Chemistry Research Journal, 2018, 3(1):60-63

Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Degradation of Lignin Substrates by Lignolytic Bacteria Isolated from Earthworm

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Abstract A research has been carried out to evaluating potency of bacteria isolated from earthworm as lignin substrates degrader. The potency of lignolytic was identified based on degradation of lignin substrates measurement using clear/diffusion zone diameters. This study showed that lignolytic bacteria isolated from earthworm produce clear/diffusion zone diameters 0.821–0.876 cm; 0.910–0.964 cm; 1.200–1.269 cm cm respectively for tannic acid, rice straw and rice bran substrates, while bacteria isolates coded EB1LG and EB3LG produced highest clear/diffusion zone diameters on all substrates measured. It was concluded that bacteria isolates coded EB1CL was potential as ellulosic substrates degrader.

Keywords Lignolytic Bacteria, Earthworm, Clear Zone Diameters, Lignin Substrates

Introduction

The decrease in productivity and efficiency of farm businesses and increasing the risk of environmental pollution from the farm are fed rich in fiber such as agricultural waste, including the weed crops allegedly due to the high content of lignocellulose feed material based on of agricultural waste which led to the nutrients contained can not be used optimally [1-2]. Howard *et al*. [3] revealed the higher the lignin content of the feed material more difficult overhauled/ broken down/digested. This is because lignin has a complex bond is very strong and physically acting as a barrier to the reform process feed material cell walls by rumen microbes. Degradation of lignin compounds can only be carried out by certain microbial enzymes from one enzyme-producing bacteria lignolitik lignase complex [3-4].

The lignolytic bacteria is a group of bacteria that are capable of producing the enzyme complex lignase consisting of lignin peroxidase/Li-P, manganese-peroxidase/Mn-P and laccase/Lac will remodel compound lignin into its constituent components [4]. The higher and balanced production and quality of the resulting enzyme complex lignase the better reshuffle lignin can be done. In the realm of the various sources of microbial consortia lignolitik can be obtained such as the gastrointestinal tract of animals, peatlands/agriculture, termites, earthworms, and source consortium mikrona other [2,5].

The earthworms are animals capable of degrading a wide range of organic materials because their intestinal tracts contain various synergistic microbial consortia such as bacteria, protozoa and micro-fungi and various enzymes such as amylase, protease, cellulase, lipase, chitinase, and urease. In addition, the mucus in the digestive tract earthworms contain various nutrients (carbohydrates, protein, minerals and organic materials, as well as various amino acids) and hormones [5]. Further disclosed earthworms microbes can degrade lignocellulose compounds and antinutrisi, producing antibiotics, fluorescent pigments, siderophores, chitinase and glucanase and various growth promoters through the dissolution of minerals, producing hormones 1-aminocyclopropane-1carboxylate (ACC) deaminase, and suppress pathogenic microbes.

Utilization of earthworms as a source of microbial inoculant consortium in the production of feed supplements bioinokulan berprobiotik proved capable of producing quality inoculant products that can enhance the productivity of ducks bali [6]. However, the research results Mudita *et al* [7-8] showed that the use of materials/resources directly natural microbial consortium not assures the stability of product quality inoculants produced. Results of research by Mudita *et al*. [9-10] indicate the utilization of pure bacterial isolates further ensure the stability of the quality of the resulting inoculant. So this research is important to be developed in order to produce superior isolate compounds that degrade lignin

Materials and Method

Isolate Source Sample Preparation

The eartworm 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.

Solid Media and Isolation

Bacteria from samples were grown in cellulolytic solid media by Hungate method [11] containing 0.02 g KH_2PO_4; 0.03 g K_2HPO_4; 0.01 g $MgSO_4$; 0.01 g $CaCl_2$; 0.10 g NaCl; 0.10 g $(NH_4)_2SO_4$; 0.10 ml Rezasurin 0.1 % solution; 0.02 g Cystein-HCl.H_2O; 0.40 g Na_2CO_3; 30.00 ml rumen liquid; 1.00 g substrate; 70.00ml Aquadest and 1.8 % Agar. Lignin substrate used were tannic acid. 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 ^oC 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 ^oC during 1-2 days. The colonies growing were selected.

Isolation of Colonies

From these inoculated tube containing the selection medium, the individual colonies of lignolytic 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 ^oC during 1-2 days. The bacteria colonies has produce clear or diffusion zone were choosen for furified by repeated streaking.

Ability of Lignin Substrates Degradation

The ability of lignin substrates degradation is determined from clear zone formed by bacteria isolates tested [11]. 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]. The clear zone diameters were measured after 24 hours of anaerobic incubation.

Results and Discussion

The results showed that the lignolytic bacteria isolates were isolated from the earthworm has the ability to return high enough to degrade lignin compounds shown with resultant clear/diffusion zone diameters 0.821–0.876 cm; 0.910–0.964 cm; 1.200–1.269 cm respectively for tannic acid, rice straw and rice bran substrates, while bacteria isolates coded EB_1LG and EB_3LG produced highest clear/diffusion zone diameters on all substrates measured (Table 1). These showed its bacteria isolates as true lignolytic bacteria. Every strain of bacteria isolates needed a spesific substrate as an energy source for growing [3-4]. This suggests that in the intestine of earthworm are various types of bacteria that have the ability to degrade cellulose compounds is high enough and capable of associating with fiber rich feed ingredients.

Table 1: Substrates Degradation of Lignolytic Bacteria Isolated from Earthworm

Bacteria Isolates	Diameter of clear zone on substrates (cm)		
	Tannic Acid	Rice Straw	Rice Bran
EB_1LG	0,872b[1]	0,960b	1,269c
EB_2LG	0,821a	0,917ab	1,228ab
EB_3LG	0,876b	0,964b	1,253bc
EB_4LG	0,806a	0,910a	1,200a
EB_5LG	0,821a	0,913ab	1,202a
EB_6LG	0,836a	0,888ab	1,202a
EB_7LG	0,829a	0,915ab	1,204a
EB_8LG	0,820a	0,899a	1,203a
SEM[2]	0,007	0,011	0,007

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 lignolytic bacteria coded EB_3LG produced the diffusion/ clear zone with highest ($P<0.05$) diameters on tannic acid and rice straw substrates, while bacteria isolate coded EB_1LG produce highest ($P<0.05$) diameters diffusion/clear zone on rice bran substrates. This indicates that bacteria isolates has different characteristically for degradation of substrates. Bacteria isolates coded EB_3LG has higher of degradation ability especially for substrates rich lignin compounds such as agricultural waste. Compared with rice bran, rice straw has higher levels of crude fiber and lignin compound. The rice straw contained 32.41 % crude fiber with 12-18 % lignin compound, while rice bran contained 18.51 % crude fiber with average 5 % lignin compounds [3, 13-14].

Based on theses data on Table 1 it appears that isolates coded EB_1LG and EB_3LG are superior candidates isolates potential to be utilized in the development of farm-based agricultural wastes. Theseare 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 [3-4]. Its bacteria isolates potential as inoculant fermenter organic material rich in cellulosic substrates such as agricultural waste.

Conclusion

The lignoytic bacteria isolates coded EB_1LG and EB_3LG have superior ability of lignin substrates were showed produced highest diameters of clear zone. 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 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.

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