

Degradation of Lignocellulosic Substrates by Bacteria Isolated from Waste Landfill

Mudita, I. M.

Laboratory of Animal Nutrition
Faculty of Animal Husbandry,
Udayana University, Denpasar,
Bali, Indonesia
muditafapet_unud@yahoo.com,
HP. 081338791005

I. W. Wirawan

Laboratory of Plant Tropical
Faculty of Animal Husbandry,
Udayana University, Denpasar,
Bali, Indonesia

A. A. P. P. Wibawa

Laboratory of Biochemistry
Faculty of Animal Husbandry,
Udayana University, Denpasar,
Bali, Indonesia

I. B. G. Partama

Laboratory of Plant Tropical
Faculty of Animal Husbandry,
Udayana University, Denpasar,
Bali, Indonesia

Abstract – A research has been carried out to evaluating potency of bacteria isolated from waste of general rubbish place landfill as lignocellulosic substrates degrader. Bacteria were isolated by Hungate selective media using combination of tannic acid (lignin), xylan, and Carboxy Methyl Cellulose/CMC (cellulose) as selective substrates. The potency of lignocellulolytic was identified based on degradation of lignocellulosic substrates measurement using clear/diffusion zone wide and lignocellulase enzyme activity. This study showed that lignocellulolytic bacteria isolated from waste landfill produce clear/diffusion zone wide 0.150 – 3.603 cm², 0.010 – 0.051 cm², 1.343 – 1.565 cm² and 0.260 – 0.435 cm² respectively for lignocellulose, lignin, cellulose and xylan substrates, while isolates coded BW 1 LC produced highest clear/diffusion zone wide on lignocellulose, lignin and xylan substrates, even though on cellulose substrates, bacteria isolates coded BW 4 LC highest clear zone wide. Meanwhile for lignocellulase enzyme activity, bacteria isolates coded BW 1 LC and BW 4 LC has highest enzyme activity at tannic acid, CMC, and xylan substrates. It was concluded that bacteria isolates coded BW1 LC and BW 4 LC were potential as lignocellulosic substrates degrader.

Keywords – Waste Landfill, Clear Zone Wide, Enzyme Activity, Lignocellulosic Substrates.

I. INTRODUCTION

The development of feeding system based on the local resources such as agricultural waste is the pillars supporting the development of sustainable and competitive animal production systems especially ruminant species in Indonesian [1]. Utilization of agricultural waste as feed will reduce the emission of pollutants in the air [2]. Although, the use of local resources waste as feed without the application of processing technology will decrease the productivity of livestock and increase pollutant emissions due to high crude fiber content in particular lignocellulose compounds and very difficult to be utilized by animals [3];[4];[5].

Most agricultural waste of crop plants are rich in lignocellulosic substrates [6];[7];[8]. Cellulose, a long chain polysaccharide made of $\beta(1,4)$ -linked glucose units, is the principal constituent of lignocelluloses. Association of cellulose with lignin, another complex polymeric molecule composed of phenylpropanoid units, form the lignocelluloses. Hemicellulose is the other major component of lignocellulose. It is a heterogeneous group of long chain polysaccharides of which basic unit are arabinose, xylose, mannose, or galactose [9]. Degradation

lignocelluloses material is a slow process and only relative narrow taxonomic range of bacteria is able to degrade such material. The ability of microorganisms to degrade lignocellulolytic material is of considerable interest both in terms of microbial ecology and biotechnology.

Degradation of lignocellulosic substrates requires the cooperative action of family of lignocellulolytic enzyme that have been classified into three complex enzyme groups: i.e lignolytic enzyme (lignase), cellulolytic enzyme (cellulase) and hemicellulolytic enzyme (hemicellulase). Lignocellulase complex enzyme can produce by certain microbe, such as lignocellulolytic bacteria. So the effort to develop lignocellulolytic bacteria is very important in optimizing utility agricultural waste as feedstuffs. The waste of general rubbish place is one source of lignocelluloses degrading bacteria isolates.

The waste of general rubbish place (landfill) contains many fiber degrading microbes (bacteria and fungi) such as lignolytic, cellulolytic, hemicellulolytic, amylolytic and proteolytic and various other microbes which high enzyme activities [10]. Pathma and Sakthivel (2012) stated that bacteria isolates in organic manure so has ability of organic material, antinutritional substrates and so function as *growth promotor*. Based on the potential of lignocellulosic substrates degradation, bacteria isolated from the dirt of general rubbish place made to explore its potential as a source of inoculum agro-waste degrader on development of competitive and sustainable livestock.

II. MATERIALS AND METHODS

Isolate Source Sample Preparation

The waste of general rubbish place landfill sample taken from the Suwung General Rubbish Place in Denpasar, Bali Province-Indonesian Country. The waste samples were used as a source of isolates taken from a pile of garbage that is going through the process of decomposition. The sample was brought to the laboratory in strict anaerobic condition for further processing. Dilution sample in series until 10^{-7} fold dilution with dilution medium formula No 14 Bryant and Burkey [11].

Solid Media and Isolation

Bacteria from samples were grown in selective solid media by Hungate method [11] containing 0,02g KH₂PO₄; 0,03g K₂HPO₄; 0,01g MgSO₄; 0,01g CaCl₄;

0,10g NaCl; 0,10g (NH₄)₂SO₄; 0,10ml Rezasurin 0,1% solution; 0,02g Cystein-HCl.H₂O; 0,40g Na₂CO₃; 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 and Carboxy Methyl Cellulose/CMC (as cellulose source). All ingredients were mixed in Erlenmeyer (except substrate that were sterilized by 5 ml aquadest in tube), pH was determined 6.8 and heated until all ingredients dissolved. The flask then transferred aseptically with oxygen-free CO₂ gas displacing all air until red color faded, closed with rubber 2 stopper, sealed, then sterilized in the autoclave at 121°C for 15 minutes. The medium in the enlenmeyer transferred aseptically to a sterile tube with oxygen free CO₂ gas displacing all air. Then 10⁻⁷ dilution colon fluid inoculated into the tube and closed with sterile cotton. The culture the incubated in 39°C during 5–7 days. The colonies growing were selected.

Isolation of Colonies

From these inoculated tube containing the selection medium, the individual colonies of lignocellolytic bacteria pricked. Bacteria isolates carefully pricked using bent platinum-iridium needle. The bacteria then transferred to plate agar medium anaerobically with gasses oxygen-free CO₂. The plate incubated at 39°C during 5 – 7 days. The bacteria colonies has produce clear or diffusion zone were chosen for furified by repeated streaking.

Ability of Lignocellulosic Substrates Degradation

The ability of lignocellulosic substrates degradation is determined from clear or diffusion zone formed by bacteria isolates tested [11]. The substrate used: CMC for cellulose, xylan for hemicellulose, tannic acid to lignin and combination of 50% CMC + 30% Xylan + 20% tannic acid for lignocellulose substrate. Each pure bacteria isolate (15 µl) was inoculated by spot method using paper disc blank 0.6 cm were placed on selective medium (solid growth medium containing 1% substrate test)[12]. Diffusion and clear zone wide were measured after 24 hours of anaerobic incubation.

Lignoselulase enzyme activity

Enzyme extract was collected from centrifuged liquid media culture in 12.000 x g for 15 minutes in 4°C. According to the substrate, extracts were tested in three kinds of substrates that contained 1% CMC powder/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 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, and 6 hour. Reduction sugar (glucose from CMC, xylose from xylan), or vanillin from tannic acid (lignin) produced from the reaction were the 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 λ 508,5 nm for glucose, 509 nm for xilosa and 279 nm for vanillin. Enzym (lignase, cellulose, and xylanase) production was estimated by using vanillin/glucose/xylose calibration curve [15]; [16]. One unit (U) of enzyme activity was

defined as 1 µmol of vanillin/glucose/xylose equivalent released per minute under standard assay condition [17]; [18].

III. RESULTS AND DISCUSSION

Ability of Lignocellulosic Substrates Degradation

The results showed lignocellolytic bacteria isolates from landfill waste capable of generating broad clear zone on lignocellulosic substrates from 0.150 - 3.603 cm², which isolates coded BW 4 LC produce highest clear zone wide (P<0.05) and isolates BW 7 LC produce lowest (P<0.05) clear zone wide. At substrate tannic acid (source of lignin), bacteria isolates from landfill waste capable of producing high clear zone wide of 0.010 - 0.051 cm², which isolates coded BW 1 LC produce highest clear zone wide (P<0.05) and isolates coded BW 5 LC produce lowest (P<0.05). At CMC substrate, bacterial isolates from landfill waste capable of producing a clear zone wide by 1.343 - 1.565 cm², which isolates coded BW 4 LC produce the highest (P<0.05) clear zone wide and isolates BW 7 LC produce the lowest (P<0.05). While the xylan substrate, bacteria isolates capable of generating landfill waste clear zone wide of 0.260 - 0.435 cm², which isolates code BW 1 LC produce the highest (P<0.05) clear zone wide and isolates BW 3 LC produce the lowest (P<0.05) (Table 1).

Table 1: Wide of clear zone (cm²) from 15 µl bacteria isolates on variety substrates

Bacteria Isolates	Ligno-cellulose	Lignin	CMC	Xylan
BW 1 LC	2,314c ¹	0,051c	1,548bc	0,435b
BW 2 LC	0,196a	0,024abc	1,527bc	0,294a
BW 3 LC	2,035c	0,042bc	1,428ab	0,260a
BW 4 LC	3,603d	0,047c	1,565c	0,419b
BW 5 LC	0,198a	0,010a	1,439ab	0,283a
BW 6 LC	0,440b	0,027abc	1,536bc	0,333ab
BW 7 LC	0,150a	0,011ab	1,343a	0,262a
SEM ²	0,22	0,01	0,02	0,02

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

Produced high clear zone wide by all bacteria isolates showed its bacteria isolates true lignocellolytic bacteria (lignocellulose degrading bacteria) and have high ability of lignocellulosic substrates degradation. These isolates isolation using combination lignin (tannic acid), cellulose (carboxy methyl cellulose/CMC) and xylan as selected substrates in bacteria isolated media. Every strain of bacteria isolates needed a spesific substrates as an energy source for growing [19]. Besides, the source of isolates derived from waste undergoing biodegradation processes so rich in lignocellulose degrading microbes including bacteria.

Based on theses data on Table 1 it appears that isolates coded BC 1 LC and BC 4 LC are the two best candidates isolates potential to be utilized in the development of farm-based agricultural wastes. These are because the ability of the formation of a clear zone reflects the ability

of substrate degradation is the result of the activity of microbial enzymes that will determine the level ability degradation of fiber component from feed material [8];[9]. Its bacteria isolates potential as innoculant fermentor organic material rich in lignocellulosic substrates such as agricultural waste.

Lignocellulase Enzyme Activity

Evaluation of lignocellulase enzyme activity from lignocellulolytic bacteria isolates were isolated from waste landfill showed at Table 2.

Table 2: Enzyme Activities from Lignocellulolytic Bacteria Isolates from Waste Landfill

Bacteria Isolate	Enzyme Activities (IU/ml) on minute to...			
	t30	t60	t180	t360
A. Lignase Enzyme Activities¹⁾				
BW 1 LC	0.060c	0.046b	0.022c	0.011b
BW 2 LC	0.044b	0.039b	0.018bc	0.009ab
BW 3 LC	0.011a	0.007a	0.013ab	0.008ab
BW 4 LC	0.056c	0.040b	0.021c	0.011b
BW 5 LC	0.008a	0.032b	0.013ab	0.008a
BW 6 LC	0.052bc	0.040b	0.020bc	0.010ab
BW 7 LC	0.013a	0.035b	0.009a	0.008ab
SEM ⁵⁾	0.002	0.003	0.001	0.001
B. Cellulase Enzyme Activities²⁾				
BW 1 LC	0.078b	2.254e	0.015c	0.008c
BW 2 LC	0.045a	1.340a	0.008a	0.005a
BW 3 LC	0.072b	2.172cde	0.012b	0.006ab
BW 4 LC	0.076b	2.221de	0.016c	0.008c
BW 5 LC	0.050a	1.670ab	0.010ab	0.061ab
BW 6 LC	0.071b	1.858bcd	0.011b	0.007b
BW 7 LC	0.052a	1.852bc	0.011b	0.006ab
SEM	0.003	0.074	0.083	0.0874
C. Xylanase Enzyme Activities³⁾				
BW 1 LC	29.581cd	18.9018d	7.431c	3.863c
BW 2 LC	11.111ab	11.507bc	4.722b	2.349a
BW 3 LC	19.868bc	12.931c	6.727c	3.112b
BW 4 LC	32.377	18.865d	6.911c	3.581bc
BW 5 LC	7.873a	9.722abc	3.728ab	1.907a
BW 6 LC	13.760ab	5.675a	3.520ab	2.349a
BW 7 LC	8.020a	7.713ab	2.502a	1.944a
SEM	2.276	0.966	0.263	0.1380

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).

Evaluation of lignocellulase enzyme activity that isolates coded BW 1 LC was significant highest (P<0.05) lignase, cellulase and xylanase enzyme activity during the period of contact with the lignocellulose substrate except cellulase enzyme activity for 180 minute and xylanase enzyme activity for 30 minute after contact with substrates, which isolates coded BW 1 LC has enzyme activity was second highest and the highest enzyme activity produced by isolates coded BW 4 LC. Bacteria isolates coded BW 5 LC, BS 3 and BW 7 LC were isolates that has the lowest lignase enzyme activity on lignocellulosic substrates after contact on lignocellulosic

substrates, respectively at the time to 30 minutes and 6 hours, 1 hour and 3 hours (Table 2). Isolates BW 2 LC has the lowest cellulase enzyme activity on lignocellulosic substrates in the entire period of contact time (Table 2). Whereas isolates coded BW 5 LC, BW 6 LC and BW 7 LC has the lowest xylanase enzyme activity on lignocellulosic substrates in the period contact for 30, 60, 180 and 360 minutes (Table 2).

In Table 2 it appears that the value of a xylanase enzyme activity produced by each isolate bacteria tends to be higher than the activity of the enzymes cellulase and lignase. This is reasonable considering the structural components of hemicellulose (xylan) tend to be easier to degrade than cellulose and lignin components. Cellulose and lignin compounds having components with very hard crystalline structure changed into components simpler and even lignin is the major limiting degradation of lignocellulosic compounds [7];[8]

On Table 2 also appears that bacteria isolates coded BW 1 LC and BW 4 LC has high lignase, cellulase and xylanase enzyme activity on all time periods contact with the lignocellulosic substrate. This indicates that its isolates (BW 1 LC and BW 4 LC) are isolates potential to be developed further, especially used in the development livestock based on agricultural waste. So basically from waste landfill has isolation high quality lignocellulolytic bacteria isolates which used as degrader of lignocellulosic compounds, especially agricultural waste

IV. CONCLUSION

Lignocellulolytic bacteria isolates coded BW 1 LC and BW 4 LC has highest ability of lignocellulosic substrates were showed produced highest wide of clear zone and lignocellulase enzyme activity. Its isolates potential to apply on optimizing the utilization of local resources based on agriculture by-product feedstuffs to support feed security of ruminant production.

ACKNOWLEDGEMENTS

The authors wish to thanks to the Directorate General of Higher Education, National Education Department, Republic of Indonesia and Udayana University through Competitive Research Grant for fund support in this experiment. Thanks are also due to Laboratory of feed and nutrition animal, Faculty of Animal Husbandry Udayana University for assistance in isolation and characterization isolates analysis.

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AUTHOR'S PROFILE



I Made Mudita

Place and date of birth: Bali-Indonesia, 10/05/1974,
 Educational background: S.Pt. Animal Husbandry, Udayana University (Indonesia), 1997, and MP; Magister of Animal Science, Udayana University, 2008. Currently undertaking doctoral education in Animal Science from the University of Udayana in

2012-now.

He is lecturer, since 2005–now at the Faculty of Animal Husbandry, Udayana University, Denpasar, Bali, Indonesia. Major research interest and Experiences: Ruminant Nutrition, Biotechnology and Microbiology. Many articles published in national journal, 2 article in international journal and 1 book “Bali Cattle. Resources of Pure Genetic from Indonesia”, Published by Udayana University Press, Bali-Indonesia, 2012 I Made Mudita, S.Pt., MP are membership in professional societies of Indonesian Nutrition and Feed Science Association, and Livestock Indonesian Scholar Association.