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The Comparison of Activator from Indigenous Decomposer for NH_3 Mitigation during the Rabbit Dung Composting

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ABSTRACT
This study aimed to compare the indigenous strain as a decomposer in the rabbit dung composting. This study was conducted by using a commercial decomposer as a control, *Pseudomonas* sp. LS3K and *Candida* sp. LS3T. Before being applied as a decomposer, the growth of *Pseudomonas* sp. LS3K and *Candida* sp. LS3T was measured. The ammonia concentration during composting was determined for 14 days, and the physical and chemical quality of the final product was observed. Statistical analysis using the completely randomized design (One-way ANOVA) and DMRT test was used in this research. The result showed that the maximum peak of *Pseudomonas* sp. LS3K and *Candida* sp. LS3T growth occurred at the 24th hour. During 14 days of the composting process, the graphical NH_3 gas emission from *Candida* sp. LS3T was lower compared to *Pseudomonas* sp. LS3K. There were no significantly differenteffects ($P>0.05$) in Rabbit dung compost's physical, chemical, and microbial quality at the end of composting. In conclusion, the *Pseudomonas* sp. LS3K and *Candida* sp. LS3T had the potency as same as with the commercial decomposer for composting the rabbit dung. *Candida* sp. LS3T had the higher potency to mitigate NH_3 gas emissions during 14 days of the rabbit dung composting.

Keywords: *Indigenous Decomposer, Aerobic composting, Rabbit dung, Pseudomonas sp. LS3K, Candida sp. LS3T.*

1. INTRODUCTION

Livestock dung is an important material resource for compost fertilizer in the environmental ecosystem and has significant ability in increasing soil quality at the Agro-ecosystem. Based on the N, P, K content, and compared with the other livestock, rabbit produces a higher quality of dung [1]. The dung is a by- product that is continuously produced as long as the livestock is alive. However, fresh dung is not recommended to be directly applied to the agricultural ecosystem because it can pollute the environment and disrupt the surrounding human ecosystem. Naturally, the dung will be decomposed by microbiological activity, but it takes a long time. A decomposer can be added as an activator to increase the decomposing process effectiveness, to optimize the composting process. The activity of microorganisms strongly influences the decomposition and stabilization of organic matter during composting. Various microorganisms such as fungi, bacteria, actinomycetes can be used to generate the composting process. There are various types of microbes that can speed up the composting process, such as *Pseudomonas* sp., lactic acid bacteria, *Actinomycetes*, *Trichoderma* sp. [2], *Bacillus* sp., *Escherichia coli*, *Enterobacter* sp. [3], *Alcaligenes* sp. [4,5], and *Arthrobacter* sp. [6,7].

During the aerobic composting process, it is possible to lose N content due to emissions in the gaseous form (NH_3), which reduces the N content of the final compost product. The utilization of an inoculant activator is one of the proper ways to inhibit the formation of gaseous ammonia by fecal microbes, accelerate the composting process, and increase various nutrients needed by plants suchas N, P, and K. *Pseudomonas* sp. LS3K and *Candida* sp. LS3T is an indigenous strain that can reduce NH_3 gas emissions from livestock waste in aerobic conditions [7–9]. It becomes interesting to study further how it serves

as an inoculant activator which accelerates the aerobic composting process, reduces ammonia gas, maintains nutrient N content during the composting process, and improves the quality of organic material the compost produced from rabbit dung.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Sample preparation

Rabbit dung was used as the primary sample material in this study, collected from a local farm in Yogyakarta, Indonesia. Firstly, rabbit dung was sieving to get pure and then crushed. The purpose of sieving and crushing in this study was to obtain homogenized particles, reduce pollutant materials, obtain smaller particles, larger surface area, and assist in the composting process.

2.1.2. Microbial inoculant as decomposer activator

In this study, *Pseudomonas* sp. LS3K and *Candida* sp. LS3T were used as the microbial inoculant for composting activator. These strains were maintenance by the Laboratory of Leather, By-Products, and Waste Technology, Faculty of Animal Science, UGM, Yogyakarta. Before applying as an inoculant activator, these bacteria were grown in the agar medium, made from 1.5 g agar powder and 1% stock solution in 100 mL water. The stock solution for 100 mL was made by 1 g meat extract, 1 g peptone, 0.5 NaCl, and aquadest (pH adjusted at 7.2 and autoclaved at 121°C, 15 psi, 15 minutes). Inoculant on the agar medium was incubated for 96 hours in the incubator at 30°C. After 96 hours of incubation, the inoculant growth was observed. Then as much as 1 ose of each strain was taken and added into 100mL of liquid medium containing 10% stock solution. The mixture of liquid medium and inoculant solution was then placed in Rotary Shaker at 120 rpm. The observation was conducted using the spectrophotometry method to characterize the growth profile during 48 hours (OD600).

2.2. Procedure and methods

2.2.1. Rabbit dung composting

In this study, the compost was made by mixing the rabbit dung and an inoculant activator. An amount of 500 g of rabbit dung was put into a plastic bottle (1.5 L), then was added by 10 ml of inoculant activator. There was a commercial activator, *Pseudomonas* sp. LS3K, and *Candida* sp. LS3T for each treatment in 3 replication, respectively. In this study, the composting procedure and mechanism were performed following the previous research [7,10]. Rabbit dung composting was performed in 14 days in aerobic conditions. The aerator was set up for supplying the air into the bottle during the composting and airflow from the bottle set into the Erlenmeyer containing 0.02 N Boric acid. During 14 days of observation, the NH_3 emission was trapped in the boric acid solution measured every 24 hours using Nesslerization methods. After 14 days, the physical and chemical quality were determined. The physical determination quality of compost included observing the color, odor, texture, and temperature. The chemical's compost quality included the moisture, pH, organic compound in total Nitrogen, Phosphor, Kalium, Carbon, and C/N ratio [11].

2.3. Analysis of data

The inoculant growth culture characteristics and NH_3 emission during the composting were analyzed descriptive using figures and graphics. The data result in physical quality, and chemical quality was analyzed with the statistical analysis used Completely Randomized Design (One-way ANOVA) and continued by Duncan's multiple range test (DMRT) if there were differences with significant effect ($P<0.05$) among the treatments [12].

3. RESULTS AND DISCUSSIONS

3.1. Growth characters of the activator culture

The growth of *Pseudomonas* sp. LS3K and *Candida* sp. LS3T on the agar medium with 1% of the nutrient medium observed for 4 days was appropriately grown (Figure 1). The growth was relatively slower compared to the previous study [7,13]. The results from the inoculation of *Pseudomonas* sp. LS3K and *Candida* sp. LS3T in agar medium with 100% stock solution, on the 18-24 hours of incubation, has grown well based on the number of colonies. The growth process of bacteria strain has been greatly influenced by the concentration of bacterial growth in the medium. Bacteria need a supply of nutrients as a source of energy for cell growth. Carbon, nitrogen, hydrogen, oxygen, sulfur, iron, phosphorus, and small amounts of other metals are the fundamental elements needed by bacteria. Lack of nutrient sources can inhibit the growth activity, and in another case, it may cause death in bacteria [14,15].

The growth characters of *Pseudomonas* sp. LS3K and *Candida* sp. LS3T could be confirmed from the increased value in the Optical Density (OD) measurement. The results of Optical Density (OD) measurements in the liquid medium (10% stock solution) can be seen in Figure 2. Growth curve of *Pseudomonas* sp. LS3K and *Candida* sp. LS3T in the liquid medium with 10% stock solution shows a different growth rate. It could be seen from the turbidity level of the liquid medium. The initial liquid medium turns from clear yellow into cloudy yellow, which is influenced by the activity of microbes. Thus the

turbidity formed can be used as an indicator of microbial growth on the liquid medium. The growth character of one bacteria in liquid media is different from other bacteria. The growth could be confirmed from the presence of turbidity (a cloudy liquid), membrane formation (a collection of cells floating on the surface of the media), and sediment (a pile of cells that settles at the bottom of the liquid culture but will dissolves when the tube is gently shaken)[14].

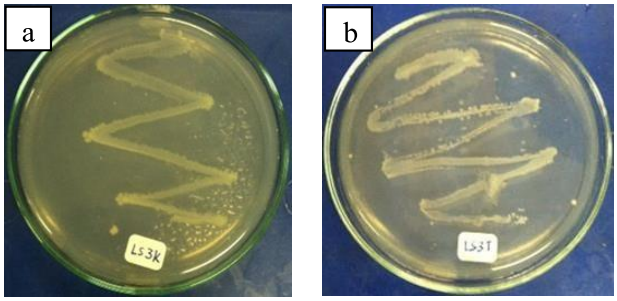

Figure 1. The growth *Pseudomonas* sp. LS3K (a) and *Candida* sp. LS3T (b) on the agar medium with 1% stock solution of nutrient medium

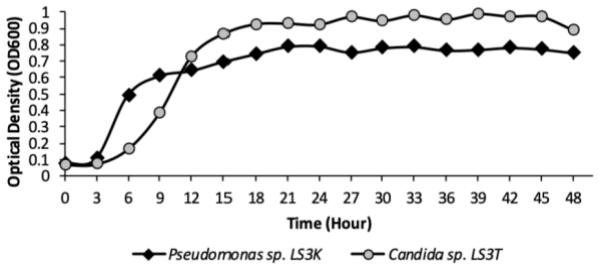

Figure 2. The growth of *Pseudomonas* sp. LS3K and *Candida* sp. LS3T on the liquid medium with 10% of nutrient medium

Based on the growth character in Figure 2, it could be seen that both of *Pseudomonas* sp. LS3K and *Candida* sp. LS3T can grow in the medium and show all growth phases, including lag, log, stationary, and death. The lag phase of *Pseudomonas* sp. LS3K and *Candida* sp. LS3T occurred at the initial 4 hours of the incubation period. The Log phase of *Pseudomonas* sp. LS3K occurred at the 4th to 24th hour and the log phase of *Candida* sp. LS3T occurs from the 4th to the 18th hour. The stationary phase of *Pseudomonas* sp. LS3K occurred at 24th to 45th hour, while *Candida* sp. LS3T occurred at 18th to 45th hour.

The death phase of *Pseudomonas* sp. LS3K and *Candida* sp. LS3T had occurred after 45th-hour incubation. There was a phenomenon of the decreasing of cell density observed. In this study, the length to reach the log and stationary phases of *Pseudomonas* sp. LS3K and *Candida* sp. LS3T was different in time and the levels of cell density. *Candida* sp. LS3T has a faster time to reach the maximum cell growth and a higher level of cell density in the liquid medium. This result was in line with the previous study [7], *Candida* sp., which was the one type of microorganism with high levels of yeast species. It is more adaptive to grow faster on the nutrient medium against *Pseudomonas* sp. Microbes' time and cell growth varied, depending on the ability to adjust to the surrounding environment and the number of nutrients available in the medium. Thus, reducing nutrients in the medium and metabolic products that may be toxic can cause the log and stationary phases. Another case may reach the death phase of microbial cells [14,15].

3.2. The potency of activator from indigenous decomposer in the rabbit dung composting

3.2.1. The NH_3 emission in the rabbit dung composting

After understanding the growth profile of *Pseudomonas* sp. LS3K and *Candida* sp. LS3T in the nutrient medium, each strain was cultivated 24 hours before applied to be an inoculant activator to decompose the rabbit dung during 14 days of aerobic composting. The observation results on NH_3 gas emissions during the composting process of rabbit dung with the addition of an activator (commercial starter, *Pseudomonas* sp. LS3K, and *Candida* sp. LS3T) could be seen in Figure 3. Based on the results, NH_3 gas emissions fluctuated daily for each type of inoculant activator used. During 14 days of observation, the fluctuation of NH_3 gas emissions using activator *Candida* sp. LS3T produces fewer emissions compared to commercial activator products and *Pseudomonas* sp. LS3K. The utilization of *Candida* sp. LS3T has the highest impact at reducing ammonia gas emissions than the blank treatment (Commercial activator) and *Pseudomonas* sp. LS3K and the blank treatment was better than *Pseudomonas* sp. LS3K in the reduction of NH_3 gas emissions. These results indicate *Candida* sp. LS3T can effectively reduce ammonia production and emission during composting rabbit dung compared to *Pseudomonas* sp. LS3K. These results align with previous studies where *Candida* sp. can reduce ammonia gas emissions more effectively than *Pseudomonas* sp. and mixture bacteria from livestock waste [7].

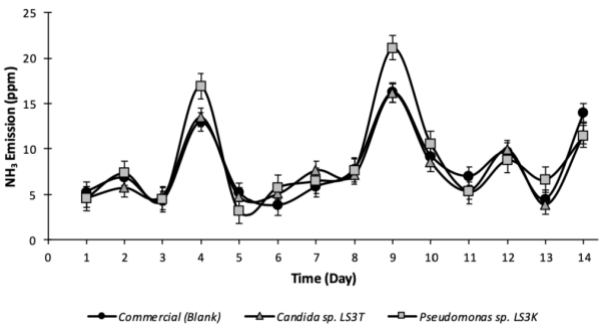

Figure 3. The fluctuation of NH_3 gas emission from rabbit dung composting during 14 days

Table 1. The physical composition of rabbit dung after 14 days of composting

Composition	Inoculant Activator		
	Commercial activator	***Pseudomonas* sp. LS3K**	***Candida* sp. LS3T**
Color	Brownish-black	Brownish-black	Brownish-black
Aroma	Like soil	Like soil	Like soil
Texture	Loose like soil	Loose like soil	Loose like soil
pH [ns]	7.0 ± 0.0	6.9 ± 0.3	6.7 ± 0.3
Temperature (°C) [ns]	27.6 ± 0.29	27.4 ± 0.67	27.4 ± 0.33

Notes:
[ns] = There were no different significant effects (P>0.05)

3.2.2. Physical quality of rabbit dung compost

Compost maturity was affected by composting time. Therefore, it is necessary to analyze physical quality parameters, including color, odor, texture, pH, and temperature. The observations on thephysical quality of rabbit dung compost after 14 days of composting can be seen in Table1. Based on Table 1, it is well known that the compost's physical quality had no significant effect (P>0,05) in the rabbit dung compost in all treatments. The physical quality of rabbit dung compost in this study is brownish-black in color, the aroma compost like the soil, the texture was loose like soil in all treatment and pH value at 6.7 – 7.0.

The physical quality of rabbit dung composting after 14 days indicated that the rabbit dung compost was matured and had met the Indonesian Standard for compost product (SNI: 19-7030-2004). Compost material has got into mature condition when it has changed in the color of brownish-black, the smell smells like the soil, loose in the texture, has a pH level of 6.5 – 8.0, and the final temperature has similar to the temperature of groundwater value (<30°C) [16–18]. The matured compost odor was like soil because the material resembles soil and is brownish-black in color, indicating stable organic matter. The final shape does not resemble the original form because it has been destroyed by natural decomposition by the microorganisms that live in the compost [16]. The composting process occurs over a wide pH range. The optimum pH for the composting process ranges from 5.5 to 7.5, and in the composting maturity, the pH will move near the neutral pH. During the composting process, changes in organic matter and the pH of the material itself by releasing acids, temporarily or locally, will cause a decrease in pH (acidification). At the same time, ammonia emission from nitrogen-containing compounds will increase the pH to the alkaline condition in the initial composting phase [19].

3.2.3. Chemical quality of rabbit dung compost

The observations on the physical quality of rabbit dung compost can be seen in Table1. Based on Table 2, it is known that the chemical composition has no significant difference (P>0,05) in the rabbit dung compost in all treatments.

Table 2. Chemicals composition of rabbit dung after 14 days composting

Composition	Inoculant Activator			
	Commercial control	***Pseudomonas* sp. LS3K**	***Candida* sp. LS3T**	**SNI: 19-7030-2004**
Moisture (%) [ns]	11.5±2.78	12.33±2.36	12.67±1.25	Max 50
Organic matter (%) [ns]	6.56±0.80	5.87±0.64	5.13±1.43	27 – 58
Total C organic (%) [ns]	3.80±0.46	3.40±0.37	2.97±0.83	9.8 – 32
Total N (%) [ns]	2.01±0.41	2.64±0.35	2.55±0.10	Min 0.4
Total P (%) [ns]	1.04±0.60	1.08±0.50	1.20±0.48	Min 0.1
Total K (%) [ns]	1.46±0.31	1.44±0.40	1.39±0.20	Min 0.2
C/N Ratio [ns]	6.55±0.83	8.15±0.56	4.53±0.71	10 – 20

Notes:
[ns] = There were no different significant effects (P>0.05)

The chemical composition of rabbit dung compost made using a commercial starter (control), *Pseudomonas* sp. LS3K and *Candida* sp. LS3T as a decomposer has met the requirements of the Indonesian Standard for compost product (SNI: 19-7030-2004) except in the variable of organic matter, total C-organic, and C/N ratio. This result has proven that the rabbit dung has potency as an organic fertilizer due to the characterization of closed compost composition standards, even as single raw materials. However, it would be better to obtain a higher nutrient content by mixing compost with other organic compounds such as sawdust. The C/N ratio is one of the essential factors as major characteristic parameters in compost processing. It is the ratio between the levels of carbon and nitrogen contained in compost that is useful for microorganisms' decomposition of organic compounds. Microorganisms need carbon and nitrogen as a source of energy in the decomposition process of organic compounds. The addition of sawdust as a filler and an auxiliary source of carbon in composting may increase the C/N ratio but is insufficient to ensure the high quality of the matured compost. Besides the C/N ratio and the macronutrient content (N, P, K, etc.), the high-quality compost for agriculture must also contain the micronutrient including Cu, Zn, Co, Mn, Fe, Etc. The high concentration of macronutrient and micronutrient content on the final compost product is challenging to obtain on the compost used single raw material without mixing with the other materials [20].

4. CONCLUSION

Based on the research, it could be concluded that the growth profile of *Pseudomonas* sp. LS3K and *Candida* sp. LS3T on Agar medium 1% stock solution has been observed after 4 days was same. The growth curve in liquid medium 10% stock solution has shown *Candida* sp. LS3T has faster growth than *Pseudomonas* sp. LS3K. The results of measuring levels of ammonia emission from rabbit dung composting showed the addition of *Candida* sp. LS3T was able to decrease the ammonia gas emissions better, and compost of all treatment has met the requirements of the Indonesian Standard for compost products.

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