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Isolation and Identification of Bacterial Composition of Composting Process

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PREFACE

Pusat Penelitian Lingkungan *Hidup - Institut* Pertanian *Bogor* (PPLH-IPB) [Center for Environmental Research - Institut Pertanian Bogor (CER-IPB)] was established in 1976. One of the Center's goal is to develop policies and concepts for natural resources and environmental management based on ecosystem characteristics, community participation, local community tradition, economic justice, and global environmental change.

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Finally, we hope this publication will be valuable and beneficial for those who have interest in Indonesia's natural resource and environmental management.

September 2009,

Kukuh Murtilaksono

Director

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Isolation and Identification of Bacterial Composition of Composting Process

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ABSTRACT

The research was aimed to identify species and characteristic of bacteria that live in Galuga waste dump site, and to compare hot composting (closed bin) and cold composting (ventilated bin). 7 bacterial genus (Lysteria, Neisseria, Kurthia, Rothia, Acinetobacter, Streptobacillus and Streptococcus) were able to be purified from Galuga waste dump site. Gram positive bacteria are Lysteria, Kurthia, Rothia. and Streptococcus. Gram negative bacteria are Neisseria, Acinetobacter, and Streptobacillus. Among 7 isolated bacteria Lysteria, Neissena, Kurthia, Rothia, Acinetobacter, and *Streptobacillus* belong to mesophilic bacteria. Meanwhile, Streptococcus is thermophilic bacteria. Composting in closed bin (hot composting) indicated better result than composting in ventilated bin (cold composting). For better cornposting process, material composting should not only leaves but also other organic materials.

1. INTRODUCTION

1.1. Background

Composting, sewage treatment, and certain types of fermentation have been practiced by humankind since the beginning of recorded history, and all these utilize microbial processes. Evidence of kitchen middens and compost piles dates back to 6000 B.C. And the more modern use of bioremediation begin over 100 years ago with the opening of the first biological sewage treatment plant in Sussex, UK, in 1891. Yet the word bioremediation is fairly new. Its first appearance in peer-reviewed scientific literature was in 1987 (Hazen, 1997).

Composting is a natural biological process, carried out under controlled aerobic conditions (requires oxygen). In this process, various microorganisms, including bacteria and fungi, break down organic matter into simpler substances. The effectiveness of the composting process is dependent upon the environmental conditions present within the composting system i.e. oxygen, temperature, moisture, material disturbance, organic matter and the size and activity of microbial populations.

Compost is the end product of a complex feeding pattern involving hundreds of different organisms, including bacteria, fungi, worms, and insects. What remains after these organisms break down organic materials is the rich, earthy substance your garden will love. Composting replicates nature's natural system of breaking down materials on the forest floor. In every forest, grassland, jungle, and garden, plants die, fall to the ground, and decay. They are slowly dismantled by the small organisms living in the soil. Eventually these plant parts disappear into the brown crumbly forest floor. This humus keeps the soil light and fluffy (http://www.compostguide.com).

Compost can be made in as little as two weeks, or it can take as much as a year, depending on the effort and attention to the process. Most organic materials, chopped or shredded into small pieces, will decompose quickly if they're kept moist and turned or stirred regularly to expose them to air. This condition can accelerate the activity of microorganism to decay organic matter. Knowing species and characteristic of bacteria is important due to compare the time needed in composting and the effectivity of composting process in different ways.

Domestic and urban solid waste is still a huge problem in Indonesia, since so far most of those wastes are discharged in landfill area. With the quantity is getting higher, this conventional handling of waste is not a good measure of handling waste anymore. Separation of waste into biodegradable and non biodegradable has not yet been a common practice in Indonesia. Composting is a simple ways of handling biodegradable solid waste. However without solid waste separation, composting of waste would never have been possible.

1.2. Objectives of the research

To identify species and characteristic of bacteria that live in compost pile. To compare type of bacteria that live in different ways of composting process. To compare the result of composting in different ways of composting.

2. MATERIALS AND METHODS

2.1. Isolation

Research was undertaken in Laboratory of Aquatic Productivity and Environment, Bogor, initiating on October 07 until October 08. Bacteria were isolated from waste dump area, Galuga, Bogor. Bacterial isolates were grown in 15 Petri disc. They were isolated from paper waste, domestic waste, plastic waste, which were already mixed with soil (Figure 1).

The research is divided into comparison of several means of composting and isolation of bacteria involved in the composting process.

2.2. Identification

Among 15 Petri disc collected, it was purified 8 bacterial isolates. A series of bacterial identification (gram stain test, motility test, and biochemistry test) was performed on each purified bacteria (Cowan, 1974 and Holt et al., 1994).Once those several tests ended, bacterial identification was conducted by following the Manual for the identification of medical bacteria (Cowan, 1974).

Figure 1. Bacteria sampling at Waste Dump Site, Galuga, Bogor.

2.3. Cultivation

Among 7 bacterial genus, 1 genus (Kurthia) was selected for cultivation and for further use as compost activator to accelerate composting process. As much as 25 ml Nutrient Broth (NA) media was sterilized, and then added Kurthia by means of Ose needle. Then incubated in room temperature for 18 - 24 hours. After one day grows, bacterial colony was counted. Kurthia colony was 2.3 x 109 CFU/ml.

2.4. Composting Preparation

Materials for composting consisted of closed bin (for hot composting), ventilated bin (cold composting), dry leaves, soil, bacterial isolate (Kurthia) (Figure4).

Ventilated bin with soil inside	Closed bin with soil inside	Dry leaves

Figure 2. Material for hot and cold composting.

2.4. Composting Process

Two type of composting was investigated, namely hot composting using closed bin and cold composting using ventilated bin. Into both bins were added until the height of 10 cm from the bin bottom. Then 1 kg dry leaves were put inside. It was followed the addition of 25 ml Kurthia isolate (total colony 2.31 x 109 CFU/ml) and 25 g urea into both bins. All materials were thoroughly mixed, and water was poured to attain wet condition. Every three days, mixing was conducted. Temperature and humidity measurement were also undertaken. During composting process, both rubbish bins were maintained always wet.

3. RESULT AND DISCUSSION

3.1. Bacteria Isolation and Identification

As much as 7 bacterial genuses were obtained from Galuga waste dump site, Those are Lysteria, Neisseria, Kurthia, Rothia, Acinetobacter, Streptobacillus and Streptococcus. Gram positive bacteria are Lysteria, Kurthia, Rothia, and Streptococcus. Gram negative bacteria are Neisseria, Acinetobacter, and Streptobacillus (Table 1 and 2). Among 7 isolated bacteria Lysteria, Neisseria, Kurthia, Rothia, Acinetobacter, and Streptobacillus belong to mesophilic bacteria. Meanwhile, Streptococcus is thermophilic bacteria.

Kurthia was cultivated for further use as costing activator. After one day cultivation, Kurthia colony was 2.3 x 109 CFU/ml.

Table 1. Result of bacterial identification (morphology, physiology, and biochemistry).

Sample Code	Catalase Test	Oxidase Test	Motility Test	Oxidative Fermentative Test	Gram stain Test	Morphological shape
S1 (Lysteria)	+		Motile	Fermentative	(+)	Rod
S2 (Neisseria)	+		Motile	Oxidative	(-)	Coccus
S4 (Lysteria)	+		Motile	Fermentative	(+)	Rod
S6 (Kurthia)	+		Motile	Fermentative	(+)	Coccus
S9 (Rothia)	+	+	Motile	Fermentative	(+)	Rod
S12 (Acinetobacter)	+		Motile	Oxidative	(-)	Coccus
S13 (Streptobacillus)			Motile	Fermentative	(-)	Rod
S14 (Streptococcus)			Motile	Fermentative	(+)	Coccus

Note: + : Positive reaction (+) : Blue, - : Negative reaction (-): Red

Bacteria are the smallest living organisms and the most numerous in compost. They make up 80 to 90% of the billions of microorganisms typically found in a gram of compost. Bacteria are responsible for most of the decomposition and heat generation in compost. They are the most nutritionally diverse group of compost organisms, using a broad range of enzymes to chemically break down a variety of organic materials.

The existence of thermophilic and mesophilic bacteria indicates that at Galuga waste dump site undergoes composting. Bacteria contribute a principle role in composting process converting organic waste into compost. Composting process is divided into three phases: 1. Mesophilic phase, 2. Thermophilic phase, and 3. Maturation phase (Trautman and Lynciw, 2008). Mesophilic bacteria take part in the onset of composting process. Therefore, the initial process of composting is called mesophilic phase. During the beginning of composting, decomposition occurs fast. Thus, temperature normally rises. It could reach 40oC or above. At this hot condition, mesophilic bacteria could not survive anymore. It was replaced by the thermophilic bacteria. High composting temperature accelerated degradation of protein, lipid, complex carbohydrate (cellulose and hemicelluloses). Once the material to be composted reduced, temperature decreased as well, then mesophilic bacteria replaced the role of thermophilic bacteria. This process is called maturation process. In general, the longer the curing or maturation phase, the more diverse the microbial community it supports.

Decomposing organisms consist of bacteria, fungi, and larger organisms such as worms, sow bugs, nematodes, and numerous others. Decomposing organisms need four key elements to thrive: nitrogen, carbon, moisture, and oxygen. All those elements are available in Galuga waste dump site that allow composting to take place. However, since waste in Galuga consists of not only organic, but also an organic material, natural composting is hindered to occur. Galuga waste dump site receives numerous variety of unsorted wastes.

Under optimal conditions, composting proceeds through three phases: 1) the mesophilic, or moderate-temperature phase, which lasts for a couple of days, 2) the thermophilic, or high-temperature phase, which can last from a few days to several months, and finally, 3) a several-month cooling and maturation phase.

3.2. Composting

Composting was conducted in closed bin as hot composting, and in ventilated bin as cold composting. Observation was performed every three days. Temperature and humidity were measured. Appearance of leaves in both bins was also observed whether they become smaller as consequence of decaying. The rate at which composting occurs depends on physical as well as chemical factors. Temperature is a key parameter determining the success of composting operations. Physical characteristics of the compost ingredients, including moisture content and particle size, affect the rate at which composting occurs. Other physical considerations include the size and shape of the system, which affect the type and rate of aeration and the tendency of the compost to retain or dissipate the heat that is generated.

Visually, hot composting indicated faster and better than cold composting. Dried leaves content less nitrogen than green leaves. Carbon to nitrogen ratio for leaves is 60 : 1. Livestock manure comprises high nitrogen. For better composting process, material composting should not only leaves but also other organic materials.

In the process of composting, microorganisms break down organic matter and produce carbon dioxide, water, heat, and humus, the relatively stable organic end product. Different communities of microorganisms predominate during the various composting phases. Initial decomposition is carried out by mesophilic microorganisms, which rapidly break down the soluble, readily degradable compounds. The heat they produce causes the compost temperature to rapidly rise.

Grass clippings and other green vegetation tend to have a higher proportion of nitrogen (and therefore a lower C/N ratio) than brown vegetation such as dried leaves or wood chips. If the nitrogen proportion is too high, the compost may become too hot, killing the compost microorganisms, or it may go anaerobic, resulting in a foul-smelling mess. The usual recommended range for C/N ratios at the start of the composting process is about 3011, but this ideal may vary depending on the bioavailability of the carbon and nitrogen. As carbon gets converted to CO_2 (and assuming minimal nitrogen losses) the C/N ratio decreases during the composting process, with the ratio of finished compost typically close to 1011 (Richard and Trautmann, 2008). In this research, dried leaves (low nitrogen content) were utilized as composting material. Therefore, the composting did not last satisfactorily.

The microorganisms are very efficient at utilizing nitrogen when that is the limiting nutrient. The smell of ammonia is an indicator that nitrogen is in excess, and carbon/energy is limiting instead. Ammonia losses are common when composting high nitrogen materials such as fresh grass clippings or manure.

Ammonia is among the most common odors found at composting facilities. Fortunately, ammonia is not a pervasive odor, so it does not require a large number of dilutions to reduce concentrations below the odor threshold. Ammonia also disperses easily, since is lighter than air (its density is 60% that of air), and does not settle in low lying areas the way hydrogen sulfide and other dense odorous compounds do. These factors make ammonia odors more prevalent on-site than off-site (Richard and Trautmann, 2008).

Another factor affecting the magnitude of ammonia volatilization is pH. NH_3 (gaseous ammonia) and NH_4^+ (aqueous ammonium ion) are in equilibrium at a pH of about 9, with higher pH's forcing more NH_4^+ into the gas form that you can smell. Thus ammonia is rarely noticed if the pH is acidic (Richard and Trautmann, 2008).

4. CONCLUSION

Seven bacterial genuses (Lysteria, Neisseria, Kurthia, Rothia, Acinetobacter, Streptobacillus and Streptococcus) were able to be purified from Galuga waste dump site. Gram positive bacteria are Lysteria, Kurthia, Rothia, and Streptococcus. Gram negative bacteria are Neisseria, Acinetobacter, and Streptobacillus. Among 7 isolated bacteria Lysteria, Neisseria, Kurthia, Rothia, Acinetobacter, and Streptobacillus belong to mesophilic bacteria. Meanwhile, Streptococcus is thermophilic bacteria.

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