equation Y=0,00635X + 0,21098 (R^2 = 0,929); the celulase (endo-glucanase and exo-glucanase) enzyme activity follow the regression equation Y=0,00622X + 0,14277 (R^2=0,972); the xylanase enzyme activity follow the regression equation Y=0,00002X + 0,20525 (R^2=0,897).

Identification of Superior Lignocellulolytic Bacteria

The superior lignocellulolytic bacteria isolates were identified with biology molecular technique [15]. Bacteria DNA was extracted using a *Genomic DNA Mini Kit (Blood/Culture cell)* follow the *Buffy Coat Protocol* (Geneaid) with some modifications such as addition of proteinase K (finally concentration of 2 mg/ml) and RNase A (finally concentration of 10 mg/mL) [16]. DNA was amplified using primer 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') [17]. The amplification of the polymerase chain reaction (PCR) reaction used with total volume of 50 µL with composition of 25 µL master mix Kappa, 2 µL Primer 27F, 2 µL Primer 1492R, 16 µL RNase and 5 µL template DNA (cDNA). Reaction programmed following on the predenaturation are 95ºC for 3 min, then followed by 30 cycles consisting of 95°C for 30 s (denaturation), 50°C for 30 s (anneling) and 72°C for 1.5 min (extension), and with 1 cycle (a final extension) at 72°C for 10 min. The amplified DNA (PCR product) were verified by electrophoresis using 1.5% agarose on TBE Buffer 1x for 40 min/100 volt with electrophoresis KIT (*Applied Biosystem*). PCR products were purified using an ultraclean PCR KIT (Mo Bio Laboratories, Inc.) and then stored at–20°C until analysis. The electrophoresis product was staining with *etium bromida* (EtBr) and visualized on *Gel Doc* (*UVITEC Cambridge*) for verification PCR product. The reading arrangement of the nucleotides on 16S rDNA is done in the *Laboratoratory of Food Science, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan.* The PCR product purified using SUPRECTM PCR *(Takara Biomedicals, Otsu, Japan)* and then reading arrangement of the nucleotides using *BigDye Primer Cycle Sequencing FS Ready Reaction Kit (Applied Biosystems)* with 3100 *Genetic Analyzer (PE Applied Biosystems)* equipment. For identification of homology arrangement of the nucleotides on 16S rDNA done with study homology BLAST in *GenBank* with the address of the site https://blast.ncbi.nlm.nih.gov/Blast.cgi#

3. RESULTS AND DISCUSSION

Isolation of Lignocellulose Degrading Bacteria

The isolation bacteria from termites using selective lignocellulose growth media has 36 bacteria isolates has degrading lignocellulose substrates consits of 10 lignocellulolytic bacteria, 7 lignolytic bacteria, 9 cellulolytic bacteria, and 10 xylanolytic bacteria (Table 1).

Table 1. The Number and Code of The Bacteria Isolates has Isolated from Termites

Bacteria Isolates	Amount and Coding Bacteria Isolates has Isolated from Termites[1]
1. Lignocellulose degrading Bacteria (Lignocellulolytic)	10 BT_1[2]LS; BT_2LS; BT_3LS; BT_4LS; BT_5LS; BT_6LS; BT_7LS; BT_8LS; BT_9LS; BT_{10}LS
2. Lignin degrading Bacteria (Lignolytic)	7 BT_1LG; BT_2LG; BT_3LG; BT_4LG; BT_5LG; BT_6LG; BT_7LG
3. Cellulose degrading Bacteria (Cellulolytic)	9 BT_1CL; BT_2CL; BT_3CL; BT4CL; BT_5CL; BT_6CL; BT_7CL; BT_8CL; BT9CL
4. Xylanose degrading Bacteria (Xylanolytic)	10 BT_1XY; BT_2XY; BT_3XY; BT_4XY; BT_5XY; BT_6XY; BT_7XT; BT_8XY; BT_9XY; BT_1XY
TOTALLY	36

Notes: 1)Coding Bacteria based on selective substrates when the growth of bacteria isolates, BTLS=bacteria isolates from termites are grown on lignocellulose substrates (mixed of tannic acid+CMC+xylan), BTLG= bacteria isolates from termites are grown on lignin (tannic acid) substrates, BTCL= bacteria isolates from termites are grown on cellulose (CMC) substrates, BTXY= bacteria isolates from termites are grown on xylanose substrates

The most lignocellulolytic bacteria can isolated from termites in accordance with the statement of Watanabe *et al.* [9]; Purwadaria *et al.* [10]; Sukartiningrum [18] reported that on body cell, saliva, and gut of termites has various of microbes (bacteria, fungi, and protozoa) and various so various of fiber degrading enzyme such as cellulase (*endo-β-D-1.4-glucanase/CMC-ase*, *exoglucanase* and *β-D-14-glucosidase*) and hemicellulase (*endo-1,4-β-xilanase* and *β-D-1,4-mannanase*). Beside that its so showed that potential of termites for starter for fermentation feedstuff rich fiber

Screening of Lignocellulose DegradingBacteria

Specific Enzyme Activities from Lignocellulose Bacteria

The evaluated of lignocellulase specific enzyme activity showed that lignocellulolytic bacteria from termites has ligninase activities 0,980–1,739 U, 0,984–1,362 U, 0,526–0,667 U, 0,308–0,390 U, 0,180–0,225 U and 0,103–0,123 U; endoglucanase activities 2,710-4,176 U, 1,565-2,140 U, 0,601-0,799 U, 0,340-0,461 U, 0,178-0,243 U and 0,088-0,130 U; exoglucanase activities 1,115-1,751 U, 0,813-1,251 U, 0,350-0,499 U, 0,222-0,314 U, 0,143-0,184 U, and 0,084-0,110 U and xylanase activities 452,232-725,959 U, 362,414-582,176 U, 142,364-226,362 U, 85,870-122,105 U, 47,089-63,795 U and 24,957-34,758 U after incubation for 30 minutes, 1 hour, 3 hour, 6 hour, 12 hour and 24 hour respectively (Table 2a-d)

Table 2a. Ligninase Specific Enzyme Activities from Termites Lignocellulolytic Bacteria

Bacteria Isolates	Ligninase Specific Enzyme Activities on Several Incubation Periods (U)					
	30 minute	1 hour	3 hour	6 hour	12 hour	24 hour
BT 1 LS	1,205abc[1]	1,086abc	0,628ab	0,361ab	0,217ab	0,119ab
BT 2 LS	1,139ab	1,216abc	0,629ab	0,357ab	0,199ab	0,110ab
BT 3 LS	1,009a	1,061ab	0,526a	0,308a	0,184a	0,103a
BT 4 LS	1,739c	1,362c	0,667b	0,390b	0,225b	0,123b
BT 5 LS	1,686bc	1,277bc	0,602ab	0,382ab	0,223b	0,122ab
BT 6 LS	1,731c	1,224abc	0,600ab	0,337ab	0,190ab	0,106ab
BT 7 LS	1,361abc	1,094 abc	0,601ab	0,345ab	0,197ab	0,108ab
BT 8 LS	1,326abc	1,080ab	0,582ab	0,321ab	0,188ab	0,107ab
BT 9 LS	1,591bc	1,166abc	0,595ab	0,336ab	0,189ab	0,105ab
BT 10 LS	0,980a	0,984a	0,574ab	0,313ab	0,180a	0,106ab
SEM[2]	0,111	0,055	0,023	0,016	0,008	0,004

Table 2b. Endoglucanase Specific Enzyme Activities from Termites Lignocellulolytic Bacteria

Bacteria Isolates	Endoglucanase Specific Enzyme Activities on Several Incubation Periods (U)					
	30 minute	1 hour	3 hour	6 hour	12 hour	24 hour
BT 1 LS	3,976b	2,084c	0,735abcd	0,401ab	0,207ab	0,115bc
BT 2 LS	3,424ab	1,713abc	0,627ab	0,340a	0,178a	0,095ab
BT 3 LS	4,105b	2,076c	0,764cd	0,400ab	0,203ab	0,115bc
BT 4 LS	4,176b	2,140c	0,799d	0,461b	0,243b	0,130c
BT 5 LS	3,681ab	2,083c	0,786d	0,440b	0,227ab	0,130c
BT 6 LS	4,160b	2,053bc	0,762bcd	0,402ab	0,205ab	0,115bc
BT 7 LS	2,710a	1,565a	0,601a	0,345a	0,179a	0,090ab
BT 8 LS	3,090ab	1,589ab	0,635abc	0,356a	0,188a	0,088a
BT 9 LS	4,028b	1,860abc	0,697abcd	0,359a	0,186a	0,104ab
BT 10 LS	4,108b	2,099c	0,769cd	0,415ab	0,211ab	0,115bc
SEM	0,241	0,097	0,027	0,016	0,011	0,005

Table 2c. Exoglucanase Specific Enzyme Activities from Termites Lignocellulolytic Bacteria

Bacteria Isolates	Exoglucanase Specific Enzyme Activities on Several Incubation Periods (U)					
	30 minute	1 hour	3 hour	6 hour	12 hour	24 hour
BT 1 LS	1,392abc	0,942abc	0,393ab	0,259ab	0,164ab	0,094ab
BT 2 LS	1,207ab	0,813a	0,350a	0,222a	0,143a	0,084a
BT 3 LS	1,638bc	1,058abc	0,457ab	0,263ab	0,163ab	0,096ab
BT 4 LS	1,751c	1,251c	0,499b	0,314b	0,184b	0,110b
BT 5 LS	1,538abc	1,049abc	0,479ab	0,282ab	0,178ab	0,108b
BT 6 LS	1,722c	1,223c	0,463ab	0,291b	0,173ab	0,100ab
BT 7 LS	1,115a	1,107bc	0,474ab	0,276ab	0,168ab	0,102ab
BT 8 LS	1,471abc	1,071abc	0,467ab	0,268ab	0,162ab	0,090ab
BT 9 LS	1,248ab	0,870ab	0,443ab	0,256ab	0,151ab	0,084a
BT 10 LS	1,378abc	0,988abc	0,416ab	0,280ab	0,173ab	0,101ab
SEM	0,094	0,053	0,026	0,014	0,007	0,004

Notes: 1) Means in the same column with different letter differ significantly (P<0,05), 2)SEM = Standard error of the treatmens and means

Table 2d. Xylanase Specific Enzyme Activities from Termites Lignocellulolytic Bacteria

Bacteria Isolates	Xylanase Specific Enzyme Activities on Several Incubation Periods (U)					
	30 minute	1 hour	3 hour	6 hour	12 hour	24 hour
BT $_1$ LS	497,690ab	362,414a	148,187a	87,701ab	47,089a	25,898ab
BT $_2$ LS	497,417ab	377,828a	154,749ab	88,230abc	47,678a	24,957a
BT $_3$ LS	471,123a	370,049a	142,364a	85,870a	47,863a	26,533abc
BT $_4$ LS	725,959d	582,176b	226,362c	122,105d	63,795b	34,758c
BT $_5$ LS	716,204cd	508,297b	213,248c	106,819abcd	56,060ab	28,862abc
BT $_6$ LS	699,033bcd	578,633b	212,048c	109,872cd	63,406b	33,874bc
BT $_7$ LS	452,332a	380,730a	194,138bc	101,313abcd	54,273ab	27,879abc
BT $_8$ LS	453,908a	376,248a	165,922ab	90,262abc	47,231a	26,434abc
BT $_9$ LS	507,373abc	381,375a	156,548ab	91,103abc	48,067a	26,691abc
BT $_{10}$ LS	702,851bcd	460,445ab	194,543bc	108,152bcd	58,460ab	30,809abc
SEM	41,884	20,374	8,949	4,409	3,023	1,719

Notes: 1) Means in the same column with different letter differ significantly (P<0,05), 2)SEM = Standard error of the treatmens and means

Produced lignocellulase (*ligninase, endoglucanase, exoglucanase,* and *xylanase*) specific enzyme activities showed that bacteria isolated from termites has high ability for degrading lignocellulose compound to simple compounds. On this research, bacteria isolates coded BT_4LS has highest specific enzyme activities for all lignocellulase (*ligninase, endoglucanase, exoglucanase*, and *xylanase*) in the all incubation time periods (Table 2). Its showed that lignocellulolytic bacteria coded BT_4LS is superior bacteria degrading of lignocellulose which potential for starter on optimise utilization agricultural waste as feed. Pasti*et al.* [19] reported from gut of termites (Termitidae) has isolated bacteria strain *Actinomycete* has high ability for degrading lignocellulose substrates, were *Streptomyces sp. EC22, Streptomyces viridosporus T7A* and *Thermomonospora fusca BD25.* Kamsani *et al.* [20] reporting that several bacteria isolated from digestive tract of termites *Bulbitermes sp.* were *Bacillus sp B1, Bacillus sp. B2,* and *Brevibacillus sp. Br3* respectively has *endoglucanase* enzyme activities (138,77; 10,02; 3,46 U/g), *exoglucanase* (10,17; 32,16; 14,19 U/g), *β-glucosidase* (2,38; 1,81; 5,45 U/g), *xylanase* (72,33; 66,33; 104,96 U/g), *lignin peroxidase* (577,03; 500,99; 648,60 U/g), *manganese peroxidase* (47,73; 41,48; 36,93 U/g) and *laccase* (45,14; 71,18; 43,4 U/g) after incubation 14 day on solid media with substrates sawdust wood meal.

Specific Enzyme Activities from Lignolytic Bacteria

The ability degrading lignin compound by lignolytic bacteria affected by ligninase enzyme activity. On this study, *ligninase* specific enzyme activities produced by lignolytic bacteria isolates from termites were 0,438-3,260 U; 3,181-3,580 U; 1,172-1,293 U; 0,639-0,702 U; 0,344-0,376 U; 0,185-0,199 U respectively after incubation on tannic acid substrates during 30 minutes, 1 hour, 3 hour, 6 hour, 12 hour and 24 hour. Bacteria isolates coded BT_5LG has highest ligninase specific enzyme activities and significant different (P<0,05) compared with BT_3LG, BT_4LG (30 minutes incubation), BT_3LG (1 hour incubation), dan BT_4LG (3 and 6 hour incubation), while incubation during 12 and

24 hour, all lignolytic bacteria isolates were not significantly (P>0,05) (Table 3).

Table 3 Ligninase Specific Enzyme Activities from Termites Lignolytic Bacteria

Bacteria Isolates	Ligninase Specific Enzyme Activities on Several Incubation Periods (U)					
	30 minute	1 hour	3 hour	6 hour	12 hour	24 hour
BT 1 LG	2,960ab[1]	3,460abc	1,273ab	0,687ab	0,368a	0,195a
BT 2 LG	3,126a	3,564c	1,289ab	0,701b	0,375a	0,198a
BT 3 LG	2,471a	3,181a	1,185ab	0,650ab	0,347a	0,186a
BT 4 LG	2,438a	3,235ab	1,172a	0,639a	0,344a	0,185a
BT 5 LG	3,260b	3,580c	1,293b	0,702b	0,376a	0,199a
BT 6 LG	2,825ab	3,527bc	1,281ab	0,693ab	0,373a	0,199a
BT 7 LG	3,018ab	3,521bc	1,253ab	0,672ab	0,372a	0,197a
SEM[2]	0,155	0,067	0,025	0,013	0,010	0,005

Notes: 1) Means in the same column with different letter differ significantly (P<0,05), 2)SEM = Standard error of the treatmens and means

Produced highest ligninase spesific enzyme activities by lignolytic bacteria isolates coded BT_5LG show that its bacteria isolate is superior ability on degradation lignin compound compored with the others lignolytic bacteria isolates from termites (Table 3). Perez *et al*. [3] said that totally degradation of lignin compounds are the response of activity of the three main extracellular enzyme such as *lignin-peroxidase/Li-P, mangan-peroxidase/Mn-P,* and *laccase/Lac.* Datta *et al.*[21] indeed said that lignin degrading bacteria can produce primarily five major extracellular enzyme including *lignin peroxidase* (LiP), *manganese-dependent peroxidase* (MnP), *versatile peroxidase* (VP), *laccase* (Lac) and *dye-decolorizing peroxidases* (DyPs). Aarti*et al.*[22] so said that in addition of the mainly extracellular enzyme, enzymes such as *aryl alcohol dehydrogenase, cellobiose, aromatic acid reductase, vanillate hydroxylase, dioxygenase* and *catalase* are also considered to play a significant role in the lignin degradation. Olsson [23] showed that several strain bacteria such as *Streptomyces sp., Thermobifida fusca, Rhodococcus jostii, Bacillus subtilis, B. lichenformis, Bacillus sp.* and *Pseudomonas flurrescens* has produce lignin degrading enzyme. On this study, identification kind of specific enzyme produce from those bacteria isolates are not done. Based on a reference related to the kind of selective substrate used is tannic acid, then allegedly kind of enzymes are produced is *Laccase* [24] or *phenol oxydase* [25; 26]

Specific Enzyme Activities from Cellulolytic Bacteria

The study of cellulose specific enzyme activities showed that cellulolytic bacteria isolates from termites has produce *endoglucanase* activities were 4,893-5,113 U; 3,623-3,857 U; 1,275-1,360 U; 0,663-0,715 U; 0,341-0,367 U; 0,173-0,187 U (Table 4a) and *exoglucanase* activities were 2,583-2,805 U; 1,461-1,780 U; 0,567-0,668 U; 0,325-0,385 U;0,174-0,202 U; 0,095-0,110 U (Table 4b) respectively on incubation during 30 minutes, 1 hour, 3 hour, 6 hour, 12 hour, and 24 hour.On its table showed that the cellulolytic bacteria coded BT_3CL has highest *endoglucanase* and *exoglucanase specific* enzyme activities on the all time periods incubation.

Table 4a. Endoglucanase Specific Enzyme Activities from Termites Cellulolytic Bacteria

Bacteria Isolates	Endoglucanase Specific Enzyme Activities on Several Incubation Periods (U)					
	30 minute	1 hour	3 hour	6 hour	12 hour	24 hour
BT_1CL	5,067abc[1]	3,763abc	1,327abc	0,693ab	0,355ab	0,181bc
BT_2CL	5,010abc	3,624a	1,274ab	0,673a	0,345a	0,176ab
BT_3CL	5,113c	3,857c	1,360c	0,715b	0,367b	0,187c
BT_4CL	4,936ab	3,613a	1,262a	0,660a	0,341a	0,173a
BT_5CL	4,942abc	3,652ab	1,272ab	0,663a	0,343a	0,175ab
BT_6CL	5,045abc	3,671ab	1,275ab	0,668a	0,342a	0,174ab
BT_7CL	5,095bc	3,796bc	1,343bc	0,711b	0,363b	0,185c
BT_8CL	4,921ab	3,623a	1,275a	0,665a	0,341a	0,173ab
BT_9CL	4,893a	3,679ab	1,295abc	0,671a	0,345a	0,173ab
SEM[2]	0,036	0,033	0,014	0,007	0,003	0,002

Notes: 1) Means in the same column with different letter differ significantly (P<0,05), 2)SEM = Standard error of the treatmens and means

Table 4b. Exoglucanase Specific Enzyme Activities from Termites Cellulolytic Bacteria

Bacteria Isolates	Exoglucanase Specific Enzyme Activities on Several Incubation Periods (U)					
	30 minute	1 hour	3 hour	6 hour	12 hour	24 hour
BT_1CL	2,756abc	1,698cd	0,623bc	0,323a	0,187bc	0,101abc
BT_2CL	2,693abc	1,461a	0,579ab	0,341abc	0,179ab	0,099abc
BT_3CL	2,805c	1,780d	0,668c	0,385c	0,202d	0,110c
BT_4CL	2,634ab	1,513ab	0,567a	0,325a	0,174a	0,095a
BT_5CL	2,625abc	1,566ab	0,577ab	0,339abc	0,177ab	0,097ab
BT_6CL	2,729abc	1,587bc	0,581ab	0,351abc	0,176ab	0,097ab
BT_7CL	2,785bc	1,718d	0,651c	0,380bc	0,198cd	0,108bc
BT_8CL	2,599ab	1,531ab	0,578ab	0,332a	0,174a	0,102abc
BT_9CL	2,583a	1,510ab	0,587ab	0,336ab	0,175ab	0,095a
SEM	0,023	0,023	0,010	0,010	0,003	0,002

Notes: 1) Means in the same column with different letter differ significantly (P<0,05), 2)SEM = Standard error of the treatmens and means

Tabel 4a-b show that cellulolytic bacteria isolates from termites produce higher endoglucanase specific enzyme activities compared than *exoglucanase* specific enzyme activities. This means that the cellulolytic bacteria isolates from termites are the first degrader of cellulose fiber to be elaborated cellulose randomly by way of breaking the hidrogen bond on crystalin/amorforous cellulose form to be single bond (oligodextrin) that will be followed by activities of exoglucanase and glucoxydase enzyme [27]. This study in line with Prabowo *et al.* [28] showed that extract of termites has highly *CMC-ase* are 0,6961-0,7638 U/mg or 7,11-33,95 fold higher compared with enzyme activity of cow rumen liquid, moreover 19,39-35,69 fold higher compared with enzyme activity of cattle rumen liquid.

Specific Enzyme Activities from Xylanolytic Bacteria

Xylanolytic bacteria isolates from termites has high xylanase specific enzyme activity were 526,228–749,306 U; 265,911-392,965 U; 133,316-172,919 U; 73,216-96,738U; 43,851-54,806 U; and 24,422-30,981 U respectively after incubation for 30 minutes, 1 hour, 3 hour, 6 hour, 12 hour

and 24 hour (Table 5).On this study, xylanolytic bacteria coded BT_8XY has highest xylanase specific enzyme activities on most of time periods incubation (Table 5). Its indicatedits bacteria is superior with best potential as starter degrading xylanose and/or others hemicellulose compounds.

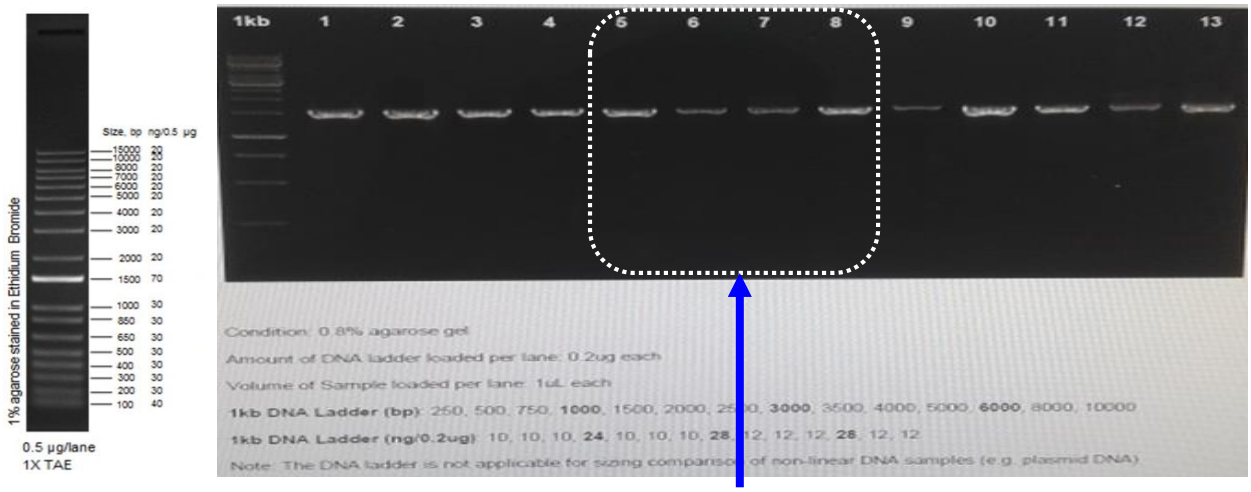
Table 5 Xylanase Specific Enzyme Activities from Termites Xylanolytic Bacteria

Bacteria Isolates	Xylanase Specific Enzyme Activities on Several Incubation Periods (U)					
	30 minute	1 hour	3 hour	6 hour	12 hour	24 hour
BT_1XY	607,89ab[1]	322,69ab	134,06a	74,14a	44,38ab	25,62ab
BT_2XY	526,23a	325,39ab	133,32a	73,22a	43,85a	24,42a
BT_3XY	742,01c	392,97c	161,23cd	91,88cd	53,21de	30,27c
BT_4XY	643,72abc	349,25bc	144,78abc	82,69abc	47,47abcd	27,28abc
BT_5XY	688,66bc	369,34bc	140,57abc	87,30bcd	50,17bcde	27,33abc
BT_6XY	561,77a	344,08bc	144,35abc	80,27abc	46,67abc	26,86abc
BT_7XY	734,21c	367,11bc	157,68bcd	87,82bcd	51,24cde	29,05bc
BT_8XY	749,31c	374,65bc	172,92d	96,74d	54,81e	30,98c
BT_9XY	531,82a	265,91a	136,85ab	76,82ab	44,71ab	25,22ab
$BT_{10}XY$	640,45abc	320,23ab	152,35abcd	84,05abcd	47,96abcd	27,02abc
SEM[2]	23,985	12,716	4,440	2,583	1,258	0,824

Notes: 1) Means in the same column with different letter differ significantly (P<0,05), 2)SEM=Standard error of the treatmens and means

Identification of Superior Lignocellulolytic Bacteria from Termites

Identification of superior lignocellulolytic bacteria isolates from termites were bacteria coded BT_4LS; BT_5LG; BT_3CL; BT_8XY with biology molecular technique showed that primer 27F can amplified the DNA superior bacteria from termites with lenght about 1492 bp (Picture 1).



Picture 1. Result of Amplification 16S rDNA from The Superior Lignocellulolytic Bacteria Isolates from Termites using Primer 27F and1492R.(1kb=Marker 1 kb DNA leader, [1-4] Rumen Bacteria Isolates, [5]BT_4LS; [6]BT_5LG; [7]BT_3CL; [8]BT_8XY; [9-13] Bacteria Isolates from Earthworm)

Based on nucleotide arrangement known the superior lignocellulolytic bacteria is *Aneurinibacillus sp. strain BT4LS* (homology 99% with Acc. No. KR063553); superior lignolytic bacteria is *Aneurinibacillus sp. strain* BT_5LG (homology 81% with Acc. No. KP980744); superior cellulolytic bacteria is *Bacillus sp. strain* BT_3CL (homology 100% with Acc. No. KX879851), and xylanolytic bacteria *Bacillus sp. strain* BT_8XY (homology 100%with Acc. No. KT981879) (Table 6).

Table 6. Result of Identification of Lignocellulolytic Bacteria Isolates from Termites

Code of Bacteria	Nucleotides Arrangement	ID Bacteria/ Homology / No. Accession
BT_4LS (lignocellulolityc)	GCTATAATGCAGTCGAGCGGACCAATGAAGAGCTTGCTCTTCGGCGGTTAGCGGCGGACGGGTGAGTAACAC GTAGGCAACCTGCCTGTACGACTGGGATAACTCCGGGAAACCGGAGCTAATACCGGATACTTCTTTCAGACC GCATGGTCTGAAAGGGAAAGACCTTTGGTCACGTACAGATGGGCCTGCGGCGCATTAGCTAGTTGGTGGGGT AACGGCCTACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGG CCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGC GTGAACGATGAAGGTTTTCGGATCGTAAAGTTCTGTTGTTAGGGAAGAACCGCCGGGATGACCTCCCGGTCT GACGGTACCTAACGAGAAAGCCCCGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGGGGCAAGCGT TGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGCTTCTTAAGTCAGGTGTGAAAGCCCACGGCTCAAC CGTGGAGGGCCACTTGAAACTGGGAAGCTTGAGTGCAGGAGAGGAGAGCGGAATTCCACGTGTAGCGGTG AAATGCGTAGAGATGTGGAGGAACACCCGTGGCGAAGGCGGCTCTCTGGCCTGTAACTGACGCTGAGGCGC GAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGTTGAGTGCTAGGTGTTG GGGACTCCAATCCTCAGTGCCGCAGCTAACGCAATAAGCACTCCGCCTGGGGAGTACGGGCGCAAGGCTGA AACTCAAAGAATTGACAGGGACCCGCACAAGCGCTGGAGCATGTGGTTTAATTCGAAGCAACGCGAGAAAC TTACCAAGGCTTGACATCCCGCTGACCCTCCCTAGAGATAGGAGCTCTTCTTCCGAGCACG	*Aneurinibacillus sp. XT-25* (99%) (KR063553)
BT_5LG (Lignolytic)	CAGTCGAGCGGACCAATTACGAGCTTGCTCATCGGTGGTTAACGCGCGAGAGTCTGACTAACACGTAGGCAA ACTTCCTGTACAACTGTGATAACTCCCGGAAACCCCAACTAATACAAAGATTCTTCTTTCATACCACATGCCCT GAATGGAAAAGACCTTTGGTCACGTACAGATGGGCCTGCGCGCACAATAGCTAGTTGGTGTGGGTAAGGGC ATACCACACGCTACGATCCGAAACACGACCTGAGACGGTGATCGGACGCACCGGTGACTGAATACCGGACC ATGACTCCTACGCCAGACCCACCATGCCAATCTTCCTGCATGGGACCAAAGTCTGACGGAGCTCGCCCCCTG AACGATAAATGTTTTCGGACCGAGAGTACTGTTGTTATAGAAGAACGCCCGGGATGACCTCACGGTCTGACG GGCCCTAATTAGAAAGACTCGCGGAACTACATGCCAACGTCCGCGGTAATACATATGGGGCGTGCGTTCCCC GGACTCATTGGGCGAACTGCGCTTGAACCGGCTTCTTAGTCAGGTGAGAAAGCCCTCGACTCACCCGTGGA GGGCCGCTTGAAACTGGGAAGCTTGAGTGCACGAGAGGAGAGCGGAATTCCACGTGTATCGATGAACTGCG AAGAGATGTGGAGGAACACCCGTGGCGAATGCGACTCTCTGGTCTGTACTGACGCTAAGGCGCGAAAGCGT CGTGAGCGAACATGATCATATACCTGCTACTCCCGCCTTAAACTCCACGCTAGAGTTGGGACTCCATCCTCTT GCGCACTACGCATACCATCCGCCGGGAATACGGCTCAAGCTGAACTCAAGGAATCATACGACCTCAAATCCT GAGCAGTGGTTAATCCATC	*Aneurinibacillus sp. Bac270* (81%) (KP980744)
BT_3CL (Cellulolytic)	TGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGGGTA ACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGGTTGTTTGAACCGCATGGTT CAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAAC GGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCC AGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTG AGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTAC CTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAG CGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTC AACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTAGCG GTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTGAGG AGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAAGTG TTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCAAGA CTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGA AGAACCTTACCAGGTCTTGACATCCTCTGACAATCCTAGAGATAGGACGTCC	*Bacillus sp. strain SAUF201* (100%) (KX879851)
BT_8XY (Xylanolytic)	GCTATAATGCAGTCGAGCGGACAGAAGGGAGCTTGCTCCCGGATGTTAGCGGCGGACGGGTGAGTAACACG TGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGAGCTAATACCGGATAGTTCCTTGAACCGC ATGGTTCAAGGATGAAAGACGGTTTCGGCTGTCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGA GGTAACGGCTCACCAAGGCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACA CGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGC CGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTGCAAGAGTAACTGCT TGCACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGG CAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCG GCTCAACCGGGGAGGGTCATTGGAAACTGGGAAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACGTGTA GCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTCTCTGGTCTGTAACTGACGCTG AGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAA GTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAGCTAACGCATTAAGCACTCCGCCTGGGGAGTACGGTCGCA AGACTGAAACTCAAAGGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACG CGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTTCCCT	*Bacillus sp. strain Suaeda B-003* (100%) (KT981879)

On the superior lignocellulolytic bacteria from termites, result of phylogeny analysis with several data bacteria isolates were isolated from termites [10, 18, 29] with nucleotide arrangement data squens refer from GenBank (www.ncbi.nlm.nih.gov), the superior lignocellulolytic bacteria from termites "*Aneurinibacillus sp. strain BT_4LS"* has high similarity (99%) with *Aneurinibacillus sp. XT-25* were has closely related phylogeny with *Bacterium strain Enterobacter aerogenes,*

Sphingobium sp. K40, Chryseobacterium kwangyangense strain Cb, Bacillus mycoides strain EGU624, Bacillus sp. strain BAB-5934 (Picture 2). This comfirms that the superior lignocellulolytic bacteria from termites has ability of degrading lignin compounds such as the role of *Bacillus mycoides strain EGU624, Bacillus sp. strain BAB-5934* [20], as well as polysaccharide degrading such as the role of *Bacillus sp., Enterobacter sp.,* and *Ochrabacterium sp.* [30].

Picture 2. Phylogeny Tree of The Superior Lignocellulolytic Bacteria Isolates from Termites analysis using *Maximum Likelihood* Method Following Tamura-Nei Model (1993) with Program MEGA 7

Based on data in *GenBank* known that *Aneurinibacillus sp. XT-25* were has high homology with the superior lignocellulolytic bacteria from termites was reported by Li, X., Xue, C,-L dan Yu, H.-Y on September 23, 2015 is bacteria cultur arable land Xitan in Yuncheng, China (https://www.ncbi.nlm.nih.gov/nucleotide/KR063553.1). *Aneurinibacillus sp.* reported have ability degrading lignocellulose compounds [31, 32, 33, 34]. Acharya and Chaudhary[31] shown *Aneurinibacillus thermoaerophilus WBS2* can produce alkali cellulase with *CMCase* activity 0,058±0,004 IU/ml (on wheat straw) and 0,081±0,011 IU/ml (on rice straw) and also *Fpase* activity

0,426±0,033 IU/ml (on wheat straw) and 0,319±0,025 IU/ml (on rice straw) when incubation on pH 9. Lotfi [32] and Chandra *et al.* [33] shown that *Aneurinibacillus aneurinilyticus* has produce lignin degrading enzyme such as *Mangan Peroksidase* and *Laccase* were degradation the chain side of lignin aromatic compound and degrading lignin with depolimerisation proses. Tsobuachi *et al.*[34] moreover said that bacteria from genus *Aneurinibacillus* has produce various enzyme such as *β-glucosidase, α-glucosidase, α-mannosidase, β-galactosidase, β-glucoronidase, oxidase, catalase, alkali-phosfatase, esterase lipase, lipase, tripsin, leucine/valin/sistin aryl amidase, α-kemotripsin, acid-phosfatase,* and *naphthol AS-BI-phosphohidrolase*. The superior lignin degrading bacteria "*Aneurinibacillus sp. strain BT$_5$LG"* closely related with *Aneurinibacillussp.Bac270* (homology 81%) (Table 6).Its in line with various research shown the strain bacteria *Aneurinibacillus sp.* are good lignin degrading bacteria isolates [3, 33]. Lotfi [32] and Chandra *et al*. [33] shown this group bacteria (*Aneurinibacillus sp*.) has ability to produce various ligninase such as *Mangan Peroksidase* and *Laccase* which has degrading side chain aromatic form of lignin and lignin depolimerisation. Based on phylogeny analysis, the superior lygnolytic bacteria from termites *"Aneurinibacillus sp. strain BT$_5$LG"* has closely relationship phylogeny with the superior cellulolytic bacteria from termites *"Bacillus sp. strain BT$_3$CL"* (Picture 2). On this picture so showed that *Bacillus sp. strain BT$_5$LG* and *Bacillus sp. strain BT$_3$CL* alsoclosely related filogeny *Enterobacter cloacae strain Razmin C, Bacillus cereus strain Razmin A, Paenibacillus sp strain Y2, Bacillus pumilus, Bacillus licheniformis.* This further comfirms that both *Aneurinibacillus sp.* or *Bacillus sp.* are the group of bacteria degrading lignin, cellulose and/or hemicellulose. Based on *GenBank* impormation shown that *Bacillus sp. Strain SAUF201* were closely related with the superior cellulolytic bacteria from termites "*Bacillus sp. strain BT$_3$CL"* had previously reported by Chen, Mou, L. and Zhang, X.on December 27, 2016 isolated from gut of *Eupolyphagasinensis* (as China traditionally medicine) in (https://www.ncbi.nlm.nih.gov/nucleotide/KX879851.1), while the xylanolytic bacteria was homologous with *Bacillus sp. Strain Suaeda B-003* previously reported by Wu, Y., Wei, X., Hao, Y., and Zhao, H. on November 30, 2016 was isolated and identified from Bacillus (https://www.ncbi.nlm.nih.gov/nucleotide/KT981879.1). This data showed that the superior cellulolytic bacteria from termites also potential function in health sector. Flint and Garner [35] said that *Bacillus sp.* is source of direct fed microbial/DFM as be probiotic and immunostimulant sources for livestock. For the superior xylanolytic bacteria from termites "*Bacillus sp. strain BT8XY*", phylogeny analysis showed that its bacteria has closely related with *Bacillus acidicola strain BT HNGu 442, Cellulomonas sp. KAR58, Cellulomonas sp strain T2 31MG-40, Paenibacillus amylolyticus strain IHBB 9270,* and *Klebsiella sp. strain R326* (Picture 2). This is in line with a statement from Wenzel *et al*. [36] was said that the bacteria *Cellulomonas, Bacillus* (except *B. cereus* and *B. megaterium)* and *Paenibacillus* were cellulolytic bacteria with the best ability of degradable cellulose and hemicellulose. Ratanakhanokchai *et al*. [37] and Tresnawati Purwadaria*et al.* [38] also

said that the group of Bacillus were xylanolytic with high enzyme activity were shown produce various xylanase complex enzyme such as *extracellular xylanase, β-xylosidase, arabinofuranosidase*, and *acetyl esterase* with specific enzyme activity were 4,8; 0,21; 0,15 dan 0,24 U/mg protein respectively. Gilbert and Hazzlewood [39] said that the *Bacillus sp.* is one of the bacteria was produce multiple enzyme of cellulase and xylanase "cellulosome" were synergistically degradation of plant cell wall compound.

4. CONCLUSION

The Lignocellulose degrading bacteria from termites has high activity degrading of lignocellulosic compounds showed that high lignocellulase specific enzyme activities. Screening of superior bacteria from termites showed that the superior lignocellulolytic bacteria is *Aneurinibacillus sp. strain BT4LS (homology 99%* withAcc. No.KR063553); superior lignolytic bacteria is "*Aneurinibacillus sp. strain BT5LG"* (homology 81% with Acc. No. KP980744); the superior cellulolytic bacteria is "*Bacillus sp. strain BT3CL"* (homology 100% with Acc. No. KX879851), and the xylanolytic bacteria "*Bacillus sp. strain BT8XY"* (homology 100%with Acc. No. KT981879).

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article

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