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By

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Partama, I. B. G., I M. Mudita, N. W. Siti, I W. Suberata, and A.A.A. S. Trisnadewi
Faculty of Animal Husbandry, Udayana University, Denpasar, Indonesia

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 since it has been consumed for 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 byproducts of many kinds of food crops, agro-industry and farm waste are sources of ruminant feed ingredients are potential alternatives.
Most natural source waste such as nonconventional waste are rich in lignocellulose materials. Cellulose, a long chain polysaccharide made of β(1,4)-linked glucose units, is the principal constituent of lignocelluloses. The association of cellulose with lignin, another complex polymeric molecule composed of phenylpropanoid units, lignocelluloses form. Hemicellulose is the other major component of lignocellulose. It is a heterogeneous 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 relative 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) mentioned that 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-1,4-glukosidase) and hemiselulase enzyme (endo-1,4-β-xilanase dan β-D-1,4-mannanase) (Purwadaria et al. 2003, 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 CaCl2; 0,10g NaCl; 0,1g (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 transferred aseptically with oxygen-free CO gas displacing all air until red color faded, closed with rubber 2 stopper, sealed, then sterilized with its content in 12 psi for 20 minutes. In warm condition, media was divided into 3 tubes. Each selective substrate then dissolved, then poured 4.5 ml each into 5 mm petri disc. Microbes source liquid (50 µl) with 10-5 dilution then were inoculated for 7-14 days in anaerobic jar filled by anaerobic generating kit. The growing colonies then were counted.

### 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 by modified Hungate method (Bachruddin, 1985) which were mixed 150 ml mineral I solution, 150 ml 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 ml aquadest in 1000 ml Erlenmeyer. Substrates that used were mixed lignin, xylan and cellulose, adjusted by each enzymes production test. All materials in Erlenmeyer then were heated 100°C for 5 minutes for homogenized along with CO gases. Temperature was sustained 45°C in water bath. An aerobic condition was reached when red color was faded. Then, 32.3 ml sodium carbonate and 16.7 ml Cystein-HCl were added. Then, the tube was closed with a rubber stopper and sealed, sterilized in 121°C for 15 minutes. Each media (according to selective substrate) was divided according to its isolates number that would be grown in 50 ml serum bottle. Isolate from solid media was dissolved in dilute solution in 0.5 λ 600 absorbent, inoculated in bottle as much as 10%, incubated in 39°C for 7 days. Growth culture media then was used as enzymes source.

### Quantitative Selection

Enzyme extract was collected from centrifuged liquid media culture in 12,000 x g for 15 minutes in 4°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 1 ml aquadest. Then mixture were shaken by fortex, enzyme activity measured in 60 minutes. Reduction of sugar (glucose from CMC, xyllose from xylan), or vanillin from lignin produced from reaction of enzyme 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 vanillin: 1 ml of sample added to 4 ml methanol, then measured absorbent with spectrophotometer in λ 508.5 nm for glucose, 509 nm for xilosa and 279 nm for vanillin.

### Research Design

The research was conducted based on qualitative and quantitative analysis. A Completely Randomized Design was used as statistical design. Isolates found used as treatment with three replication and lignocellulase, cellulase, xylanase, and ligninase as parameters being 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.

<table>
<thead>
<tr>
<th>No</th>
<th>Species</th>
<th>Isolate Source</th>
<th>Bali Cattle Rumen Content Waste</th>
<th>Termites</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lignocellulolytic bacteria</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Lignolytic Bacteria</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Cellulolytic Bacteria</td>
<td>5</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Xylanolytic Bacteria</td>
<td>5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TOTAL</td>
<td>14</td>
<td>9</td>
<td></td>
</tr>
</tbody>
</table>

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 showed that 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 isolate 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 that BCR Ligsll 4 and BCR Ligsll 2 bacteria isolates has higher quality and most potencial as lignocellulose inocullant/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 Hydrogen bond in cellulose crystallin structure (α1,4 glucoside 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 Activities from Lignocellulose Degrading Bacteria From Bali Cattle Rumen Content Waste.

<table>
<thead>
<tr>
<th>No</th>
<th>Isolate of Mikroba</th>
<th>Lignase Enzyme Activities $^{1)}$</th>
<th>Cellulase Enzyme Activities $^{2)}$</th>
<th>Xylanase Enzyme Activities $^{3)}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>t5</td>
<td>t10</td>
<td>t15</td>
</tr>
<tr>
<td>1</td>
<td>BCR Ligsll 1</td>
<td>0.1265d$^a$</td>
<td>0.3295b</td>
<td>0.2009c</td>
</tr>
<tr>
<td>2</td>
<td>BCR Ligsll 2</td>
<td>0.5164b</td>
<td>0.4071ab</td>
<td>0.3880a</td>
</tr>
<tr>
<td>3</td>
<td>BCR Ligsll 3</td>
<td>0.1978c</td>
<td>0.3692ab</td>
<td>0.2821b</td>
</tr>
<tr>
<td>4</td>
<td>BCR Ligsll 4</td>
<td>0.6440a</td>
<td>0.4416a</td>
<td>0.2174c</td>
</tr>
</tbody>
</table>

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 column with different letter differ significantly ($P<0.05$).

### Table 3. Cellulase activities from cellulose degrading bacteria.

<table>
<thead>
<tr>
<th>No</th>
<th>Isolate of Bacteria</th>
<th>Exo-glucanase $^{1)}$ (U/ml) on minute to...</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>t5</td>
</tr>
<tr>
<td>1</td>
<td>BCR CMC 1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>BCR CMC 2-1</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>BCR CMC 2-2</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>BCR CMC 2-3</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>BCR CMC 3</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>BR CMC 2</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>BR CMC 3-1</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>BR CMC 3-2</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>BR CMC 3-3</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>BR CMC 3-4</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>BR CMC 3-5</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>BR CMC 3-6</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>BR CMC 3-7</td>
<td>0</td>
</tr>
</tbody>
</table>

Notes: 1) exo-glucanase analysis using substrates Avicel-cellulose mikro crystallin, 2) Endo-glucanase analysis using substrates CMC powder, 3) Mean in the same column 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 β-1,4, bond and end side chain with a-1,2 or α-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.

<table>
<thead>
<tr>
<th>No</th>
<th>Isolate of Bacteria</th>
<th>Xylanase Activities (U/ml) on minute to...</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>t5</td>
</tr>
<tr>
<td>1</td>
<td>BCR Xy 1</td>
<td>44,7940cd'</td>
</tr>
<tr>
<td>2</td>
<td>BCR Xy 2</td>
<td>69,8124ab</td>
</tr>
<tr>
<td>3</td>
<td>BCR Xy 3</td>
<td>77,5386a</td>
</tr>
<tr>
<td>4</td>
<td>BCR Xy 4</td>
<td>56,1994bc</td>
</tr>
<tr>
<td>5</td>
<td>BCR Xy 5</td>
<td>30,8131d</td>
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<tr>
<td>6</td>
<td>BR Xy 1</td>
<td>85,6328a</td>
</tr>
</tbody>
</table>

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 isolate BR Xy 1.

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