PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 12 (9): 851-853, 2013
ISSN 1680-5194

Potency of Indigenous Bacteria from Oil Palm Waste in Degrades Lignocellulose as a Sources of Inoculum Fermented to High Fibre Feed

Yunilas[1], Lili Warli[2], Yetti Marlida[2] and Irsan Riyanto[2]
[1]Department of Animal Husbandry, Faculty of Agriculture, University of North Sumatera, Medan, Indonesia
[2]Department of Animal Nutrition, Faculy of Animal Husbandry, Andalas University, Padang, Indonesia

Abstract: This study aimed to test the ability of indigenous bacteria derived from oil palm waste to degrade lignocellulose as sources of inoculum fermented to high fibre feed. The selection is based on the highest ratio of clear zone the colony grow for 24 h (Kluepfel, 1988). The study we got 10 isolates that could degrades lignocellulose, but 5 among it has a better ability to degrades lignocellulosa with the ratio of clear zone (index hydrolysis) there are YL.B1 (2.9), YL.B2 (2.7), YL.B7 (2.6), YL.B8 (1.7) and YL.B9 (2.5). YL.B1 is the best isolates to degrades lignocellulosa, it shown by the highest hydrolysis index value rather than other isolates. Isolates YL.B1 is indigenous bacteria from oil palm waste, it has the potential to degrade the fiber (lignocellulose) and can be used as a source of inoculum fermented for to high-fiber feed.

Key word: *Indigenous bacteria*, oil palm waste, high-fiber feed

INTRODUCTION

Indigenous bacteria are bacteria that are exploited from the substrate itself that has optimal ability to degrade the substrate. Through the exploration of indigenous bacteria will produce multi-enzyme that an important role in feed processing. Feed processing using the indigenous bacteria will optimize the ability of rumen microorganisms to digest high fiber feed. Multi enzymes contained in the indigenous bacteria from oil palm waste will have a higher ability to degrade lignocellulose of oil palm waste itself.

The Cellulosome is merging of *cellulose-binding domain* (CBD) and *xilan-binding domain* (XBD) into a multi-enzyme with high molecular weight that are found in some of an aerobic cellulolytic microorganisms. The cellulosome is cellulase complex of several cellulolytic bacteria or molds that work in synergy with different characteristics (Bayer *et al*., 1994). Multi-enzymes are very efficient in the degradation of cellulose and hemicellulose. The main products of cellulose degradation from cellulosome mold is glucose, while from the cellulosome bacteria is cellobiose. Cellulosome fungi of debility more than cellulosome bacteria such activity of synergy between components and hydrolysis activities of the cellulose and hemicellulose (Dashtban *et al*., 2009).

Cellulose by cellulosome and others cellulolytic microbes will be degraded to cellobiose and glucose. Xylan by xylanolytic microbes will be degraded into soluble sugars and then by saccharolytic microbes be degrade again become simpler sugars. Furthermore, a simple sugar that will be utilized by other microbes to produce other products such as ethanol, acetone, methane and so on. Besides, it would have resulted with vitamins, nutrients and protective agents (Bayer *et al*., 1994).

Lignin degrading enzymes (ligninolytic) consists of laccase (polyphenol oxidase), lignin peroxidase (Li-P) and manganese peroxidase (Mn-P). All three are multi extracellular enzymes that play a role in lignin depolymerization process (Widyastuti *et al*., 2007). Hemicellulose which is the second component is the most heterogeneous polymers of pentose (xylose, arabinose), hexose (mannose, glucose, galactose) and sugar acids (Saha, 2004). The main sugar residues constituting the xylan, mannan, galactan and glucan (Fengel and Wegener, 1995).

In plants, cellulose coated with a polymer that consists mainly of xylan and lignin. Xylan can be degraded by xylanase, but very difficult degraded lignin. If xylan and lignin is removed, the cellulose can be degraded by cellulases from cellulolytic bacteria or mold to produce cellobiose and glucose. Cellobiose system often serves to inhibit the action of cellulases and cellulolytic process will quickly stop when no other saccharolytic microbes in the ecosystem. Cellobiose excess generated will be utilized by microbes saccharolytic so that microbes can continue cellulolytic cellulose degradation (Bayer *et al*., 1994).

MATERIALS AND METHODS

Isolation of indigenous bacteria: As source of indigenous isolates used oil palm waste (oil palm frond, palm carnel cake and sluge). One gram each of samples collected aseptically were serially diluted in distilled water from 10^{-3} to 10^{-5} and spread plated on

Corresponding Author: Yunilas, Department of Animal Husbandry, Faculty of Agriculture, University of North Sumatera, Medan, Indonesia

modified selective agar medium containing (0,5 g pepton, 0,5 g yeast agar, 0,1 g K_2HPO_4, 0,02 g $MgSO_4$. $7H_2O$, 1 g Na_2CO_3, 20 g agar, 0,25 g CMC, 0,25 g xylan, 0,25 g lignin, 0,25 g mannan) in 1000 mL distilled water. The plates were incubated at 37°C for 1-2 days and the growing bacteria colonies were sub-cultured to obtain pure cultures. The isolat finded to be Purified in the medium of NA (Cappuccino and Sherman, 1987).

Qualitative screening of ligninolytic producing from indigenous bacteria: Lignocellulose producing bacteria were screened on modified selective agar medium (Basal Salt Medium) containing 0,2% KNO_3, 0,1% K_2HPO_4, 0,05% $MgSO_4$.7H2O, 0,05% NaCL, 0,001% $FeSO_4$, 0,03% $CaCO_3$, 1,8% agar and CMC, xylan, mannan or tanat acid. Plates were spot inoculated with spore suspension of pure cultures and incubated at 37°C. After 48 h, plates were flooded with 1% Congo red solution for 15 min.The diameter of zone of decolorization around each colony was measured. Hydrolysis index was determined and expressed by the ratio between the diameter of the degradation halo and the diameter of the colony (Khokhar *et al*., 2012) and than identified. Bacterial identification was based on colony morphology, microscopic observation using staining reaction and biochemical tests using the Polymerase Chain Reactions (PCR)-sequencing method (Cappuccino and Sherman, 1987). Colony morphology observation includes observation on the shape and color colonies (Pelczar and Chan. 1988). All experiments were carried out in triplicate and the mean and standard deviation values were calculated using the MS Excel program.

RESULTS AND DISCUSSION

Isolated from oil palm waste obtained 10 isolates of cellulolytic and 5 of them showed better growth than others. Then from the five isolates (YL.B1, YL.B2, YL.B7, YL.B8 and YL.B9) the ability of selected lignocellulosic to degrades qualitative Fig. 1.

Based on the test results are lignocellulose activity on qualitative (Fig. 1) appears five isolates of successfully remodel lignocellulose in a modified selective media are characterized by a clear zone. Clear zone formed around colonies of bacteria showed that the bacteria success to hydrolyze of lignocellulose contained in the media.

Presence of bacterial extracellular enzymes, causing lignocellulose hydrolyzed into peptides and simple monomers. In the hydrolysis process that would perfectly produced glucose, while the majority will be generated disaccharide cellobiose.

Wide clear zone isolates ranged from 19.00-27.50 mm with a hydrolysis index ranges from 1.7-2.9 Table 1. Results obtained qualitative selection that isolates YL.B1 most extensive clear zone compared to other isolates with diameter 27.50 mm, so that YL.B1 isolates can be expressed as a potential enzyme producing isolates based on the highest hydrolysis index than other isolates were obtained.

The resulting clear zone is lower than *Bacillus amyloliquefaciens* (27.85 mm) but higher than that of *Bacillus coagulans* (15.40 mm) in the CMC medium (Wizna, 2007). Observations demonstrate the ability of isolates to degrade xylan, mannan and cellulose is more powerful, but very weak ability to degrade lignin (Tabel 2). These results are consistent statement (Bayer *et al*., 1994) that xylan can be degraded by xylanase enzyme, but very difficult degraded lignin.

Based on observations, it appears that $YL.B_1$ isolates is very efficient in breaking down carbohydrates compounds present in the substrate. The results showed that the isolates YL.B1 has the potential to produce a wide range of particular enzymes cellulase, hemicellulase/xylanase, mananase and lignin degrading enzymes (lignocellulose).

From isolates that have the most extensive clear zone (YL.B1) then be identified. Morphological observations appear isolates creamy whitish, with a circular shape, surface konvex the entire edge of the colony, a short form of stem cells (cocoid) and a negative gram stain test. Biochemical test results showed that these isolates are not able to use citrate as a carbon source, facultative anaerobic, bacteria cannot ferment sugars, the bacteria are motile by flagella, did not produce gelatinase enzyme, the bacteria has the amylase enzyme which can hydrolyze starch (starch), bacteria have the catalase enzymes, bacteria do not produce H_2 and CO_2 gas and avoid the formation of H_2S (hydrogen sulfite).

Tests conducted are not sufficient to determine the species of these isolates, because of the variation in test results between species. Then to determine the

Table 1: Diameter of the clear zone and hydrolysis index from isolates on medium CMC, xylan, lignin and mannan (48 h)

No.	Isolates	Clear zone (mm)	Hydrolysis Index (HI)
1.	YL.B1	27.50	2.9
2.	YL.B2	26.00	2.7
3.	YL.B7	25.50	2.6
4.	YL.B8	19.00	1.7
5.	YL.B9	24.25	2.5

Hydrolysis index = ratio between the clear zone/colony diameter

Table 2: Extracellular Enzyme Activity Test of Isolates YL.B1 In Medium Containing Substrates CMC, Xylan, Lignin and Mannan Qualitative

Medium containing substrates	Clear zone
Cellulose (CMC)	++
Xylan (from beechwood)	+++
Mannan (bean gum)	+++
Lignin (tanat acid)	+

+ Means there's enzyme activity

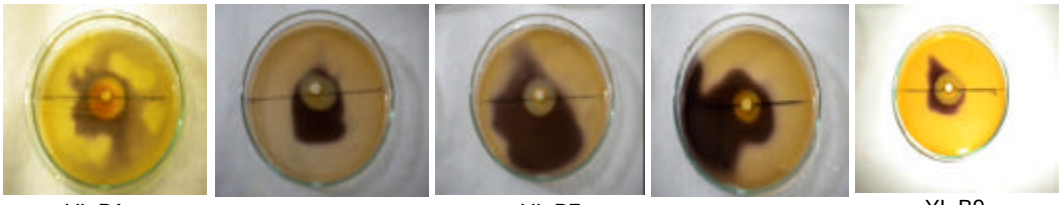

Fig. 1: Test results of Q ualitative Activity of ligninolytic of Bacteria Isolates on Medium CMC, Xylan, Lignin and Mannan (48 h)

species identification of isolates YL.B_1 performed by 16S rRNA gene sequences as the most effective approach is to determine the bacterial taxonomic analysis of 16S rRNA molecules. From the analysis of the partial 16S rRNA gene sequences of isolates YL.B1 showed that these isolates belong to the species *Bacillus* sp.

Morphological observations on YL.B1 (*Bacillus* sp.) in line with the statement (Barrow and Feltha, 1993) that the *Bacillus* sp., is a rod-shaped bacteria, classified as Gram-positive bacteria in young cultures, motile (non-motile reactions sometimes occur), produces spores that are usually resistant to heat, are aerobic (some species are facultative anaerobes), catalase positive and oxidation varies. Each species differences in the use of sugar, some do and some not ferment. According to (Corbin, 2004), *Bacillus* sp., colonies have a common characteristic whitish beige in color and can be spherical and irregular on the incubation period 24-48 h.

Conclusion: Based on qualitative testing, chemical testing and molecular analysis of the 16S rRNA YL.B1 isolates (*Bacillus* sp.) is an indigenous bacteria from oil palm waste could potentially degrade lignocellulose and can be used as a source of inoculum fermented to high-fiber feed, especially in the processing of oil palm waste.

REFERENCES

Barrow, G.I. and R.K.A. Feltha, 1993. Cowan And Steel's Manual for the Identification of Medical Bacteria. Published By The Press Syndicate Of The University Of Cambridge, United Kingdom.

Bayer, E.A., E. Morag and R. Lamed, 1994. The *Cellulosome*-A *Treasure-Trove* for Biotechnology. TIBTECH, 12: 379-386.

Cappuccino, J.G. and N. Sherman, 1987. Microbiology a Laboratory Manual; 2 th ED., California. The Benjamins Columning Publishing Company.

Corbin, B.D., 2004. *Identification and Characterization Bacillus thuringiensis*. J. Bacteriol., 186: 7736-7744.

Dashtban, M., H. Schraft and W. Qin, 2009. Fungal Bioconversion of Lignocellulosic Residue: Opportunities and Perspectives. Int. J. Biol. Sci., 578-595.

Fengel, D. and G. Wegener, 1995. *Kayu: Kimia, Ultrastruktur, Reaksi-reaksi.* Diterjemahkan oleh Hardjono Sastrohamidjoyo. Cetakan I, Gajah Mada University Press, Yogyakarta. Hal., 124-154.

Khokhar, I., M.S. Haider, S. Mushtaq and I. Mukhtar, 2012. Isolation and screening of highly cellulolytic filamentous fungi. J. Applied Sci. Environ. Manage., 16: 223-226.

Pelczar, M.J. and E.C.S. Chan, 1988. *Dasar-dasar Mikrobiologi*, Jilid 1. Ratna Siri Hadioetomo, Teja Imas, S. Sutarmi Tjitrosomo, Sri Lestari Angka, penerjemah. Jakarta: Penerbit Universitas Indonesia. Hal., 81-91.

Saha, B.C, 2004. *Lignocellulose Biodegradation and Application in Biotechnology*. US Government Work. Am. Chem. Soc., 2-14.

Widyastuti, H., Siswanto and dan Suharyanto, 2007 *Optimasi pertumbuhan dan aktivitas enzim lignolitik Omphalina sp dan Pleurotus ostreatus pada fermentasi padat.* Menara Perkebunan, 75: 93-105.

Wizna, Potensi, 2007. *Bacillus amyloliquefaciens* Isolat Serasah Hutan dalam Peningkatan Kualitas Pakan Campuran Empelur Sagu dan Isi Rumen dan Implikasinya Terhadap Produktifitas Ternak Unggas.