IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 4, Issue 1 (Jan. - Feb 2018), PP 50-53
www.iosrjournals.org

Degradation of Cellulosic Substrates By Bacteria Isolated From Earthworm

*[1]Sutama, I N. [2]S., I M. Mudita,[3] I W. Suberata
Corresponding Author: Sutama,

Abstract: *A study had been carried out to evaluating degradation ability of cellulosic substrates by bacteria isolated from earthworm. Bacteria were isolated by hungate selective media with Carboxy Methyl Cellulose/CMC as selective substrates. The potency of cellulolytic was identified based on degradation of cellulosic substrates measurement using clear/diffusion zone diameters and cellulase (endoglucanase and exoglucanase) enzyme activities. This study showed that cellulolytic bacteria isolated from earthworm coded EB_1CL has produce highest clear/diffusion zone diameters on CMC and rice bran substrates and with highest endoglucanase enzyme activity, while bacteria isolate coded EB8CL has produce highest clear/diffusion zone diameters on avicel and rice straw substrates and with highest exoglucanase enzyme activity. was concluded that bacteria isolates coded EB1CL and EB8CL was potential as cellulosic substrates degrader.*
bacteria isolates

Keywords: *Cellulolytic Bacteria, Earthworm, Clear Zone Diameters, cellulosic Substrates*

Date of Submission:10-01-2018 Date of acceptance: 26-01-2018

I. Introduction

Most agricultural residues and waste of crop plants are rich in cellulosic material. Cellulose are a long polysaccharide made of β (1,4)-linked glucose units and forms a crystaline and amorf structure Degradation lignocelluloses material is a slow process and only rellative narrow taxonomic range of bacteria is able degrade such material. Degradation of cellulosic substrates requires the cooperative action of a family of cellulolytic enzymes that have been classified into three mayor groups; endoglucanases (EC3.2.1.4), exoglucanase (EC3.2.1.91) and glucosidases (EC3.2.1.21) (Sirisena and Manamendra, 1995). Cellulase complex enzyme can produce by certain microbe, such as cellulolytic bacteria. So the effort to develop cellulolytic bacteria is very important in optimizing utility agricultural residues and waste as feedstuffs. The earthworm is one source of celluloses degrading bacteria isolates. Earthworms exist in many humid habitats and are considered to be one of the most important organisms among soil invertebrates. They have beneficial effects on soil productivity by improving the physical and chemical properties of soil. Earthworm burrowing creates soil pore spaces, thus increasing water conductivity and air transport. They promote soil aggregation by depositing casts rich in glycoproteins, polysaccharides, bacteria, and clay, and also affect soil profile formation by mixing litter layers with soil surface layers, which act to deepen humus layers with soil depth. Part of the beneficial effects of earthworms on soil structure and development is attributed to their feeding activities. Their consumption pattern involves the breakdown and incorporation of large amounts of mineral soil and organic matter, which contain a variety of microorganisms that contribute to the degradation of organic materials.

II. Meterials And Method

2.1 Isolate Bacteria Sources Sample Preparation

The eartworm was being isolate bacteria sources sample. The sample taken from the Research Farm of Animal Husbandry Faculty, Udayana University, Bukit Jimbaran, Badung Regency, Bali Provence-Indonesian Country. The sample was brought to the laboratory and produced as 10% earthworm dilution use NaCl 0,9% solution. This dilution as the sources bacteria isolates.

2.2 Solid Bacteria Growth Media and Isolation Activities

Bacteria from samples were grown in cellulolytic solid media by Hungate method (Ogimoto and Imai, 1981) countaining 0,02g KH_2PO4; 0,03g K_2HPO4; 0,01g MgSO4; 0,01g CaCl4; 0,10g NaCl; 0,10g (NH4)2SO; 0,10ml Rezasurin 0,1% solution; 0,02g Cystein-HCl.H_2O; 0,40g Na_2CO_3; 30,00 ml rumen liquid; 1,00g substrate; 70,00ml Aquadest and 1,8% Agar. Cellulose substrate used were Carboxy Methyl Cellulose/CMC. 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 CO_2 gas displacing all air until red color faded, closed with rubber 2 stopper,

sealed, then sterilized in the autoclave at 121^{o}C for 15 minutes. The medium in the enlenmeyer transferred aseptically to a sterile tube with oxygen free CO_2 gas displacing all air. Then 10^{-7} dilution colon fluid inoculated into the tube and closed with sterile cotton. The culture the incubated in 37^{o}C during 1-2 days.The colonies growing were selected.

2.3 Isolation of Colonies

From these inoculated tube containing the selection medium, the individual colonies of cellulolytic bacteria pricked. Bacteria isolates carefully pricked using bent platinum-irridium needle. The bacteria then transferred to plate agar medium anaerobically with gasses oxygen-free CO_2. The plate incubated at 37^{o}C during 1 - 2 days. The bacteria colonies has produce clear or diffusion zone were choosen for furified by repeated streaking.

2.4 Ability of Cellulosic Substrates Degradation

The ability of cellulosic substrates degradation was determined from clear zone formed by bacteria isolates tested (Ogimoto and Imai, 1981). Each pure bacteria isolate (15µl) was inoculated by spot method using paper disc blank 0.6 cm were placed on selective medium (solid growth medium containing 1% substrate test) (Subbarao, 1993). The clear zone diameters were measured after 24 hours of anaerobic incubation.

2.5 Cellulase enzyme activity

Enzyme extract was collected from centrifuged liquid media culture in 12.000 x g for 15 minutes in 4^{o}C. According to the substrate, extracts were tested in two kinds of substrates that contained 1% CMC powder/avicel micro crystalline in 50 mM acetate buffer and pH 5.5 respectively for endoglucanase and exoglucanase enzyme activity. 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) produced from the reaction were the enzyme activities (Efiok, 1996). For sugar reduction:1 ml of sample was added to 3 ml DNS reagent and 1 ml aquadest (Miller, 1959), then measured the absorbent by spectrophotometer in λ 508,5 nm production was estimated by using glucose/ calibration curve (Adney and Baker, 2008; Ghose, 1987). One unit (U) of enzyme activity was defined as 1 µmol of glucose equivalent released per minute under standard assay condition (Irfan *et al.*, 2012; Lo *et al*., 2009).

III. Results And Discussion

3.1 Ability of Cellulosic Substrates Degradation

The results showed that the cellulolytic bacteria isolates were isolated from the earthworm has the ability to return high enough to degrade cellulosic compounds shown with resultant clear/diffusion zone diameters 1,039 – 1,098 cm; 0,973 – 0,991 cm; 1,010 – 1,060 cm; 1,239 – 1,351 cm respectively for CMC, avicel, rice straw and rice bran substrates, while bacteria isolates coded EB_1CL produced highest clear/diffusion zone diameters on CMC, rice bran substrates and significant different with bacteria isolates coded EB_2CL (on CMC), EB_2CL; EB_5CL; EB_6CL and EB_8CL (on rice bran). While bacteria isolates coded EB8CL produced highest clear/diffusion zone diameters on avicel and rice straw substrates and significant different with EB_2CL; EB_4CL; EB_5CL; and EB_6CL (on avicel), EB_2CL; EB_5CL and EB_6CL (on rice straw) (Table 1). These showed that bacteria isolates coded EB_1CL and EB_8CL were superior cellulolytic bacteria with higher ability of cellulosic substrates degradation.

Table 1. Substrates Degradation of Cellulolytic Bacteria Isolated from Earthworm

Bacteria Isolates	Substrates Degradation			
	CMC	Avicel	Rice Straw	Rice Bran
EB_1CL	1,098b[1]	0,990c	1,053cd	1,351 c
EB_2CL	1,039a	0,976ab	1,010a	1,239 a
EB_3CL	1,093ab	0,988c	1,051bcd	1,331 bc
EB_4CL	1,093b	0,975ab	1,046 bcd	1,320 bc
EB_5CL	1,073ab	0,974ab	1,026 ab	1,280 ab
EB_6CL	1,089ab	0,973a	1,028 abc	1,289 ab
EB_7CL	1,092ab	0,984bc	1,042 bcd	1,306 bc
EB_8CL	1,093ab	0,991c	1,060 d	1,296 b
SEM[2]	0,011	0,002	0,006	0,010

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

On Table 1 showed that bacteria isolate coded EB_8CL produced the diffusion/clear zone with highest diameters on avicel and rice straw substrates. This indicates that bacteria isolates with coded EB_8CL have a higher ability to degrade crystalline cellulose substrates. The avicel micro crystalline substrates are sources of cellulose which are rich of crystalline compounds. Similarly than avicel, rice straw so rich of crystalline cellulose. Compared with rice bran, rice straw has higher levels of crude fiber and cellulose compound. The rice straw contained 32.41% crude fiber with 32 -35% cellulose compound, while rice bran contained 18.51% crude fiber with average 27% cellulose compounds (Wahyono and Hardianto, 2007; Howard *et al.,* 2003 and Baig *et al*.,2016). The high ability of avicel and rice straw substrates degradation by bacteria isolate coded EB8CL is believed to be due to the high activity of cellulase enzyme especially *exo-glucanase* enzyme activity owned by its bacteria isolates (Table 2). While the high degradation ability of CMC and rice bran substrate by bacteria isolate coded EB_1CL is believed as a result of high *endo-glucanase* enzyme activity possessed by its bacteria isolate (Table 2). Enzyme *endo-glucanase* will randomly degrade cellulose in both amorforous and crystalline components (Howard *et al*., 2003). Perez *et al.* (2002) revealed that *endo-glucanase* is the first enzyme to degrade cellulose compounds, especially amorforous cellulose components. Based on theses data on Table 1 it appears that isolates coded EB_1CL and EB_8CL were the 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 cellulosic substrate degradation is the result of the activity of microbial enzymes that will determine the level ability degradtion of fiber component from feed material (Howard *et al.,* 2003; Perez *et al*., 2002). Its bacteria potential as innocullant fermentor organic material rich in cellulosic substrates such as agricultural waste.

Cellulolase Enzyme Activity

The study of cellulase enzyme activity from cellulolytic bacteria isolates were isolated from earthworm showed that all bacteria isolates has high cellulase enzyme activity both endoglucanase and exoglucanase (Table 2). The bacteria isolate coded EB_1CL has significant highest ($P<0,05$) of *endoglucanase* enzyme activity for all time periode measured except on 30 minute incubation at CMC substrates were 0,0587 U, 0,1012 U and 0,0516 U respectively on 10, 20 and 60 minute incubation. Eventhough on 30 minute incubation at CMC substrates, bacteria isolate coded EB_6CL has highest endoglucanase enzyme activity was 0,0844 U but were not significant different with EB_8CL, EB_1CL and EB_3CL. Bacteria isolate coded EB_2CL has lowest of *endoglucanase* enzyme activity for all time period incubation (Table 2a). This show that cellulase enzyme activity from cellulolytic bacteria isolate were isolated from earthworm responsible for the hydrolysis of cellulose through the breakdown of hydrogen bond in cellulose structure (α1,4 glukoside bond) from cellulose componds. CMC-ase (*endoglucanase*) are proposed to initiate attack randomly at multiple internalsites in the amorphous regions of the cellulose fibre (Howard *et al.,* 2003; Perez *et al.*, 2002) and bacteria isolates coded EB_1CL and EB_6CL were superior cellulolytic bacteria with higher of endoglucanase enzyme activity. On Table 1 so showed that its bacteria has produced higher diffusion/clear zone diameters on CMC and rice bran substrates which indicated high degradation ability of amorphous cellulose compounds.

Table 2. Enzyme Activities from Cellulolytic Bacteria Isolates of Earthworm

Bacteria Isolate	**Enzyme Activities (U) on minute to...**			
	t10	**t20**	**t30**	**t60**
a. Endo-Glucanase Enzyme Activities[1)]				
EB_1CL	**0,0587b**	**0,1012b**	0,0801bc	**0,0516c**
EB_2CL	**0,0311a**	**0,0713a**	**0,0559a**	**0,0324a**
EB_3CL	0,0510ab	0,0921b	0,0753bc	0,0473bc
EB_4CL	0,0338a	0,0901ab	0,0724b	0,0455bc
EB_5CL	0,0419ab	0,0975b	0,0739b	0,0424b
EB_6CL	0,0408ab	0,0971b	**0,0844c**	0,0463bc
EB_7CL	0,0408ab	0,0898ab	0,0720b	0,0462bc
EB_8CL	0,0500ab	0,0947b	0,0761bc	0,0495c
SEM[2]	0,0046	0,0040	0,0019	0,0014
b. Exo-Glucanase Enzyme Activities[2)]				
EB_1CL	**0,0735b**	0,0649c	0,0702e	0,0466cd
EB_2CL	0,0438ab	**0,0350a**	0,0559cd	**0,0324a**
EB_3CL	0,0595ab	0,0542bc	0,0631de	0,0432c
EB_4CL	0,0268a	0,0394ab	**0,0446a**	0,0373b
EB_5CL	0,0381ab	0,0496abc	0,0476ab	0,0424c
EB_6CL	**0,0253a**	0,0530bc	0,0546bc	0,0463cd
EB_7CL	0,0485ab	0,0487abc	0,0656e	0,0452cd

EB_8CL	0,0655ab	**0,0659c**	**0,0709e**	**0,0481d**
SEM^2	0,0085	0,0036	0,0016	0,0009

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

On *exoglucanase* activity, the cellulolytic bacteriaa isolates has exoglucanase enzyme activity 0,0253 – 0,0735 U, 0,0350 – 0,0659 U, 0,0446 – 0,0709 U, 0,0324 – 0,0481 U respectively for 10, 20, 30 and 60 minutes after incubation on the avicel micro crystalline substrate. The bacteria isolate coded EB_8CL has shown highest (P<0,05) exoglucanase enzyme activity on all time periods incubation measured except on 10 minute incubation (Table 2b). This show that bacteria has responsible for hydrolysis of crystalline compounds cellulose to simple compounds "glucose". Based on the cellulase enzyme activities value obtained, the bacteria isolates coded EB_1CL and EB_8CL have higher quality and most potential as cellulosic substrates degrader with high enzyme activity which show a high ability of bacteria isolates to degrade cellulosic compounds as well as a variety of fiber rich feedstuffs to simple nutrients as sources of energy for animals/livestock. It is feasible that the isolates most potential to be used as fermentor/degrader of material/feed resources rich in crude fiber and cellulose such as agriculture waste product.

IV. Conclussion

The cellulolytic bacteria isolates coded EB_1CL and EB_8CL has higher ability of degradation cellulosic substrates were showed produced highest diameters of diffusion zone and cellulase enzyme activity. Its isolates potential to apply on optimizing the utilization of local feed resources based on agriculture by-product.

Acknowledgements

The authors wish to thanks to the Udayana University through Group 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.

References

[1]. Adney, B. dan J. Baker. 2008. Measurement of Cellulase Activities. Laboratory Analytical Procedure (LAP). Technical Report. National Renewable Energy Laboratory. 1617 Cole Boulevard Golden, Colorado. 1-8
[2]. Efiok, B. J. S. 1996. Basic Calculation for Chemical and Bniological Analysis. AOAC International, Maryland, USA
[3]. Ghose, T. K. 1987. Measurement of Cellulase Activities. International Union of Pure and Applied Chemistry. Pure and Appl. Chem., 59 (2); 257-268
[4]. Howard, RL, Abotsi E., Rensburg JVEL., and Howard S. 2003. Lignocellulose Biotechnology: Issues of Bioconversion and enzyme Production. African Journal of biotechnology 2:6002-619. Available from: URL: Http://www.vtt.fi/inf/pdf [cited 2008, February 25]. 2(12): 602-619
[5]. Miller, G. L. 1959. Use of Dinitrosalisylic Acid Reagent. Method for Determination of Reducing Sugar. Anal. Chem. 31: 426 – 428
[6]. Ogimoto, K. And Imai S. 1981. Atlas of Rumen Microbiology. Japan Scientific Societies Press, Tokyo. 231 p
[7]. Perez, J., J. Munoz-Dorado, T. De la Rubia, and J. Martinez. 2002. Biodegradation and Biological Treatment of Cellulose, Hemicellulose and Lignin; an overview. Int. Microbial, 5 (2): 53-62
[8]. Sirisena, D. M., and T. P. Manamendra. 1995. Isolation and characterization of cellulolytic bacteria from decomposing rice straw. J. Natn.Sci.Coun, Sri Lanka 23 (1); 25-30
[9]. SubhaRao, N.S. 1993. Biofertilizers in Agriculture and Forestry. 3rd ed. International Science Publisher, New York. (Xi) p 242
[10]. Wahyudi, A., dan Z. Bachruddin. 2005. Aktivitas Enzim Selulase Ekstraseluler Bakteri Rumen Kerbau, Sapi, Kambing dan Domba pada Beberapa Kultur Fermentasi: Upaya Mendapatkan Starter Probiotik bagi Ternak Ruminansia. Proseding Seminar Nasional. Pengembangan Usaha Peternakan Berdaya Saing di Lahan Kering. Edisi Pertama. Fakultas Peternakan Universitas Gadjah Mada. Hal: 342-347

Sutama, I N."Degradation of Cellulosic Substrates By Bacteria Isolated From Earthworm." IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) 4.1 (2018): 50-53.