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Research Article

Isolation and identification on microorganism decomposers of Palu local cow manure of Central Sulawesi, Indonesia

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Abstract: Microbial decomposers are living things posessing an important role in outlining materials derived from organic compounds entering the environment as plant nutrients so that they arereusable by the greenery. This study was aimed to isolate and identify types of microbial decomposers from Palu local cow manure of Central Sulawesi, Indonesia. The results showed that in Palu local cow manure there were three types of microbial decomposers namely *Lactobacillus sp*, A*ctinomycetessp* and *Aspergillus sp*. In Actinomycetes sp., the colony growth was very slow and firmly attached to the media surface after incubated for 7 days. It showed formation of mycelium spreading on the media surface with a serrated edge. Aspergillus sp.had morphological characteristics formed on media as follows: green-yellowcolonies; like-furtextures; green conidia; radiatconidia arrangement, uniseriat, fialidwhich almostfilled the entiresurface of vesicles; like-ballroundvesicle; coarse, thick-walled, and dark greenconidiophores.

***Keywords**: Actinomycetes sp., Aspergillus sp., Lactobacillus sp., microbial decomposers*

Introduction

Utilizing manure from cow dung to increase the productivity of agricultural lands today is continuosly pursued in accordance with (i) the constant decline in the levels of soil organic matter, (ii) the productivity decline in larger agricultural land which undergoes a leveling-off (Nuhung, 2006), and (iii) the ongoing price increase of synthetic chemical fertilizers. Manure is largely known, and able to be produced by farmers or farmer groups with a simple technology (small-medium scale).

Currently, various sources of organic matter and microbial decomposers are locally available, so that business opportunities in composting are widely open. Challenges in the use of microbial decomposers of local cow manure in Palu mainly lies in the effectiveness and efficiency of decomposers found in Palu local cow manure.
Microorganism decomposers are living things possesing an important role in restoring materials derived from organic compounds entering the environment so that it is reusable by the greenery. So far, in order to increase their farm productivity in Indonesia, most farmers rely on inorganic fertilizers (FAO, 2005). However, the dependence in using chemical fertilizers and their improper application results in the rising shortage of soil nutrients, the disturbance of soil reaction, the imbalance development of nutrients in plants, the increase of plant susceptibility to pests and diseases, the reduction of soil organic matter, the harm toward the lives of the microorganisms benefiting to soil, the reduction of modules in legume root nodules of plants and mycorrhiza association as well as the increase of environmental pollution (Savci, 2012). Such conditions will be limiting factors to sustain yields in a long term.

Effects from the continuous application of chemical fertilizers in large quantity without organic supplements have triggered a new interest inthe system of plant nutrition application. The management of integrated nutrient is an integral part of sustainable agriculture to meet human needs without damaging the environment and to conserve land resources. This model of agricultural system has received much attention from researchers and farmers.

According to Aziz et al. (2012) the soil fertility management system combining the use of inorganic and organic fertilizers contributes to improve agricultural yields, protect the environment and prevent land degradation. The soil fertility management system can also include organic fertilizers, green manure, green algae, bio-fertilizer, and crop rotation with legumes by minimizing the use of chemical fertilizers to produce optimum yields without damaging the soil fertility.

Efforts to increase the production of corn and soil quality by applying organic fertilizer is very important to develop. It is because the source of any organic materials (litter, compost, manure, green manure, organic mulch, cover crops and other organic materials) plays an important role in improving, enhancing and maintaining the sustainable land productivity. According to Carter (2001) and Magdoff and Van Es (2009), organic materials can improve the soil physical and biological properties; also,it can improve soil chemical properties such as reducing the solubility of aluminum, increasing the availability of N, P, S in the soil, and improving soil CEC through an active carboxyl group. They can act accordingly when they have been decomposed completely.

Green manure is a fertilizer which consists of whole green plants (e.g. salviniales plant Azolla), grass, non-legume and legume, crop residues (straw, stover, corn cobs, sugarcane, and coconut fiber), or only parts of the plant such as the remaining of stems and root-stumps over the top of green plants for animal feed (Agus and Widianto, 2004; Suratmini 2009). The speed and maturity of green manure decomposition are controlled by many factors, such as humidity, temperature, aeration, soil texture, presence of limestone and mineral salts, time, environmental conditions, the characteristics of material (type, chemical components and size), and the presence of appropriate organisms (Pieters, 2006).

The level of decomposition can be determined by measuring carbon dioxides, ammonia production and accumulation of nitrates. In addition, it can also be obtained by measuring the stability or maturity, bulk density, organic matter content, water holding capacity, pH, C / N ratio, total N, and phosphorus (Pandy and Shukla, 2006).

Applying cow manure as organic fertilizers for composting forage provides better results on plant growth when compared to inorganic ones (Vukobratović et al., 2008; Esmaeilian et al., 2012; Haghighat et al., 2012). Similar studies have also been carried out on rice cultivation (Kayeke et al., 2007; Jeon et at., 2011), potatoes (Davis et al., 1996) and maize (Baiwa et al., 2002; Tejada, et al., 2008; Dhima et al., 2009; Wijesinghe et al., 2009; Odhiambo, et al., 2010; Maobe et al., 2010; Talgre et al., 2012; da Silva et al., 2012). The objective of this study was to isolate and identify microbial decomposers from the manure of Palu local cow.

Materials and Methods

The study was conducted at the Laboratory of Plant Protection, Faculty of Agriculture, Tadulako University, Palu from April to June 2014. Ten grams of Palu local cow manure was placed into a beaker glass containing sterile water and filled up to 100 mL. Next, it was shaken to reach homogeneous state, then the soil solution and distilled water were separated. To make 10^{-2} dilution, the suspension was taken from 1 mL solution, inserted into the test tube containing 9 mL of sterile water and in the vortex. The process was continued by taking the 10^{-2} solution to obtain 10^{-3}dilution and was continuously diluted to reach 10^{-7}. Each test tube was marked with a label.

The microorganism suspension took 1 mL from dilution 10^{-4} to 10^{-6} by a sterile pipette and it was spread on the surface of Nutrient Agar (NA) medium. It was then flattened by drigalski and avoided in order to not be scratched or lifted. The petri dish containing microorganism cultures was then labeled (containing the name, origin of cow manure, day and date of isolation, and then incubated for 2-3 days at the temperature of 37ºC).

The microorganism suspension of 1 Ose dilution was obtained aseptically, then streaked onto the tip of ose as smooth as possible over the GYP medium which was added 1% of $CaCO_3$ and 10 ppm of sodium azide. The petri dish which already contained the microorganism cultures were then incubated for 48 hours at the temperature of 37º C. Results of decomposer microorganism isolation of manure from Palu local cow was then identified for its content of lactic acid bacteria.

The identification of isolate species *Lactobacillus* was performed by the growth test at different temperatures (15°C and 45°C), and different pH (pH 3.5 and 9), the acid test on the ability of acid formation from various carbon sources, and peptidoglycan type-test. In addition, the colouring test was conducted by Gram staining test with four reagents, namely crystal violet liquid, iodine solution, alcohol and safranin. Catalase test used 3% H_2O_2 solution. The test results were applied to determine *Lactobacillus* bacterial species as referred by the book of Bergey's Mannual of Systematic Bacteriology (David, 2001).

Actinomycetes identification was conducted by growing isolated colonies. The sample,1 Ose,was taken and diluted in 1 mL of distilled water and inoculated by a pour plate on the medium of Starch-casein Agar (SCA) and Raffinosa histidine (RHA) and the addition of chloramphenicol as an antifungal. The inoculated medium was incubated at 28^0 C for 2 weeks (Ambarwati, 2007).

Colonies that grew on media was observed. Each colony which had different shape was isolated on SCA media to obtain pure isolates and they were grown by streak plate. Isolates were incubated for 2 weeks at 28^0 C. Those, which were suspected of being *Actinomycetes* members, were observed when forming mycelium and colour of isolates. The purified isolates were inoculated on Oat meal agar media by taking one ose isolate and being grown by spread plate on the same media. The inoculated medium was incubated at 28^0 C for 2 weeks. The colour of grown isolates wasobserved.

Aspergillus sp is categorized as a group of fungus. Therefore, it was grown on medium Potato Dextrose Agar (PDA) for identification. The suspension as the dilution result was taken 1 mL, grown on PDA, and incubated at room temperature for 7 days. The identification was done by looking at the formation of colony colour, hyphae, conidiophores and conidia. (Afzal et al., 2013).

Results

The results of microbial identification grown on NA and PDA media provided three potential types of microbes as green decomposers. They were *Lactobacillus*, *Actinomycetes* and *Aspergillus sp*. Identification results on the microbial to genus level taken from the manure showed *Lactobacillus* based on the series of confirmatory test (Table 1).

Observations on *Actinomycetes* colonies growing on PDA showed they were white, not shiny, small in diameter (1.2-3 mm) (Figure 1). The colony growth was very slow and firmly attached to the media surface after incubated for 7 days. The observation result using a microscope showed the mycelium formation spreading on the media surface with a serrated edge.

Table 1. Testing *Lactobacillus* bacteria from manure

Testing	Result
Circular colony morphology	+
Growth at the temperature of 15^{o}C	-
Growth at the temperature of45^{o}C	+
Growth at the pH 3.5	+
Growth at the pH 9	-
Acid formation onSucrose	+
Acid formation onMaltose	-
Acid formation onGlucose	+
Test of Gram	+
Test ofCatalase	-
Form of Basil	+

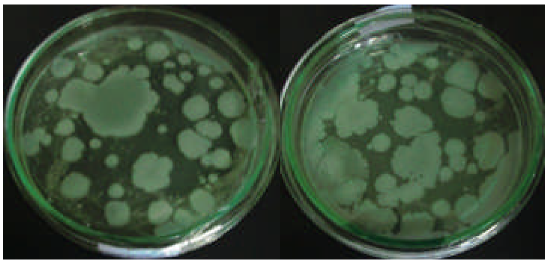

Figure 1. *Actinomycetes* colonies growing on PDA media.

Aspergillus sp. had the morphological features formed on the media as follows: green-yellow colonies; like-fur textures; green conidia; radiat conidia arrangement, uniseriat, fialid that almost filled the entire surface of vesicles; like-ball round vesicle; coarse, thick-walled, and dark green conidiophores (Figure 2).

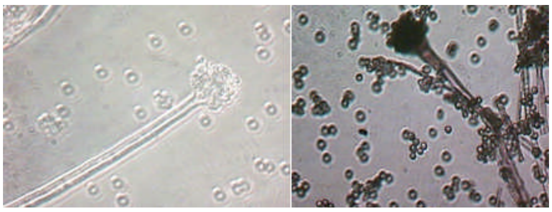

Figure 2. Conidia of *Aspergillus sp* was observed under a microscope with 400x magnification

Discussion

Results of laboratory analysis revealed that within the manure as the source of microbes there were three types of microbial decomposers, namely *Lactobacillus, Actinomycetes* and *Aspergillus sp*. Identification results were obtained by some identifiers indicating that bacteria could only grow at the temperature of 45°C instead of low temperatures.

Lactobacillus could grow at high pH instead of low pH (acidic). It is presumably because the cell wall of bacteria undergoes lysis when the environmental conditions are very acidic, so the growth is hampered even unable to grow in these conditions (Guerra et al., 2006).

Acid formation in various carbohydrate sources were found in the media by using glucose and sucrose. According to Ray and Daeschel (1992), the microbes will multiply rapidly and become a large population on the decomposed substrate containing sufficient sugar. Then, the glucose was further hydrolyzed through glycolysis or Emden-Meyerhof-Parnas (EMP). Glycolysis is a breakdown of glucose into pyruvate or lactic acid. It is the main path of glucose utilization, occuring in the cytosol of all bacteria cells with the aim to generate energy (ATP).

Results of Gram staining on the bacterial isolates showed purple colour, meaning that the entry in the Gram was positive. The formation of purple colour on Gram-positive bacteria occurred since the main constituent component of cell wall in Gram-positive bacteria was peptidoglycan. Thus, it was able to bind the violet crystalpaint. Lactic acid bacteria were included in the class of Gram-positive bacteria (Stamer, 1979).

The catalase test showed a positive reaction to this test, which was indicated by the appearance of air bubbles in the Durham tube. The occurrence of this air bubble formation indicated O_2 gas formation from H_2O_2 breakdown by catalase enzyme of the bacteria, based on the reaction: $2H_2O_2 \rightarrow 2H_2O + O_2$. Lactic acid bacteria are those incapable of producing the catalase enzyme (Stamer, 1979).

According to Sembiring et al. (2000) the results on the use of Oatmeal Agar media can be directly observed since they reveal the formation colour of aerial mycelium, the pigmentedvegetative mycelium and the diffused pigment colour. Nanjwade et al. (2010) stated that the morphology of *Actinomycetes* growing on media can support to identify the characteristics of actinomycetes. However, such information is not applicable specifically on the level of genus.

Conclusion

The result of isolation and identification of microbial decomposers in the manure of Palu local cow provides three types of microbes namely *Lactobacillus*, *Actinomycetes*, and *Aspergillus sp*. The availability of cow composted manure in the field can be used to improve the physical, chemical and biological characteristics of soil because it contains microbial decomposers.

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