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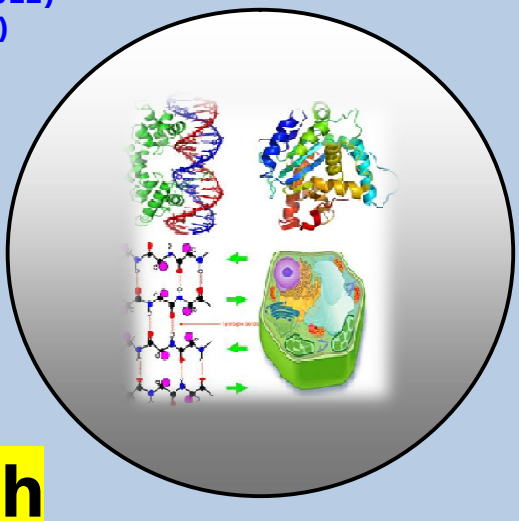

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ABSTRACT

A research has been carried out to evaluate the potency of lignocellulose degrading bacteria isolated from bali cattle rumen content waste and termites.Those animals were chosen sinceit hasbeen consumedfor its low quality crude fiber as the main energy sources. Lignocellulose degrading bacteria were isolated by Hungate selective media,using lignin (tannic acid), xylan, and cellulose as selective substrates. The best lignocellulose degrading bacteria then was determined by enzyme activity by isolate. It showed that lignocellulose degrading bacteria could be found in Bali cattle rumen content waste and termites. In bali cattle rumen content waste found 4 isolate lignocellulolytic, 5 isolatecellulolytic, and 5 isolatexilanolytic bacteria. Even though from termites found 8 isolate cellulolytic and 1 isolate xilanolytic. Enzyme activity evaluation showed first and second highest lignocellulolytic, cellulolytic, and xylanolytic activities from bali cattle rumen content waste isolates were reached by BCR Ligsll 2and isolate BCR Ligsll 4, isolate BCR CMC 2-2and isolate BCR CMC 2-3, BCR Xy 2 and BCR Xy 3. Meanwhile from termites first and second highest cellulolytic, and xylanolytic activities were reached by BR CMC 3-7 and BR CMC 3-2, isolate BR Xy. It can be concluded that nine isolate bacteria has highest enzyme activity chosen as nonconventional waste degrader for bali cattle feed production.
Key word: Bacteria, Lignocellulose, Nonconventional Waste, Rumen Content Waste and Termites.

INTRODUCTION

The development of feeding system based on the local resources is the pillars supporting the development of sustainable and competitive animal production systems, especially ruminant species in Indonesia (Ginting, 2004). The residues, waste and byproductsof many kinds of food crops, agro-industry and farm waste are sources of ruminantfeed ingredients are potential alternatives.

Most natural source waste such as nonconventional waste are rich in lignocellulose materials. Cellulose, a long chain polisacharide made of β(1,4)-linked glucose units, is the principal constituent of lignocelluloses. Theassociation of cellulose with lignin, another complex polimeric molecule composed of phenilpropanoid units, lignocelluloses form. Hemicellulose is the other major component of lignocellulose. It is a heterogeneus group of long chain polysaccharides in which basic unit are arabinose, xylose, mannose, or galactose (Ishihara, 1980). Degradation lignocelluloses material is a slow process and only rellative narrow taxonomic range of bacteria is able degrade such material. The ability of microorganisms degrade lignocellulolytic material of considerable interest in terms of microbial ecology and biotechnology. Lignocellulose degrading bacteria has an important role in energy supply for ruminants. Ruminants are able to convert low quality feed in rumen because its role of lignocellulolytic bacteria. Bali cattle rumen content waste is an animal slaughterhouse waste could be as a source of microorganisms such as lignocellulolytic bacteria (Clarke and Bauchop, 1977). Kamra (2005) mentionedthat rumen microbe of ruminants in tropic area including bacteria (10^{10}–10^{11}colony/ml, were 50 species), ciliated protozoa (10^{4}–10^{6}cpu/ml, were 25 species), and anaerobic fungi (10^{3}-10^{5} zoospore/ml, were 5 jenis). Even though, termites are known to thrive on lignocellulolytic materials such as: barks, woods and plant materials (Nakashima *et al.*2002). Termites are among the most important lignocellulose-digesting insects and possess a variety of symbiotic microorganisms in their hindguts such as bacteria (Konig 2006). Termites have the ability to digest wood that contains high fiber, due to the enzyme activity produced by microbe such as bacteria (Cook and Gold, 2000).Termites has microbe at all body cell and various fiber degrading enzyme such as cellulase complex enzyme (*endo-β-D-1.4-glukanase/CMC-ase*, *aviselase*, *eksoglukanase* and *β-D-14-glukosidase)* and hemiselulase enzyme (*endo-1,4-β-xilanase* dan *β-D-1,4-mannanase*) (Purwadaria et al. 2003[ab],2004). Degradation of lignocelluloses material requires the cooperative action of family of lignocellulolytic enzymes that classified into three major groups: complex lignases, complex cellulases, and complex hemicellulolytic.

MATERIAL AND METHODS

Isolate Sources

The bacteria were isolated from fresh sample of bali cattle rumen waste and termites. Bali cattle rumen sample were take from Antang-South Sulawesi slaughter house. Meanwhile termites taken from degrading wood in area Hasanuddin University Rusunawa Complex.

Solid Media and Isolation

Microbes from all samples were grown in solid media by Hungate method (Ogimoto and Imai, 1981): weigh 0,02g KH2PO4; 0,03g K2HPO4; 0,01g MgSO4; 0,01g CaCl4; 0,10g NaCl; 0,10g (NH4)2SO; 0,10ml Rezasurin 0,1% solution; 0,02g Cystein-HCl.H2O; 0,40g Na2CO3; 30,00ml rumen liquid; 1,00g substrate;70,00ml Aquadest and 1,8% Agar. Selective substrate used were lignin, xylan and cellulose.All ingredients were mixed in Erlenmeyer (exceptsubstrate that were sterilized by 5 ml aquadest intube), pH was determined 6,8 and heated until allingredients dissolved.

The flask then transferredaseptically with oxygen-free CO gas displacingall air until red color faded, closed with rubber 2stopper, sealed, then sterilized with its content in12 psi for 20 minutes. In warm condition, mediawas divided into 3 tubes. Each selective substratethen dissolved, then poured 4,5 ml each into 5mm petri disc. Microbes source liquid (50µl) with 10-5 dilution then were inoculated for 7-14days in anaerobic jar filled by anaerobicgenerating kit. The growing colonies then werecounted.

Qualitative Selection

The lignin degrader bacteria was selected qualitatively based on the diffusion zone diameter that formed around colony (Subbarao, 1993:Samingan,1998: Martani, 2003). While xylan and cellulose degrading bacteria were selected by measured clear zone around colony (Ogimoto and Imai, 1981). Each isolate was inoculated by spot method on nutrient agar that contain 1% tannic acid (Subbarao, 1993). Cellulose and xylan degrader were isolated according clear zone around colonies on nutrient agar that contain 1% cellulose and 1% xylan respectively (modified Hungate method in Ogimoto and Imai, 1981). Diffusion and clear zone were measured after 7 days of anaerobic incubation.

Liquid Media

Isolates were grown in liquid media bymodified Hungate method (Bachruddin, 1985)which were mixed 150 ml mineral I solution, 150ml mineral II solution, 1 ml rezasurin 0,1% (w/v),2,00g substrate, 400 ml rumen liquid extract, 2 gyeast extract as enrichment nutrient, and 250 mlaquadest in 1000 ml Erlenmeyer. Substrates thatused were mixed lignin, xylan and cellulose,adjusted by each enzymes production test. All materials in Erlenmeyer then were heated 100^{o}Cfor 5 minutes for homogenized along with COgas. Temperature was sustained 45^{o}C in waterbath. An aerobic condition was reached when redcolor was faded. Then, 32,3 ml sodium carbonateand 16,7 ml Cystein-HCl were added. Then,the tube was closed with a rubber stopper and sealed,sterilized in 121^{o}C for 15 minutes. Each media(according to selective substrate) was divided according to itsisolates number that would be grown in 50 mlserum bottle. Isolate from solid media wasdissolved in dilute solution in 0,5 λ 600 absorbent,inoculated in bottle as much as 10%, incubated in 39^{o}C for 7 days. Growth culture media then wasused as enzymes source.

Quantitative Selection

Enzyme extract was collected from centrifuged liquid media culture in 12.000 x g for15 minutes in 4^{o}C. Based on the substrate, extracts tested in three kinds of substrates contain:1% CMC powder/Avicel/xylan/Tannic Acid (as source of lignin) in 50 mM acetate buffer and pH 5,5. Each substrate liquid in buffer was taken 8 ml, added with 1 ml enzymes source, and 1ml aquadest. Then mixture were shaken by fortex, enzyme activity measured in 60 minutes. Reduction of sugar (glucose from CMC, xylose from xylan), or vanillin from lignin produced from reaction ofenzyme activities (Efiok, 1996). Sugar reduction such as:1 ml of sample was added to 3 ml DNS reagent and 1 ml aquadest (Miller, 1959), for vanilin: 1ml of sample added to 4 ml methanol, then measuredabsorbent with spectrophotometer in λ 508,5 nm for glucose, 509 nm for xilosa and 279 nm for vanilin.

Research Design

The research was conducted based on qualitative and quantitative analysis. A CompletelyRandomized Design was used asstatistical design. Isolates foundusedas treatment with three replication and lignocellulase, cellulase, xylanase, and ligninase as parametersbeing observed.

RESULT AND DISCUSSION

Isolation of Lignocellulolytic Bacteria

Isolated bacteria from bali cattle rumen waste reached 4 lignocellulolytic isolates, 5 cellulolytic isolates, and 5 xylanolytic isolates. Meanwhile,termites has isolated 8 cellulolytic isolates and 1 xylanolytic isolates (Table 1).

Table 1. Number Of Lignocellulolytic Bacteria from Isolate Source.

No	Spesies	Isolate Source	
		Bali Cattle Rumen Content Waste	Termites
1	Lignocellulolytic bacteria	4	0
2	Lignolytic Bacteria	0	0
3	Cellulolytic Bacteria	5	8
4	Xylanolytic Bacteria	5	1
	TOTAL	14	9

Cellulose and xylanose degrading bacteria could be found from all sample, but lignocellulose degrading bacteria only found from bali cattle rumen waste (Table 1). Could isolated lignocellulose degrading bacteria in bali cattle rumen content show consorsium microbe on bali cattle rumen higher than termites. This condition may affect higher capacity derived from termites of Bali cattle rumen.

Quantitative Lignocellulolytic Activity

The study showedthat lignocellulose degrading bacteria from bali cattle rumen has lignase activities 0,1156 – 0,6440 unit/ml after contact 5 – 20 minute with substrats. BCR Ligsll 4 Isolate highest lignase activities and significant different (P<0,05) on minute to 5' and 10', even though on minute to 15' and 20', BCR Ligsll 2 isoalte was the highest lignase activities and significant different (P<0,05) than all isolate. Evaluation of cellulase activities from these isolates showed isolate BCR ligsll 4 has highest cellulase activities (P<0,05) on minute to 10 and 20 were 0,549 U/ml and 0,224 U/ml respectively, but on minute to 5 and 15 all bacteria isolates were similar cellulase activities. Meanwhile,highest xylanase activities from lignocellulose degrading bacteria isolates found by BCR ligsll 4 bacteria isolate on 5 minute contact with 55,0037 U/ml substrates (P<0,05), even though on 10 up to 20 minute produced by BCR Ligsll 2 bacteria isolate of 32,6527 U/ml, 85,1729 U/ml and 47,1394 U/ml respectively (Table 2). Based on the enzyme activities value, it was found thatBCR Ligsll 4 and BCR Ligsll 2 bacteria isolates has higher quality and most potencial as lignocellulose innocullant/fermentor. The evaluation on endo-glucanase activities from cellulose degrading bacteria isolates did not reach values up to 20 minutes contact between extract enzymes on substrates. These case may be effected by time duration for minimum enzyme of isolate bacteria degrading substrates and Hidrogen bond in cellulose crystallin structure (α1,4 glukoside bond) can not crumbled. Exo-glucanase activities from cellulose degrading bacteria isolates from bali cattle rumen waste and termites so could not activities values.These isolates recent can degrade avicel/cellulose micro crystallin after 10' until 20' minutes contact (see in Table 3).

Table 2. Enzyme Activies from Lignocellulose Degrading Bacteria From Bali Cattle Rumen Content Waste.

No	Isolate of Mikroba	Enzyme Activities (IU/ml) on minute to...			
		t5	t10	t15	t20
Lignase Enzyme Activities[1)]					
1	BCR Ligsll 1	0,1265d[4]	0,3295b	0,2009c	0,1317b
2	BCR Ligsll 2	0,5164b	0,4071ab	0,3880a	0,1944a
3	BCR Ligsll 3	0,1978c	0,3692ab	0,2821b	0,0256d
4	BCR Ligsll 4	0,6440a	0,4416a	0,2174c	0,1156c
Cellulase Enzyme Activities[2]					
1	BCR Ligsll 1	0,7490a	0,3825b	0,3741a	0,1776b
2	BCR Ligsll 2	0,8106a	0,3552b	0,3860a	0,1828b
3	BCR Ligsll 3	0,6518a	0,3576b	0,4138a	0,2091ab
4	BCR Ligsll 4	0,8026a	0,5492a	0,3370a	0,2240a
Xylanase Enzyme Activies[3]					
1	BCR Ligsll 1	36,6078ab	21,2472b	51,6924b	41,3447a
2	BCR Ligsll 2	6,0706b	32,6527a	85,1729a	47,1394a
3	BCR Ligsll 3	15,2686b	13,5210c	50,0981b	41,9886a
4	BCR Ligsll 4	55,0037a	4,8749d	76,3429a	30,6751b

Notes: 1) Lignase analysis using Tannic Acid substrates, 2) Cellulase (Endo-glucanase) analysis using CMC powder substrates, 3) Xylanase analysis using Xylanose substrates, 4)Mean in the same colom with different letter differ significantly (P<0,05).

Table 3. Cellulase activities from cellulose degrading bacteria.

No	Isolate of Bacteria	Exo-glucanase[1] (U/ml) on minute to...			
		t5	t10	t15	t20
1	BCR CMC 1	0	0,1346e[3]	0,0911f	0,0574g
2	BCR CMC 2-1	0	0,1406e	0,1443c	0,0869bcd
3	BCR CMC 2-2	0	0,4373a	0,3785b	0,0661efg
4	BCR CMC 2-3	0	0,1415de	0,4377a	0,0765def
5	BCR CMC 3	0	0,1554cde	0,0676g	0,0961bc
6	BR CMC 2	0	0,0473f	0,0854fg	0,1015b
7	BR CMC 3-1	0	0,2041bcde	0,1010ef	0,1013b
8	BR CMC 3-2	0	0,2368b	0,1334cd	0,0775def
9	BR CMC 3-3	0	0,2219bc	0,1202de	0,0638fg
10	BR CMC 3-4	0	0,2170bcd	0,1182de	0,0809cde
11	BR CMC 3-5	0	0,1435de	0,0884f	0,0829cd
12	BR CMC 3-6	0	0,1440de	0,1205de	0,0710defg
13	BR CMC 3-7	0	0,1664bcde	0,1129e	0,1889a

Notes: 1) exo-glucanase analysis using substrats Avicel-cellulose mikro crystallin, 2) Endo-glucanase analysis using substrats CMC powder, 3) Mean in the same colom with different letter differ significantly (P<0,05).

Cellulose degrading bacteria isolate from bali cattle rumen waste and termites has exo-glucanase activities on minute to 10 until 20 were 0,0473 – 0,4377 U/ml. BCR CMC 2-2 cellulolytic bacteria isolate from bali cattle rumen waste has highest exo-glucanase on minute to 10 was 0,4373 U/ml and significantly (P<0,05) with the whole of them. On minute to 15, BCR 2-3 isolate has highest exo-glucanase activities of 0,4377 U/ml. Even though, on minute to 20, BR CMC 3-7 bacteria isolate has highest exo-glucanase activities was 0,1889 U/ml. Even though on minute to 20', isolate BR CMC 3-7 from termites has highest exo-glucanase activities. At table 3 so showed cellulose degrading bacteria from termites that has high enzyme activities was BR CMC 3-2 isolate were 0,2368 U/ml and 0,1334 U/ml respectively contacts with substrates on minute to 10' and 15'. Xylan is main carbohydrate that form hemicellulose, consist of xylosa polymer and other sugar with ß-1,4, bond and end side chain with a-1,2 or a-1,3 bonds (Peres *et al.*, 2002). Xylanase enzyme activities from xylanose degrading bacteria isolate bali cattle rumen waste and termites showed at Table 4. At table showed isolate BR Xy 1 from termites has highest (P<0,05) enzyme activities on minute to 5 and 20 were 85,6328 U/ml and 46,1507 U/ml. Even though on minute to 10 and 15, isolate BCR Xy 2 from bali cattle rumen waste has highest (P<0,05) enzyme activities were 93,2211 U/ml and 33,6951 U/ml.

Table 4. Xylanase activities from xylanose degrading bacteria.

No	Isolate of Bacteria	Xylanase Activities (U/ml) on minute to...			
		t5	t10	t15	t20
1	BCR Xy 1	44,7940cd[1]	71,5140b	4,3844c	34,6532b
2	BCR Xy 2	69,8124ab	93,2211a	33,6951a	45,8747a
3	BCR Xy 3	77,5386a	78,6884ab	16,0351b	44,3111a
4	BCR Xy 4	56,1994bc	75,5611ab	17,6294b	16,8092d
5	BCR Xy 5	30,8131d	59,0048b	29,6480a	26,0072c
6	BR Xy 1	85,6328a	14,4868c	4,9975c	46,1507a

Notes: 1) Xylanase analysis using substrats xylanose, 2) Mean in the same colom with different letter differ significantly (P<0,05).

CONCLUSION

Isolation of lignocelulose degrading bacteria from bali cattle rumen waste found 4 lignocellulolytic bacteria, 5 cellulolytic bacteria, and 5 xylanolytic bacteria. Even though from termites could isolated 8 cellulolytic bacteria and 1 xylanolytic bacteria. Isolate BCR Ligsll 2 and isolate BCR Ligsll 4 has highest first and second lignocellulolytic enzyme activities. First and second highest cellulase activities were isolate BCR CMC 2-2 and isolate BCR CMC 2-3 from bali cattle rumen waste, even though from termites were isolate BR CMC 3-7 and isolate BR CMC 3-2 . First and second highest xylanase activities were isolate BCR Xy 2 and isolate BCR Xy 3 from bali cattle rumen waste, even though from termites was isolateBR Xy 1.

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