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Determination of Potential Bacteria from Five Different Types of Green Biomass Enriched Liquid Organic Fertilizer for Developing Bio-decomposer

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Bacteria plays significant roles in the production of liquid organic fertilizer (LOF) by degrading green biomass and other solid materials. This experiment aimed to determine the genus of bacteria present in five different types of green biomasses enriched LOF which were separately prepared and non-aerobically incubated for six weeks. Those green biomasses are (1) *Gliricidia sepium*, (2) *Leucaena leucocephala*, (3) *Ageratum conyzoides* L., (4) *Eichhornia crassipes* and (5) banana corms. Samples from each biomass-enriched LOF were grown in nutrient agar medium to be isolated for further determination. Bacteria identifications are conducted using Gram-staining technique and Bergey's Manual. Qualitative screening for cellulolytic bacterial isolates was conducted by streaking on the cellulose Congo Red agar media as indicated by its clear zone formations. Results indicated that at the genus level, bacteria of *Pseudomonas, Staphylococcus* and *Bacillus* were the predominant identified groups in all green mass enriched LOFs. Gram-negative *Pseudomonas* was identified in four enriched LOFs of *Ageratum, Musa, Eichhornia and Gliricidia*. Gram-negative *Staphylococcus* was found in *Leucaena* enriched LOF. Furthermore, gram-positive *Bacillus* was found in Ageratum, *Eichhornia* and *Gliricidia* enriched LOF, while gram-positive *Staphylococcus* was identified in *Musa* and *Leucaena* enriched LOF. Cellulolytic test indicated that gram-positive *Staphylococcus* of *Musa* enriched LOF and gram-negative *Pseudomonas* groups had much higher cellulolytic ability than *Bacillus* of *Eichhornia* enriched LOF. In conclusion, both gram-positive *Staphylococcus* of *Musa* enriched LOF and gram-negative *Pseudomonas* groups are the promising bacteria to be used for developing bio-decomposer. Further research should be addressed in developing carrier media for those promising bacteria for bio-decomposer.

Key words: liquid organic fertilizer; cellulolytic bacteria; bio-decomposer;

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Introduction

The use of liquid organic fertilizer (LOF) in organic vegetable production is crucially important to improve nutrient absorption by the crops since solid organic fertilizer often takes longer time to be available for the crops. Fahrurrozi *et al.* (2015; 2016) and Muktamar *et al.* (2016) have developed such LOF for organic sweet corn production in closed agriculture production system (CAPS) station in Rejang Lebong highland, Bengkulu Province, Indonesia. The quality of LOF, including its nutrient contents, is determined by the nature of composing materials and how those materials get composted. Fahrurrozi *et al.* (2017) reported that the type of green mass is very important aspect to be considered since each type of green mass has different nutrient status which eventually affect the LOF nutrients. Another important aspect for LOF production is the presence of decomposing microorganisms, including bacteria (Haytova, 2013) which affect LOF nutrient contents through its roles as chemical decomposers in the process breaking down organic matter. According to Lee (2016), bacteria are microorganisms that able to degrade organic materials into nutrient and organic fertilizer.

Bacteria are microscopic one-celled organisms that are found everywhere and can be harmful or beneficial. Benefitting bacteria include those are used in decomposition organic matter. Research conducted Strom (1985) focused on the roles of *Bacillus* spp. in composting of solid organic wastes successfully identified the species of *Bacillus* that played roles in composting. Chandna *et al*. (2013) reported that both Gram-positive and Gram-negative bacteria are involved in the process of decomposition of organic matter belonged to the order *Burkholderiales, Enterobacteriales, Actinobacteriales* and *Bacillales*, which includes genera *e.g. Staphylococcus, Serratia, Klebsiella, Enterobacter, Terribacillus, Lysinibacillus Kocuria, Microbacterium, Acidovorax* and *Comamonas.* Some other genera or species of bacteria might be found elsewhere since bacteria growth and population dynamic are environmentally controlled.

Developing a bio-decomposer for LOF used in closed production system must integrate local bacteria with solid materials used for composing LOF taken from similar environment. With respect to developing LOF for organic sweet corn production in closed agriculture production system, it is very important to find local degrading bacteria. This experiment aimed to determine the genus of bacteria present in five different types of green biomasses enriched LOF.

Materials and Methods

Sample preparation

Five sources of liquid organic fertilizer (LOF) using local green biomasses were separately prepared as suggested by Fahrurrozi *et al.* (2015;2016) and Muktamar *et al.* (2016). However, in this LOF production, external effective microorganisms are not included. Five local green biomasses were (1) *Gliricidia sepium* (Jacq.) Kunth ex Walp., (2) *Leucaena leucocephala* (Lamk.) de Wit, (3) *Ageratum conyzoides* L., (4) *Eichhornia crassipes* (Mart.) Solms, and (5) banana corms were collected from Closed Agriculture Production System (CAPS) Research Station (1.015 m above sea level at 3 °, 27', 30.38" South Latitude and 102°. 36', 51.33'' East Longitude).

Isolation of Bacteria

An aliquot of 1 ml of each bacterium isolate which was grown in Potato Dextrose Broth (PDB) medium for 24 hours was taken and mixed with ultrapure water (9 ml), into reaction tubes (1cmx9cm), and then kept diluted until 10^{-7}. Each time of dilution process, suspension was fortexed for 30 seconds. As much as 1 ml of each 10^{-6} and 10^{-7} suspensions was placed into petri dish and dripped with 45 ^{0}C Plate Count Agar (PCA) medium. The mixture was agitated to ensure the bacterium isolates get well diluted with the growing medium. The petri dish was securely covered and flipped and then incubated in room temperature. Two days later, each sample were screened by removing a single colony grown from initial isolates and streaked in another petri dish, with the same growing medium for microscopic characterization and stored at slanted nutrient agar at room temperature. Microscopically identifications of bacteria at the genus level were conducted by using Gram-staining technique and Bergey's Manual (Holt *et al.*, 1994).

Cellulolytic screening

Screening for cellulolytic bacterial isolates was conducted using 1% Congo red indicators (El-Sersy *et al*., 2010). Screening of cellulolytic bacteria was conducted by using modified carboxymetil cellulosa (CMC) media (modified from Hasanah and Saskiawan, 2015) which is composed 10 g CMC, 0.2 g $MgSO_4 7H_2O$, 0.02 g KNO_3, 0.4 g $CaCL_2\,2H_2O$, 2 g yeast extract, and 0.8 g blended powdery solid agar. All those materials were homogeneously diluted in 1 *l* of ultrapure water and boiled over medium heat and then simmering until

thickened. Each bacterium isolate was dripped with 0.1% (weight/volume) Congo red solution and then placed into tube test of 1 cm x 10 cm as much as 6 ml and securely covered with polyethylene plastic (0.6 µm thickness). All tubes were sterilized with autoclave in 1.5 *psi* for 20 minutes at 121 ^{0}C. Once the pressure get back to zero, take the tube tests from autoclave, and placed tubes vertically until thickening for incubation at room temperature. The ability of bacteria to degrade cellulose was expressed by observing the clear zone formation in the sample tubes at 2 weeks after incubation and 5 weeks after incubation. Changing the color of CMC media from red to clear indicates cellulolytic enzyme activities of observed bacteria. The higher clear zone appeared in the tubes, the higher cellulolytic ability of this bacteria to degrade green biomasses.

Results and Discussion

Results indicated that at the genus level, gram-negative *Pseudomonas* was identified in four enriched LOFs of *Ageratum, Musa, Eichhornia and Gliricidia*. Gram-negative *Staphylococcus* was found in *Leucaena* enriched LOF. Furthermore, gram-positive *Bacillus* was found in *Ageratum, Eichhornia* and *Gliricidia* enriched LOF, while gram-positive *Staphylococcus* was identified in *Musa* and *Leucaena* enriched LOF (Table 1). It appeared that bacteria of *Pseudomonas, Staphylococcus* and *Bacillus* were the predominant identified groups in all green mass enriched LOFs.

Table 1. Genus of bacteria identified in five different of liquid organic fertilizers

No	Genus of bacteria	Sources of green biomass Liquid Organic Fertilizer				
		Ageratum conyzoides	***Musa* sp. corms**	***Leucaena leucocephala***	***Eichhornia crassipes***	***Gliricidia sepium***
1	*Pseudomonas*	*Gram negative*	*Gram negative*		*Gram negative*	*Gram negative*
2	*Bacillus*	*Gram positive*	-	-	*Gram positive*	*Gram positive*
3	*Staphylococcus*	-	*Gram positive*	*Gram negative & Gram positive*	-	-

Microscopical observation revealed that genus of *Pseudomonas* was characterized with circular colony and twisted amoeboid cell, white in color, bacilli shaped (single bacillus and diplobacilli and streptobacilli). It also observed that the cell size of gram-negative *Pseudomonas* genus from *Ageratum* enriched LOF was 1.6 x 0.7 µm and those of *Musa* enriched LOF

was 1.3 x 0.8 µm. Meanwhile the cell size of gram-negative *Pseudomonas* genus from *Eichhornia* enriched LOF was 0.55 x 0.4 µm and those of from *Gliricidia* enriched LOF were 1.7 x 1.2 µm and 1.5 x 0.8 µm.

The colony of genus *Bacillus* was characterized with circular and filamentous amoeboid, yellow and white in color as well as single bacillus and diplobacilli and streptobacilli in shape. The cell size of gram-positive *Bacillus* genus from *Agreratum* enriched LOF was 1.3 x 0.8 µm, 0.08 x 0.05 µm from those of *Eichhornia* enriched LOF, and 1.5 x 1.0 µm of those from *Gliricidia* enriched LOF.

Furthermore, the colony of gram-positive *Staphylococcus* was characterized with round, irregular and filamenteus in shape, white color of colony and streptococci arrangement. The cell size of gram-positive *Staphylococcus* from *Musa* enriched LOF was 0.5 x 0.5 µm and the size of those from *Leucaena* enriched LOF was ≤ 0,5 µm.

These three promising bacteria, *Pseudomonas*, *Staphylococcus* and *Bacillus*, might have different ability in degrading organic materials used in composing LOF. These groups were further compared its cellulolytic ability to degrade green biomasses. Cellulolytic test as observed at weeks after incubation indicated that gram-positive *Staphylococcus* of *Musa* enriched LOF and *Bacillus* of *Eichhornia* enriched LOF had much higher cellulolytic ability than gram-negative *Pseudomonas* group. However, after 5 weeks of incubation, there was a changing in cellulolytic ability where gram-positive *Staphylococcus* of *Musa* enriched LOF and gram-negative *Pseudomonas* groups had much higher cellulolytic ability than those of from gram-positive *Bacillus* of *Eichhornia* enriched LOF (Figure 1).

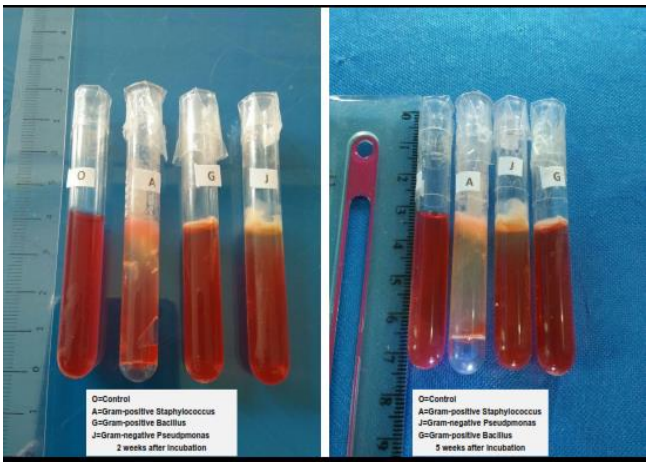

Figure 1. Clear zone formation of selected samples of bacteria for cellulolytic screening after 2 weeks of incubation (left) and 5 weeks after incubation (right)

All these three thermophilic bacteria have been reported to play significant roles in composting organic materials (Ahlawat and Vijay, 2010). The superiority of *Staphylococcus* to *Bacillus* and *Pseudomonas* was recorded to have better ability to degrade organic wastes. Compost prepared with *Staphylococcus* sp. was lighter than that of *Bacillus* sp. and had lower total dissolved solids along with conductivity. According to Hefnawy *et al*., (2013), both *Staphylococcus* sp. and *Bacillus* sp. were the dominant species during the initial composting process, especially *Staphylococcus aureus, S. xyloseus* and *Bacillus subtilis, B. brevis,* as well as *B. polymyxa.* Previously, Hassen *et al.* (2002) also reported that the dominant roles of *Staphylococcus* during the composting of municipal solid waste. Shaini and Jayasree (2017) also concluded the superiority of *Staphylococcus* in composting organic wastes.

This experiment also revealed that there was a change cellulolytic ability of *Staphylococcus, Pseudomonas* and *Bacillus* genus over the time of incubation. After two weeks of incubation, it was noted that cellulolytic ability of *Staphylococcus* was the highest, followed by *Bacillus* and *Pseudomonas*. After five weeks of incubation, cellulolytic ability *Staphylococcus*, remained the highest, however, cellulolytic ability of *Pseudomonas* was higher than *Bacillus*. The latter implied that *Pseudomonas* takes longer time to degrade solid organic matters than *Bacillus* does. According to Weller (2007), *Pseudomonas* spp. are common bacteria in agricultural soils and have many traits that make them well suited as bio-decomposer, especially *Pseudomonas fluorescens* (Hamastuti *et al.*, 2012; Raden *et al*., 2017) and *P. cellulosa* (Anindyawati, 2010). In addition, both *Pseudomonas spp*. and *Bacillus spp.* are the most efficient phosphorous solubilizing microorganisms. Such trait indicates the capability in solubilizing inorganic phosphorus from insoluble compound. According to Manullang and Rusmini (2015) and Raden *et al*., (2017) both *Bacillus* sp. dan *Pseudomonas* sp. are generally used as effective decomposers in producing organic fertilizers.

In conclusion, genus of *Staphylococcus*, *Bacillus* and *Pseudomonas* predominant bacteria present in green biomasses enriched liquid organic fertilizer. The genus of *Staphylococcus* had the highest cellulolytic ability compared to those of *Pseudomonas* and *Bacillus.*

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