The role of microbes in organic material decomposition and formation of compost bacterial communities

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Abstract. The application of microbial decomposers and bio-stimulant into agriculture wastes influence the abundance of microorganisms and their biological diversity of the compost. The study was conducted to determine the role of microbial in the decomposition of agricultural wastes that produces compost and its effect on the diversity and function of microbes that inhabited the compost product. Agricultural wastes used straw, leaves and grass from the Cibinong Science Center-Botanical Garden. Decomposer microbes used fungi (Trichoderma), and bacteria (Azospirillum brasilense and Agrobacterium sp.). The composting process was conducted anaerobically and the compost beds were reversed every 7 days. The diversity and function of inhabiting microbes resulted from the composting process was determined and identified by conducting microbial isolation and 16S rRNA genes identification. The ability of the microbes as decomposers was also analyzed. The results showed that the genus Bacillus tended to dominate. The microbial consortia have a high enzyme activity to degrade material waste agriculture. Of the three sources of compost used, the grass produced a better compost, forming a diverse and beneficial bacterial community. Therefore, it can be stated that the use of microbial decomposers for composting the agriculture wastes is an appropriate method for forming the beneficial microbial ecosystems.

1. Introduction

The Cibinong Science Center-Botanical Garden (CSC-BG) area produces organic waste/agricultural biomass waste abundantly and daily in the form of broad-leaf leaves that fall and grass. The lack of proper management of these organic material wastes, especially agricultural wastes, causes environment pollution. The wastes should be further handled and processed to produce valuable resources, such as compost fertilizer for plant nutrients. This research was conducted to support zero waste activities in the Cibinong CSC-BG area, so as to create a bioresources-based and environmentally friendly area with a sustainable bioorganic environment.

Compost is humus-like substance from conversion of organic wastes which can augment chemical, physical, and biological soil properties. Compost is useful for improving the whole crop production, soil quality and fertility, crop nutrition, freshwater resources, food safety, biodiversity, and microenvironment. Composting is the biological decomposition and stabilization of organic matter under anaerobic conditions with microbial succession, and the final product is also free from pathogens and plant seeds [1].

The quality of the product of composting depends on some factors such as humidity, temperature, and pH. Moreover, the substrate utilized and the microbiota have an important role on compost formation. Agricultural solid wastes from CSC-BG, such as grass, fallen leaves and straw have a high
content of organic matter. Ratio of C/N for rice straw, leaves of trees, and grass was 80, 40-80, 20, respectively. The C/N ratio shows the intensity of microorganisms in the decomposition of available organic matter and compost stability over time. Composting process will reduce the C/N ratio to support soil productivity. The composition of agricultural biomass which consists mainly of cellulose can be a good substrate for microbial decomposers. The suitability of organic material composition and types of microbes in the decomposition process is related to the production of nutrients that can be utilized by plants [2].

It has been explained in the previous paragraph that the stability and quality of compost products is dependent on the microbiota [3]. Some researchers related to the use of microbial inoculants for the purpose of accelerating composting and improving the final product have been done. The research that observed the application of microbial inoculants (Bacillus and Actinobacteria) in the composting of vegetable products could improve the quality of agricultural products by increasing the final maturation of the compost [4]. However, more information about the new microbiota formed is needed to be studied.

The potential microbial consortium will work more optimally in the degradation of the substrate because it produces diverse enzymes. The use of potential microbial decomposers is very important in the process of biomass degradation to produce high-value-added compost [5]. The microbes have a role to minimize ecological imbalance and to maintain nutrient flow from one system to another [6]. The potential microbes that have been characterized as decomposers, including the fungal and bacterial groups (Trichoderma, Azospirillum brasilense and Agrobacterium sp.), were selected to explore the effects of microbes in the composting process. The microbes have been characterized as having lignin, protein and cellulose degrading activities, Indole-3-Acetate Acid (IAA) producing capability, as well as nitrogen fixation and phosphate solubilization activity. Their potential activity as decomposers have been studied in our previous research (data not published).

Application of microbial decomposer and compost biostimulant into the agriculture wastes has an influence on the abundance of microorganisms and their biological diversity of the compost. The study was conducted to determine the role of microbial decomposer in the decomposition of agricultural wastes that produces compost and its effects on the diversity and function of microbes that inhabit the compost product.

2. Materials and Methods

2.1 Material

The raw material used in this study were rice straw, wide drop leaf litter (mahogany, guava, and mango leaves) and pruning grass available around the CSC-BG area. The wastes were collected in the garden and dried for 1 month before being processed into compost. The average of temperature, humidity, and pH of the three raw materials were 32.15°C, 74.78%, and 7.72, respectively.

We used 2 compost inocula, that were Plant Symbiotic Microbes (PSM) inoculum and commercial inoculum (Pro). PSM inoculum consists of 3 microbes, namely Azospirillum brasilense, Agrobacterium sp. and Trichoderma sp. Two bacterial isolates, Azospirillum brasilense and Agrobacterium sp. were maintained on slants of nutrient agar for 2 days. One loop full of each bacterium was inoculated in 50 ml modified peptone-succinate-salts (MPSS) liquid medium as starter and incubated with shaking for 2 days. Two percent of broth inoculum (10⁶ CFU/ml) of each isolate was individually inoculated into 5L MPSS broth medium and incubated with shaking for 2 days [7]. One fungal isolate, Trichoderma sp. was grown in the media of Potato Dextrose Agar (PDA) in petri dishes for 3-5 days. One of petri dish with full of Trichoderma sp. was inoculated in 400 g of sterile rice that has been added with 200 ml of distilled water to a sterile baking pan. The pan was covered with sterile baking paper and incubated for 5-7 days. Rice media that have been overgrown with Trichoderma sp., was dried in an oven at 50°C for 2 days [8].

Commercial starter inoculum (Pro) consists of Aspergillus sp., Trichoderma harzianum DT 38, Trichoderma harzianum DT 39, and decomposer bacteria. This starter inoculum was ready to use by
mixing it directly into the compost material. The starter inoculant requirements for the composting process are presented in the following table (Table 1).

Table 1. The treatment of composting process

| No. | Treatment                          | Dose                                      |
|-----|------------------------------------|-------------------------------------------|
| 1   | Pro Commercial + Straw (J1)        | 393.6 g pro + 60 kg straw                |
| 2   | PSM Inoculum + Straw (J2)          | 180 g *Trichoderma* + 2.4 L bacterial inoculum + 240 ml molasses + 60 kg straw |
| 3   | Pro Commercial + Leaves (D1)       | 656 g pro + 100 kg leaves                |
| 4   | PSM Inoculum + Leaves (D2)         | 300 g *Trichoderma* + 4 L bacterial inoculum + 400 ml molasses+100 kg leaves |
| 5   | Pro Commercial + Grass (R1)        | 656 g pro + 100 kg grass                 |
| 6   | PSM Inoculum + Grass (R2)          | 300 g *Trichoderma* + 4 L bacterial inoculum + 400 ml molasses + 100 kg grass |

2.2 Composting process
The three types of raw materials (plant biomass), upland rice straw, wide drop leaf litter wastes and pruning grass, were identified as substrate J, D, and R, respectively. Each biomass waste was collected, chopped and processed to produce compost. Each compost bed was made from 60 kg of rice straw, 100 kg of wide leaf litter, and 100 kg of grass, each divided into 3 layers. Each layer (20 kg for straw and 33 kg for leaves and grass) was inoculated with microbial decomposer inoculum [9]. The dimension of each stack or bed of compost was 2m x 1m x 60cm. Composting was conducted anaerobically for 90 days under controlled temperature and humidity. The compost beds were reversed every 7 days. All experiments were conducted in triplicate. The quality of the final compost product was compared with SNI 19-7030-2004 standards [10].

2.3 Sampling and analysis
At the final composting process, every sample (10 g each) was taken and diluted with 90 ml of sterile distilled water and then was shaken for 1 hour. One ml of sample solution was put into 9 ml of sterile distilled water and then was made into serial dilutions (10^1 to 10^9). A total of 100 µl of sample solution from 10^4 to 10^9 of dilution were plated into the nutrient agar (NA) medium in the petri dish, then flattened with a spatula, and incubated until the microbes grew. The grown bacteria were counted for their number of colonies and purified until they got a single colony. Bacterial isolates that were successfully cultured were measured for their hydrolytic potentials, particularly the amylase, protease, and cellulose activity. Standard procedures were utilized for the calculation of enzymatic activity according to Baehaki and Budiman [11] and Patil et al [12]. Enzymatic activity index was determined using equation 1.

\[ IE = \frac{X1 - X2}{X2} \]  

IA/IS/IP = Enzymatic (Amylolytic, cellulolytic and proteolytic) activity index  
X1 = diameter of clear zone  
X2 = diameter of colony

The potential isolates that have ability as decomposer were identified by 16S rRNA gene sequence analysis. Amplification of the 16SrRNA gene was achieved by amplify the genomic DNA from selected bacteria that used as template for PCR amplification with the following universal primers, 27F (5’-AGA GTT TGA TCC TGG CTC AG-3’) and 1492 R (5’-GTT TAC CTT GTT ACG ACT T-3’). The PCR method was carried out using GoTaq Green PCR kit and the PCR products were then directly sequenced.
The complete sequencing results were built by using DNA Baser suite and used for further nucleotide BLAST analysis (https://blast.ncbi.nlm.nih.gov/).

Moisture content and temperature of each sample compost product was determined using thermohygrometer (HTC-2) by plugging the tool into the sample compost. The pH of each compost sample was measured by taking 10 grams of compost sample and then diluting it with 100 ml of distilled water. The acidity level of solution was measured by pH meter (TPS Digital pH meter).

The chemical composition of samples i.e: Total P (Bray), Total K (Olsen), Ca and Mg (Morgan Wolf), Organic C (Walkley and Black), and Total N (Kjeldahl) were analyzed in the soil analysis laboratory, Research Center for Biology, Indonesian Institute of Sciences.

3. Results and Discussion

3.1 The characteristics of compost product

Three isolates (Azospirillum brasilense and Agrobacterium sp., and Trichoderma sp.) were selected based on their hydrolytic activity [13]. The selected microbes were repeatedly tested for their ability to determine their involvement in the organic decomposition processes and their ability in compost microbiota formation. Our inoculum (PSM) compared with commercial inoculum (Pro). Inoculation by microbes was needed to achieve successful and prompt composting [14]. The characteristics of compost product that were inoculated with PSM and pro inoculum are presented in Table 2.

| No | Code sample | Parameters |
|----|-------------|------------|
|    |             | P-Total | K-Total | Ca (%) | Mg (%) | C organic (%) | N-Total (%) | C/N Ratio |
| 1  | J1          | 2.83    | 2.75    | 0.074  | 0.067  | 27.74    | 1.59       | 17.45     |
|    | Note        | √       | √       | √      | √      | √        | √          |
| 2  | J2          | 3.29    | 2.71    | 0.158  | 0.068  | 24.36    | 1.52       | 16.03     |
|    | Note        | √       | √       | √      | √      | √        | √          |
| 3  | D1          | 2.37    | 2.61    | 0.556  | 0.066  | 25.34    | 1.18       | 21.53     |
|    | Note        | √       | √       | √      | √      | √        | √          |
| 4  | D2          | 2.24    | 2.56    | 0.226  | 0.066  | 21.61    | 1.23       | 17.53     |
|    | Note        | √       | √       | √      | √      | √        | √          |
| 5  | R1          | 12.12   | 2.6     | 0.586  | 0.067  | 31.34    | 2.81       | 11.16     |
|    | Note        | √       | √       | √      | √      | √        | √          |
| 6  | R2          | 9.01    | 2.72    | 0.069  | 0.068  | 30.59    | 2.83       | 10.81     |
|    | Note        | √       | √       | √      | √      | √        | √          |
| 7  | SNI 19-7030-2004 | >0.10  | >0.20   | <25.5  | <0.6   | 9.8-32   | >0.4       | 10-20     |

Note: √ = in accordance with SNI 19-7030-2004 standard, J1=Compost product from raw material of straw with Pro inoculum, J2= Straw with PSM inoculum, D1= Leaves with Pro inoculum, D2= Leaves with PSM inoculum, R1= Grass with Pro inoculum, R2= Grass with PSM inoculum. Pro = commercial inoculum, PSM = Plant Symbiotic Microbes-inoculum.

Based on the parameters from the final composting result, both inoculum treatments, PSM inoculum and Pro inoculum, produce compost quality in accordance with SNI 19-7030-2004 standards. Both inocula are proven to reduce the C/N ratio of raw materials to produce compost that can be used for plant nutrition. Based on the results, PSM inoculum was able to reduce the C/N ratio greater than commercial inoculum. This is due to the added microbes are effective in converting C-organic from raw materials and the high degradation activity of inoculants. Trichoderma, one of the microbial inoculants,
could stabilize the C/N ratio, increased nitrogen (N), phosphorus (P), and potassium (K) in compost [15].

The C/N ratio value can be influenced by the type of material used for compost. Based on the results, the C/N ratio of raw materials from highest to lowest of raw materials, leaves (80), rice straw (80) and grass (20) respectively, after the process of composting the C/N ratio of leaves (17.53-21.53), rice straw (16.03-17.45) and grass (10.81-11.16). During composting, the values of C/N ratio were decreased. The decrease in the C/N ratio is caused by an overhaul of organic material so that it becomes available, and release of CO₂ into the air by microorganisms. This organic acid content decreases and the total N-level rises due to the formation of ammonium into nitrate during decomposition [16]. The higher the C/N ratio of compost (product) indicates that the organic material has not been completely decomposed. On the other hand, the lower C/N value of compost indicates that organic material has decomposed and is almost becoming compost.

| No | Treatment | Weight of raw material (kg) | Weight of compost product (kg)* | Yield (%)*¹ |
|----|-----------|-----------------------------|--------------------------------|-------------|
| 1  | J1 (Pro)  | 60                          | 44.1 ± 3.15                    | 73.5 ± 5.25 |
| 2  | J2 (PSM)  | 60                          | 32 ± 3.6                       | 53.3 ± 6    |
| 3  | D1 (Pro)  | 100                         | 78 ± 2.78                      | 78 ± 2.78   |
| 4  | D2 (PSM)  | 100                         | 63 ± 3                         | 63 ± 3      |
| 5  | R1 (Pro)  | 100                         | 30 ± 2.64                      | 30 ± 2.65   |
| 6  | R2 (PSM)  | 100                         | 31 ± 2.29                      | 31 ± 2.29   |

Note: J1=Compost product from raw material of straw with Pro inoculum, J2= Straw with PSM inoculum, D1= Leaves with Pro inoculum, D2= Leaves with PSM inoculum, R1= Grass with Pro inoculum, R2= Grass with PSM inoculum. Pro = commercial inoculum, PSM = Plant Symbiotic Microbes-inoculum. *¹ values are expressed as mean ± SD (n=3).

Weight loss was observed during the composting process (Table 3). The reduction in weight occurred because of the decomposition process. The raw materials of compost released carbon dioxide (CO₂), energy, water, plant nutrients, and resynthesized organic carbon compound. The similar results were reported by Gautam et al. [17] who observed weigh loss of biomass over a 45-day period.

3.2 Molecular analysis during composting
The influences of the inoculant on bacterial succession over the composting process could be observed in Figure 1. The treatment presented different profiles of the number of microbes and their activity. This indicates that there was a dominance of certain bacteria species within the general bacterial community during the composting process.
**Figure 1.** The number of culturable bacteria from the raw material (rice straw) of compost. (A). Enzymatic activity index of bacterial isolates, (B). The number of isolates based on their activity. JR=bacterial isolates from raw material of straw, IS = Index of Celullose, IA = Index of Amylase, IP = Index of Protease, No act = No activity.

**Figure 2.** The number of culturable bacteria from the raw material (leaves) of compost. (A). Enzymatic activity index of bacterial isolates, (B). The number of isolates based on their activity. DL=bacterial isolates from raw material of leaves, IS = Index of Celullose, IA = Index of Amylase, IP = Index of Protease, No act = No activity.
Figure 3. The number of culturable bacteria from the raw material (grass) of compost. (A). Enzymatic activity index of bacterial isolates, (B). The number of isolates based on their activity. RT= bacterial isolates from raw material of grass, IS = Index of Celullose, IA = Index of Amylase, IP = Index of Protease, No act = No activity.

Figure 1, 2, and 3 show the indigenous microbes that inhabit each the raw material of compost. Some of indigenous microbes have the hydrolytic enzymatic too, particularly cellulase, amylase, and protease activity. The number of culturable indigenous bacterial is varied based on the type of raw material. The number of culturable bacterial from grass was the highest. The bacterial community in the raw material of compost involved in composting process together with bacterial inoculum which was added in process.
Figure 4. The number of culturable bacteria from the compost product of Straw. (A). Enzymatic activity index of bacterial isolates, (B). The number of isolates based on their activity. J = bacterial isolates from compost product of straw, IS = Index of Celullose, IA = Index of Amylase, IP = Index of Protease, No act = No activity.

Figure 5. The number of culturable bacteria from the compost product of leaves. (A). Enzymatic activity index of bacterial isolates, (B). The number of isolates based on their activity. D = bacterial isolates from compost product of leaves, IS = Index of Celullose, IA = Index of Amylase, IP = Index of Protease, No act = No activity.
Based on Figure 4, 5, and 6, the number of culturable bacteria from compost product increased and varied based on the type of raw materials. Microbial inoculant increases the microbial population that leading to the fundamental transformation and changes and cause the production of important enzymes [18]. It is revealed that the biomass is processed by the synergy of cellulase enzymes that are secreted by microbial inoculum and indigenous microbes. The succession of a microbial community during composting, reflects their degradative capacity for the compost mix. The composting efficiency increases with increasing of solid and liquid inoculums and is usually affected by the combination of native microbes [19].

**Table 4. Identification of bacterial isolates from raw materials of compost based on 16S rRNA gene sequence analysis**

| No | Bacterial Isolate | Raw material | Identify as                     |
|----|-------------------|--------------|---------------------------------|
| 1  | J 1               | Straw        | *Bacillus haynesii*             |
| 2  | J 2.1             | Straw        | *Bacillus* sp.                  |
| 3  | J 2.2             | Straw        | *Pseudomonas plecoglossicida*   |
| 4  | J 3               | Straw        | *Paracoccus communis*           |
| 5  | DL 1.1            | Leaves       | *Pseudomonas* sp.               |
| 6  | DL 2.1            | Leaves       | *Bacillus megaterium*          |
| 7  | DL 2.2            | Leaves       | *Bacillus* sp.                  |
| 8  | DL 2.3            | Leaves       | *Bacillus flexus*               |
| 9  | DL 3.1            | Leaves       | *Bacillus flexus*               |
| 10 | DL 3.2            | Leaves       | *Bacillus safensis*             |
| 11 | DL 4              | Leaves       | *Bacillus aerius*               |
| 12 | DL 5.1            | Leaves       | *Chryseobacterium cucumeris*    |
| 13 | DL 5.2            | Leaves       | *Bacillus* sp.                  |
| 14 | RT 9              | Grass        | *Bacillus aryabhattai*          |
| 15 | RT 10             | Grass        | *Bacillus sonorensis*           |
Table 5. Identification of bacterial isolate from compost product based on 16S rRNA gene sequence analysis

| No | Bacterial isolate | Raw material + inoculum | Identify as               |
|----|-------------------|-------------------------|--------------------------|
| 1  | J 1.1             | Straw + pro             | Bacillus safensis        |
| 2  | J 1.3             | Straw + pro             | Bacillus haynesii        |
| 3  | J 1.5             | Straw + pro             | Bacillus sp.             |
| 4  | J 1.6             | Straw + pro             | Bacillus megaterium      |
| 5  | J 3.1             | Straw + PSM             | Bacillus safensis        |
| 6  | J 3.2             | Straw + PSM             | Pseudomonas plecoglossicida|
| 7  | J 3.4             | Straw + PSM             | Paracoccus communis      |
| 8  | J 3.5             | Straw + PSM             | Bacillus cereus          |
| 9  | J 3.7             | Straw + PSM             | Bacillus megaterium      |
| 10 | J 3.8             | Straw + PSM             | Bacillus megaterium      |
| 11 | DL 1.1            | Leaves + pro            | Bacillus megaterium      |
| 12 | DL 1.2            | Leaves + pro            | Bacillus sp.             |
| 13 | DL 1.3            | Leaves + pro            | Bacillus toyonensis      |
| 14 | DL 1.5            | Leaves + pro            | Bacillus sp.             |
| 15 | DL 1.6            | Leaves + pro            | Bacterium sp.            |
| 16 | DL 1.7            | Leaves + pro            | Bacillus megaterium      |
| 17 | DL 1.8            | Leaves + pro            | Bacillus sp.             |
| 18 | DL 3.1            | Leaves + PSM            | Bacillus flexus          |
| 19 | DL 3.2            | Leaves + PSM            | Bacillus sp.             |
| 20 | DL 3.3            | Leaves + PSM            | Bacillus flexus          |
| 21 | DL 3.4            | Leaves + PSM            | Bacillus safensis        |
| 22 | DL 3.5            | Leaves + PSM            | Bacillus subtilis        |
| 23 | DL 3.6            | Leaves + PSM            | Bacillus safensis        |
| 24 | DL 3.7            | Leaves + PSM            | Bacillus aerius          |
| 25 | DL 3.8            | Leaves + PSM            | Chryseobacterium cucumeris|
| 26 | DL 3.9            | Leaves + PSM            | Chryseobacterium indologenes|
| 27 | R 1.1             | Grass + pro             | Bacillus sp.             |
| 28 | R 1.2             | Grass + pro             | Bacillus subtilis        |
| 29 | R 1.5             | Grass + pro             | Bacillus sp.             |
| 30 | R 1.7             | Grass + pro             | Bacillus safensis        |
| 31 | R 3.1             | Grass + PSM             | Delftia sp.              |
| 32 | R 3.2             | Grass + PSM             | Bacillus sp.             |
| 33 | R 3.3             | Grass + PSM             | Bacillus aryabhattai     |
| 34 | R 3.4             | Grass + PSM             | Bacillus sonorenseis     |
| 35 | R 3.5             | Grass + PSM             | Ochrobactrum intermedium |
| 36 | R 3.6             | Grass + PSM             | Bacterium                |
| 37 | R 3.7             | Grass + PSM             | Bacillus megaterium      |
| 38 | R 3.8             | Grass + PSM             | Bacterium                |
| 39 | R 3.9             | Grass + PSM             | Bacillus sp.             |

The variation of microbiome depends on environmental conditions, composition of the raw materials and nutrient supplements, and interaction among all these factors. Any variation in the structure of the microbial community is likely to show the functional properties of compost produced. The microbes
that we applied as inoculums at the beginning of the composting process cannot be found in the compost product. This was probably because of the competition with native inhabitants of the composts and colonization efficiency of microbial communities. Due to inability to survive in a competitive environment, some of microbial inoculant sometimes becomes disappear. Other species of bacteria that were different from those used as inoculant may have been more abundant over the composting process.

The identified bacteria from raw material were dominated by the genus of Bacillus. These species was plentiful in the initial phase of the process and was responsible for cellulose degradation. Composting microbiota depends on its own competitive effectiveness and its survival capacity and environmental conditions are mostly responsible for microbial fluctuations during the composting process. In the compost product there are some microbial groups which are detected frequently in moderate or high number. These groups constitute the composting resident microbiota [20]. The availability of dominant bacteria, Bacillus, in the initial process and in the final product suggests that it is a part of composting resident microbiota.

4. Conclusions

PSM and commercial inoculant produce compost quality in accordance with SNI 19-7030-2004 standards. PSM inoculated compost attains better compost quality and maturity, based on C/N ratio. The addition of microbial inoculum was found to influence the degradation of organic material and the formation of bacterial community in the compost. The highest microbial community is found in the raw material from grass, the grass-dwelling microbes have high degrading enzyme activity, resulting in the lowest C/N ratio. The presence of dominant bacteria, Bacillus, in the initial process and the final product suggests that it is a part of composting resident microbiota. The understanding about the compost microbial ecology along with the diversity and function of inhabiting microbes is needed for the application of soil microbial technology in the rhizosphere. The addition of microbial inoculant was found to influence the bacterial community of the compost and involved in each the stage of the composting process.

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