Effect of lignocellulolytic microorganisms isolated from the peel of cassava, rice straw, and sawdust for the composting process of rice straw

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Abstract. The composting process will take a long time if only rely on indigenous decomposing microorganisms. This study explored the source of the isolates as bioactivator of composting. The purpose of the present study was to isolate lignocellulolytic microorganisms from the peel of cassava, rice straw (RS), and sawdust and to investigate the effect of the lignocellulolytic microorganisms for the composting process of RS. The research was conducted by two steps. The first step was isolation of bacteria and fungi from the peel of cassava, RS, and sawdust by using CMC, Lignolytic selection, and Omeliansky media, and isolation general bacteria and Actinobacteria from the one-week-old RS compost by using media of NA and SCA. The second step was application of the mixed isolates obtained from the first step as bioactivator for the composting of RS. As the control treatment, the other composting of RS was also set up with no addition of bioactivator. After 60 days composting, the results showed that RS compost product from the composting using bioactivator indicated higher quality with C/N ratio, total N, P2O5, and K2O namely 15.2, 1.44%, 1.40%, and 1.86% comparing with control that were 23.2, 1.12%, 1.19%, and 1.63%, respectively.

1. Introduction
The composting process is microbial aerobic decomposition of solid organic matter that passes through a thermophilic phase [1,2]. Application of compost can affect soil fertility and health, by increasing the availability of nutrients, both macro-nutrients such as C, N, P, K [3] and Mg [4] as well as micro-nutrients such as Fe, Mn, and Zn [5], improving soil physical quality of porosity, aggregation and soil capacity to bind water [6], increasing the population of general microorganisms and beneficial microorganisms in the soil such as phosphate solubilizing microorganisms [7], and increasing microbial biomass [8].

The quality of compost depends on the materials and technology used during the composting process [9]. Compost that is not processed properly, can cause conditions that will harm the soil and plants. A study reported that immature compost contains toxic compounds that inhibit the growth of plant seeds [10]. One of the efforts to speed up the composting process and to improve the quality of compost is by adding bioactivator.

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Several studies on the effect of bioactivator showed that application of selected microorganisms as bioactivator gave the significant acceleration of composting process and improved the compost quality. The use of bioactivator consisting of a consortium of bacteria, actinobacteria and mesophilic and thermophilic fungi on composting post-harvest tomato plants (lacking fruits) and pruning pine chips (50:50 w/w) resulted in longer 5 days of period of thermophilic phase compared to the control [11]. The higher microbial activity was indicated by a higher percentage of lignin and cellulose degradation and a faster decrease in the C/N ratio in the inoculated compost. Inoculated compost took 20 days to reach a C/N ratio of 20, while compost control reached a C/N ratio of 20 on day 45 of composting [12]. Another study also reported that compost treated with bioactivator had C/N ratio of 16 and higher nutrient content of N 1.85 %, P 8.2 mg/g, and K 10.2 mg/g higher than the uninoculated compost that having C/N ratio of 19.8 and N 1.60 %, P 5.7 mg/g, K 9.8 mg/g respectively [13].

Based on the importance of the application of bioactivators in accelerating the composting process and improving the quality of compost, this study was conducted to isolate lignocellulolytic bacteria and fungi from several sources and to examine their capability as bioactivator in the composting process of RS.

2. Materials and methods

2.1. Preparation of the sources of isolates

Sources of isolates of lignocellulolytic microorganisms and general bacteria were obtained from the outer peels, inner peels and vines of cassava, the mixture of mahogany and sengon sawdust in a ratio of 1:1, dried RS, and one week old RS compost. Composting RS as a source of isolates was carried out using modification method of Cahyani et al. [14] with no addition of chemical fertilizer. In short, the RS compost was set up by preparing 40 kg of dried RS, then soaked in a water bath for about 2 minutes, removed, and arranged layer by layer to form a pile with a size of 50x50x50 cm. When the RS compost pile was one week old and the center of the pile reached a temperature of about 60°C, a compost sample was taken from the center of the compost pile and used as one of the sources of lignocellulolytic isolates.

2.2. Preparation of media

The selective media used for the isolation of lignocellulolytic bacteria and fungi were CMC (Carboxymethyl Cellulose), Lignolytic selection media, and Omeliansky media, while for the isolation of general bacteria and actinobacteria from one week old RS compost sample used NA (Nutrient Agar) and SCA (Starch Casein Agar) media. The chemical composition for CMC media is as follow: 0.25 g K$_2$HPO$_4$, 0.25 g KH$_2$PO$_4$, 0.5 g (NH$_4$)$_2$SO$_4$, 0.05 g MgSO$_4$.7H$_2$O, 0.05 g CaCl$_2$, 3 g NaCl, 0 0.05 g Yeast Extract, 5 g cellulose, 9 g agar and 500 mL distilled water for 500 mL needs [15]. Lignolytic selection media made from 0.5 g K$_2$HPO$_4$, 0.25 g KCl, 0.25 g MgSO$_4$.7H$_2$O, 0.05 FeSO$_4$, 2.5% lignin, 10 g agar and 500 mL aquadest for 500 mL needs [16]. Omeliansky media made from 0.5 g (NH$_4$)$_2$SO$_4$, 0.5 g KH$_2$PO$_4$, 0.25 g MgSO$_4$.7H$_2$O, 1 g CaCO$_3$, 0.05 g NaCl, 7.5 g agar and 500 mL aquadest for 500 mL needs [17]. SCA media made from 5 g soluble starch, 0.15 g casein (vitamin free), 1 g KNO$_3$, 0.025 g MgSO$_4$.7H$_2$O, 1 g K$_2$HPO$_4$, 1 g NaCl, 0.01 g CaCO$_3$, 0.005 g FeSO$_4$.7H$_2$O, 9 g agar and 500 mL of distilled water for the needs of 500 mL [18]. NA media made from 3.5 g of beef extract, 5 g of peptone, 10 g of agar and 500 mL of distilled water for 500mL needs [15].

2.3. Isolation of lignocellulolytic bacteria and fungi

Isolation of lignocellulolytic bacteria and fungi from samples of outer peels, inner peels and vines of cassava, the mixture of mahogany and sengon sawdust in a ratio of 1:1, dried RS, and isolation of general bacteria and actinobacteria from samples one week old RS compost were carried out using the spread plate method. Isolation was done separately for each type of sample. As amount of 10 g sample was used for the dilution series in 0.85% physiological saline solution. Inoculation on CMC, lignolytic selection media, and omeliansky agar media were taken from 10$^{-1}$, 10$^{-2}$, 10$^{-3}$ dilutions, while for NA and SCA agar media were taken from 10$^{-2}$, 10$^{-3}$, and 10$^{-4}$ dilutions. In addition, several small pieces of each
sample were also inoculated to the respective media. After incubation for a week at room temperature, the population density and morphology colony were observed.

2.4. Purification and propagation of isolates on liquid media

Purification was carried out by selecting isolates based on the diversity of colonies in each medium. Each type of colony was taken to be purified on the same agar medium with two replications. One set of replicate was propagated in liquid culture from the same medium to be used as a bioactivator, while another set of replicate was maintained as stock cultures. Liquid cultures were incubated at room temperature for 2 weeks by shaking to 2 hours/day. The calculation of the population density of the liquid cultures were carried out before applying to the compost.

2.5. Set up RS compost and application of bioactivator

RS material used for the composting was characterized with the content of total C 40.8 % and total N 0,5 %. A total of 100 kg of dried RS was chopped with a length of 3 – 5 cm, soaked in a basin of water and arranged into two piles with the size of each pile of about 60 x 60 x 60 cm³. The first compost pile was a control treatment, while the second compost pile was treated with the addition of the culture of bioactivator. After two weeks of piling, the two compost piles were turned and moistened. At the same time of this turning the bioactivator was applied on the compost treatment. The bioactivator contained mixed isolates from five types of media @250 mL with population density of $10^{15}$ CFU/mL that were diluted in 10 L of water. Incubation of the two compost piles was continued until two months, with twice turning on day 14 and on day 28.

2.6. The observed and measured variables

In the isolation stage of lignocellulolytic bacteria and fungi, the observed variables were morphology colony and population density of each medium. During the composting process of two piles of RS compost, the control, and the treatment of bioactivator, the observation was conducted for temperature, moisture content, and pH. The chemical analysis of the product of two RS compost consisted of Total C, Organic Matter content, Total N, C/N ratio, P₂O₅, and K₂O.

3. Results and discussion

3.1. Isolates of lignocellulolytic bacteria and fungi

In total there were 55 colony diversities found on 3 media of CMC, lignolytic selection media, and omeliansky (Table 1), namely 25 types of bacterial colonies and 30 types of fungal colonies, which were the results of the isolation of lignocellulolytic microorganisms obtained from the sources of the outer peels, inner peels and vines of cassava, the mixture of mahogany and sengon sawdust with a ratio of 1:1, and dried RS. The diversity of fungal and bacterial colonies was determined from the variety of the colony morphology that consisted of shape, elevation, surface, opacity, margin, and color. The population density of each type of colony was calculated. The growth of isolates from the mixture of mahogany and sengon sawdust on Omeliansky media showed the highest colony diversity and population density compared to other isolate sources, namely for bacteria there were 3 types of colonies with a total population density of $8.17 \times 10^{4}$ CFU/ml and for fungi there were 5 types of colonies with a total population density of $1.12 \times 10^{4}$ CFU/ml (Table 1).

The results of the isolation of general bacteria and actinobacteria from one week old RS compost on NA and SCA media were obtained 3 and 2 types of bacteria colonies, respectively. It is important to be noted, that the morphology of bacterial colonies on SCA media was the same as those grown on NA media, but the population density of each colony was higher on SCA media than on NA media (Table 1). Based on the colony morphology, bacteria growing on NA and SCA media belonged to the Actinobacteria group. This is consistent with previous studies which also showed that actinobacteria were the dominant decomposer microorganisms in the thermophilic phase detected by the PLFA method.
[14] and the PCR-DGGE method followed by sequencing [19]. The existence and role of actinobacteria as decomposers in the thermophilic phase of composting has been widely reported [19,20].

Table 1. Colony diversity and population density of isolated lignocellulolytic bacteria and fungi

| No | Source of Isolate                      | Media           | Bacteria/Fungi | Number of Colony Diversity | Population Density (CFU/ml) |
|----|----------------------------------------|-----------------|----------------|---------------------------|-----------------------------|
| 1  | Outer peels of cassava                 | CMC             | Bacteria       | 3                         | $0.27 \times 10^4$          |
|    |                                        | Lignolytic      | Bacteria       | 2                         | $0.3 \times 10^4$           |
|    |                                        | selection media | Fungi          | 2                         | $1.5 \times 10^4$           |
|    |                                        | Omeliansky      | Bacteria       | 1                         | $1 \times 10^4$             |
|    |                                        |                 | Fungi          | 2                         | $0.04 \times 10^4$          |
| 2  | Inner peels and vines of cassava       | CMC             | Bacteria       | 3                         | $3.2 \times 10^4$           |
|    |                                        |                 | Fungi          | 4                         | $0.05 \times 10^4$          |
|    |                                        | Lignolytic      | Bacteria       | 4                         | $1.03 \times 10^4$          |
|    |                                        | selection media | Fungi          | 1                         | $0.00001 \times 10^4$       |
|    |                                        | Omeliansky      | Bacteria       | 2                         | $1.04 \times 10^4$          |
|    |                                        |                 | Fungi          | 2                         | $0.01 \times 10^4$          |
| 3  | The mixture of mahogany and sengon sawdust in a ratio of 1:1 | CMC             | Bacteria       | 1                         | $0.01 \times 10^4$          |
|    |                                        |                 | Fungi          | 3                         | $0.05 \times 10^4$          |
|    |                                        | Lignolytic      | Bacteria       | 4                         | $1.03 \times 10^4$          |
|    |                                        | selection media | Fungi          | 2                         | $0.37 \times 10^4$          |
|    |                                        | Omeliansky      | Bacteria       | 3                         | $8.12 \times 10^4$          |
|    |                                        |                 | Fungi          | 5                         | $1.12 \times 10^4$          |
| 4  | Dried rice straw                      | CMC             | Bacteria       | 1                         | $0.0001 \times 10^4$        |
|    |                                        |                 | Fungi          | 3                         | $0.68 \times 10^4$          |
|    |                                        | Lignolytic      | Bacteria       | 3                         | $0.71 \times 10^4$          |
|    |                                        | selection media | Fungi          | 1                         | $0.0008 \times 10^4$        |
|    |                                        | Omeliansky      | Bacteria       | 3                         | $0.67 \times 10^4$          |
|    |                                        |                 | Fungi          | 1                         | $0.1 \times 10^4$           |
| 5  | One week old rice straw compost       | NA              | Bacteria       | 3                         | $21.18 \times 10^4$         |
|    |                                        | SCA             | Bacteria       | 2                         | $368.44 \times 10^4$        |

CMC: Carboxymethyl Cellulose; SCA: Starch Casein Agar; NA: Nutrient Agar

Each colony type of all isolates was subcultured on agar media and on liquid media according to the respective media. The cultures growth on agar media were used for stock isolates, whereas the culture growth on liquid media were used for bioactivator to be applied in composting RS. Some purified isolates are presented in Figure 1 a-g. Three types of isolates from the mixture of mahogany and sengon sawdust on omeliansky media are presented in Figure 1 e-g, namely the isolates of SD-O-1-f, SD-O-2-f, and SD-O-4-b.
Figure 1. Lignocellulolytic microorganism isolates: (a) Isolate OP-C-1-f (fungi isolated from outer peels of cassava on CMC media), (b) Isolate SD-C-2-f (fungi isolated from sawdust on CMC media), (c) Isolate SD-L-4-b (bacteria isolated from sawdust on Lignolytic selection media), (d) Isolate OP-L-3-f (fungi isolated from outer peels of cassava on Lignolytic selection media), (e) Isolate SD-O-1-f (fungi isolated from sawdust on Omeliansky media), (f) Isolate SD-O-2-f (fungi isolated from sawdust on Omeliansky media), (g) Isolate SD-O-4-b (bacteria isolated from sawdust on Omeliansky media).

3.2. Temperature, moisture content, and pH during the composting process
Table 2 showed that on day 7 the two compost piles reached the highest temperature of 63-64°C and then on day 14 the temperature decreased to 44°C in the control and 43°C in the treatment piles. Thus, the two compost piles had relatively the same temperature patterns during the first 14 days. The bioactivator was applied to the pile of compost treatment on day 14. The effect of the addition of bioactivator was observed on day 21, the compost treatment increased the temperature to 52°C while the compost control decreased the temperature to 41°C. The thermophilic phase in the pile of compost treatment was observed until day 28, and then the temperature gradually decreased to 33°C. This finding indicated that application of bioactivator resulted in the extension of thermophilic phase in the composting process. In previous studies, the extension of the thermophilic phase could occur due to the addition of ammonium sulfate in the second week of composting of RS [14]. The thermophilic phase in the composting process is very important for the elimination of the harmful pathogens and viruses [2, 14, 19–21].

Table 2. Temperature, moisture content, and pH during the composting process of RS

| No. | Treatment                  | D0   | D7   | D14  | D21  | D28  | D60  |
|-----|----------------------------|------|------|------|------|------|------|
| 1   | Temperature                |      |      |      |      |      |      |
|     | Compost Control            | 28.0°C | 63°C | 44°C | 41°C | 38°C | 32°C |
|     | Compost + Bioactivator     | 28.1°C | 64°C | 43°C | 52°C | 45°C | 33°C |
| 2   | Moisture content           |      |      |      |      |      |      |
|     | Compost Control            | 98%  | 95%  | 85%  | 420% | 379% | 326% |
|     | Compost + Bioactivator     | 95%  | 91%  | 83%  | 415% | 363% | 315% |
| 3   | pH                         |      |      |      |      |      |      |
|     | Compost Control            | 7.1  | 8.7  | 8.7  | 8.2  | 7.5  | 7.4  |
|     | Compost + Bioactivator     | 7.1  | 8.8  | 8.8  | 8.4  | 7.6  | 7.5  |

Turning of the piles was conducted on day 14 and on day 28. Data on day 14 and on day 28 were observed before turning.
On the first day of composting the moisture content of two compost piles were similar of 98% and 95% (Table 2), and then decreased gradually until day 14 (before turning) to 85% and 83%. After turning on day 14, the moisture content increased on day 21 to 420% and 415%. The pattern of moisture content between the two compost piles were similar from the first day until the end of composting.

The initial pH of RS compost was the same of 7.1 and after undergoing the composting process for 14 days the pH increased sharply to 8.7 and 8.8 (Table 2). Significant increase of pH in the second and third weeks to the range of 8.7-8.8 was also found in previous studies [14]. The increase of pH in the composting can occur due to intensive ammonia production in the early weeks of the decomposition process [22]. After D21 both compost piles decreased in pH towards neutral until the end of composting (D60).

3.3. Chemical characteristics of compost products

During the composting process, RS undergoes a decomposition process which means there was a decrease in organic matter and total C. In Table 3, it is clear that there is a decrease in the total C in the compost products compared to the initial RS material which had total C 40.8 %. Compost with bioactivator treatment had a greater decrease in total C than in the compost control. At the end of the composting, the product of compost treated with bioactivator indicated total C 21.82 %, while compost control was 26.01 %. Inoculation of selected microorganism in the composting process can increase the rate of decomposition of organic matter, this result is in line with research conducted by Xu and Li [23] that in the composting of pig manure and RS with an initial total C of 40.28 %, after 20 days of composting, there was a greater decrease of total C in the compost inoculated with Bacillus subtilis, Bacillus licheniformis, Phanerochaete chrysosporium, Trichoderma koningii, and Saccharomyces cerevisiae by 24.54 % compared with 16.75 % for compost control.

Total N, P2O5, and K2O in the two compost products is presented in Table 3. Total N, P2O5, and K2O of compost product with bioactivator treatment was higher than the compost control, 28.57 %, 17.65 %, and 14.11 %, respectively. This study showed that the addition of bioactivator had an effect on accelerating the rate of decomposition in the composting process that result in the producing of the slightly higher of total N, P2O5, and K2O compared to the compost control. The two compost products meet the quality standard for solid organic compost product according to the Decree of the Minister of Agriculture of the Republic of Indonesia 261/KPTS/SR.310/M/42/2019 which determine that the standard of the total macro nutrients (N + P2O5 + K2O) of solid organic fertilizer is minimal 2 %. Several other studies reported that to increase the nutritional content of compost products, it was necessary to add other compost materials such as composting rice straw with poultry manure and biochar could increase the total N content of compost up to 2.4 % [24]. The addition of rock phosphate and waste mica could increase the total P and total K became 3.2 % and 2.6 % compared to control compost which had a total P of 0.5 % and a total K of 1.41 % [25].

Table 3. Total C, organic matter content, total N, C/N ratio, P2O5, and K2O of compost products

| Treatment                  | Nutrient content at the end of composting (D60) |
|----------------------------|-----------------------------------------------|
|                            | Total C (%)    | Organic Matter (%) | Total N (%) | C/N  | P2O5 (%) | K2O (%) |
| Compost Control            | 26.01          | 44.84             | 1.12        | 23.2 | 1.19     | 1.63    |
| Compost + Bioactivator     | 21.82          | 37.62             | 1.44        | 15.2 | 1.40     | 1.86    |

As shown in Table 3, the compost product with the bioactivator treatment had a lower C/N ratio than the compost control. The C/N ratio of compost product with bioactivator treatment was 15.2 that was achieved in 2 months of the composting process. The quality standard for the C/N ratio of organic fertilizer according to the Decree of the Minister of Agriculture of the Republic of Indonesia 261/KPTS/SR.310/M/42/2019 is ≤ 25. This fact indicated that the present composting process of RS with the addition of bioactivator was estimated to reach a C/N ratio of ≤ 25 in less than 2 months. Another similar study reported that the composting process of 80 kg of RS and 288 kg of cow dung
inoculated with *B. lincheniformis* 1-1v and *B. sonorensis* 7-1v (separately or as mixed cultures) only took 45 days to reach the C/N ratio ≤ 25, while the uninoculated compost took 59 days [21].

The results of the present study was different compared with the previous study on composting process of RS by Cahyani et al. [14] which took 145 days of composting to reach the C/N ratio of compost products ≤ 25, because the composting process of 300 kg of RS was carried out in winter with the temperature in the first 75 days below 10°C and without the addition of bioactivator, but there was the addition of ammonium sulfate on day 14 of composting. Zhang et al. [26] reported that environmental factors such as temperature and humidity affect microbial activity in the composting process.

4. Conclusion
Isolation of lignocellulolytic bacteria and fungi from the mixture of mahogany and sengon sawdust in a ratio of 1:1 on Omeliansky media resulted the highest colony diversity and population density compared to the other sources of outer peels, inner peels and vines of cassava, and dried RS. On the other side, the isolation of general bacteria and actinobacteria from one week old RS compost on NA and SCA media, indicated that the morphology of colonies growing on SCA media were the same type with those that grown on NA media, but the population density was higher on SCA media. The application of the mixed cultures from those all isolated lignocellulolytic bacteria and fungi as bioactivator in the composting of RS resulted in the extension of the period of thermophilic phase, increasing the rate of composting, and improving the quality of compost. After 60 days composting, the RS compost product with bioactivator treatment indicated C/N ratio of 15.2, total N 1.44 %, P₂O₅ 1.40 %, and K₂O 1.86 %, showing higher quality compared to the compost control that indicated C/N ratio of 23.2, total N 1.12 %, P₂O₅ 1.19 %, and K₂O 1.63 %. Future studies are needed to explore more sources to find more microbial isolates that potential to be used as bioactivators for composting.

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