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Isolation and Identification of Mold in Banana Bunches and Their Potential as Bioinoculants to Accelerate Decomposition of Household Organic Waste

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ABSTRACT
Large populations and final storage areas cause an increase in organic waste. Efforts are needed to improve waste decomposition. Mold is one of the microorganisms that can break down organic waste. This study aims to 1) isolate and identify molds from 8 banana bunches, 2) calculate mold populations. Banana bunches were taken from Madiun Regency. Isolation and mold conversion were carried out at the Biology Laboratory of the Department of Microbiology, Faculty of Science and Technology, Airlangga University, Surabaya. Isolation was carried out by making suspension of 8 banana bunches in which each suspension was inoculated on a petri dish with medium Potato Extract Agar. Subsequently it was incubated at 30°C for 3-7 days. After that the mold colonies were observed macroscopically and micrococisically. After observing the mold colonies, identification and calculation of the population of each species were carried out using the Total Plate Count (TPC). From the results of the study, it was found 10 species of fungal, *Aspergillus niger,Aspergillus penicilloides, Aspergillus oryzae, Rhizopus nigricans, Rhizopus oryzae, Mucor piriformis, Fucarium chlamydosporium, Penicillium citrinum, Penicillium citrinum, Penicillium chrysogenum, Penicillium expantium.* The TPC of each species were 6.2 x 10^7 CFU/ml, 5.4 x 10^7 CFU/ml, 4.9 x 10^7 CFU/ml, 4.2 x 10^7 CFU/ml, 3.6 x 10^7 CFU/ml, 4.1x 10^7 CFU/ml, 4.1 x 10^7 CFU/ml, 3.3 x 10^7 CFU/ml, 3.8 x 10^7 CFU/ml, 4.1 x 10^7 CFU/ml. Insulated molds have the potential as bioinoculant acceleration of decomposition organic waste due to rapid growth.

Keywords: *Mold, Banana bunches, Bioinoculant, Decomposition, Organic waste.*

1. INTRODUCTION

Large population accompanied by increased activity results in an increase in the volume of waste. The resulting waste will be disposed of at the landfill (TPA). Nowadays it is difficult to find land for landfills so this case can lead to higher waste dumps. Waste dumps will cause environmental problems. Large volumes of waste disposal have the potential to release methane gas which can increase greenhouse gas emissions and contribute to global warming. The waste dump will produce leachate which can cause contamination of groundwater around the landfill. Leachate from the landfill has an impact on groundwater sources so that groundwater around the landfill should not be used for drinking water supplies unless handled according to drinking water standards. [2] Leachate contains heavy metals and organic contaminants. It has a potential source of groundwater pollution that makes groundwater unusable for household needs [3].

The waste generated is generally organic waste. Based on the composition and physicochemical characteristics, 59% of the waste is organic waste [4]. By looking at the composition of the waste which is mostly organic material, waste management by composting method is an alternative to process waste. Organic waste is a type of waste that can decompose naturally. Natural decomposition takes 3 to 4 months [5], so this method is not effective in reducing waste dumps. The addition of biodecomposers will accelerate the decomposition process in composting. Microorganisms act as biodecomposers in the process of decomposition of organic waste. Mold is one of the microorganisms that can decompose organic waste [6].

This study aims to isolate and identify molds from banana bunches and also to determine its potential as a bioinoculant. Banana bunches are one part of a banana tree that physically has a hard texture. In the banana chip industry and processed foods made from bananas, banana bunches are a problem because it is difficult to decompose. Related to this, there has been no research on the isolation of fungi from banana bunches.

2. MATERIAL AND METHODS

2.1. Sampling

Banana bunch samples were taken from gardens around community houses in Madiun Regency, East Java, Indonesia. Harvested bananas were cut and some of the bunches were put in plastic and then taken to the laboratory. Banana bunches taken were from Ambon, Rojo Temen, Kepok, Rojo Ketan (Milk), Cavendis (Morosebo), Marlin, Ulin and Mas bananas.

2.2 Mold isolation

Banana bunches were put into different boxes and coded A, B, C, D, E, F, G, and H and then left for a week so there was a growth of microorganisms. After banana bunches already have mold on them, the suspension was made by inoculating into erlenmenyer containing 100 ml of sterile distilled water. 1 ml of the sample suspension was diluted to 10^{-6}. All suspensions resulting from dilution were inoculated on petri dishes with Potato Dextrose Agar (PDA) media added with chloramfenical. Furthermore, the suspension was incubated at 30^0C for 3-7 days where the growth of mold colonies was then observed in the media.

2.3. Purification of mold colonies

The growing mold colonies were purified by cutting the mold and putting it into new media. PDA media were added with chloramfenical to a petri dish which was then incubated at room temperature for 3 to 7 days. The pure and growing colonies were inoculated on slanted Agar in a test tube containing PDA media.

2.4 Identification of molds

Molds that have been isolated and purified were identified by observing them macroscopically and microscopically. Macroscopic observations include the upper part, color of the colony, the surface shape of the mold colony (cotton, puder, bludru, wax), the line from the center of the colony towards the edge of the colony, and drops of exudate. Lower observations include zoning: a circular line from the center of the colony to the edge of the colony and the color of the colony. Microcospic observations of mold includes whether isolated hyphae, pigmented hyphae: (hyaline is colorless / blue when given staining, dark (greenish brown or blackish, grayish black), hyphoid hyphae or not, simple asexual spores (Arthrospora, Blastospora, Klamidospora, blackish gray), hyphoid hyphae or not, simple asexual spores (Arthrospora, Blastospora, Klamidospora, Sporangiospora).

2.5 Calculation of Total Plate Count (TPC) of molds

To find out the growth of mold, mold population was calculated using the Total Plate Count (TPC) method. 10 gram banana bunches were then inoculated into 90 ml of sterile distilled water and mixed until homogeneous and then diluted to 10^{-7}. Each suspension from the dilution results was taken as much as 1 ml and then inoculated into a sterile petri dish. Next 1 ml of chloramphenicol and 15 ml was added to the PDA media then homogenized. After the media becomes solid, incubation was carried out at 30^0C for 3 x 24 hours. The next process was counting the number of mold colonies that grow on petri dishes and multiplied by the dilution factor.

3. RESULT AND DISCUSSION

From the isolation and identification of molds from 8 banana bunch samples, it was found 5 genera consisting of 10 species namely *Aspergillus* 3 species, *Penicillium* 3 species, *Rhizophus* 2 species, *Fusarium* 1 species and *Mucor* 1 species. Ten species of the mold are *Aspergillus niger*, *Aspergillus penicilloides*, *Aspergillus oryzae*, *Rhizopus nigricans*, *Rhizopus oryzae*, *Mucor piriformis*, *Fusarium chlamydosporum*, *Penicillium citrinum*, *Penicilliun chrysogenum*, *dan Penicillium expansum*. Lavendis banana bunches are the most common mold species, that is 8 species, while there are at least 3 species of ambon bananas. The results of the isolation and mold identification of each banana bunch are presented in Table 1.

From Table 1 it is known that the molds of the genera *Aspergillus* and *Penicillium are* found in all banana bunches. *Aspergillus* and *Penicillium* are saprophytic molds that are cosmopolitan in nature and are found in various types of habitats [6]. Aspergillus is a mold that belongs to the class of Ascomycetes which can be found everywhere and grows as saprophyte in

Table 1. Results of isolation and mold identification of each banana bunch

Sample	Mold / Fungi
Ambon banana bunches	*Aspergillus penicilloides, Aspergillus oryzae, Penicillium citrinum*
Rojo Temen bunches	*Aspergillus niger , Aspergillus oryzae, Penicillium chrysogenum, Penicillium expansum, Fusarium chlamydosporum*
Kepok bunches	*Aspergillus penicilloides, Aspergillus oryzae, Penicillium chrysogenum, Penicillium expansum, Fusarium chlamydosporum*
Rojo Ketan (Susu) bunches	*Aspergillus niger , Aspergillus penicilloides, Aspergillus oryzae, Penicillium chrysogenum, Penicillium expansum*
Lavendis (Morosebo) bunches	*Aspergillus niger , Aspergillus penicilloides, Aspergillus oryzae, Penicillium citrinum, Penicillium chrysogenum, Penicillium expansum, Rizophus nigricans, Mucor piriformis*
Marlin bunches	*Aspergillus niger , Aspergillus oryzae, Penicillium chrysogenum, Penicillium expansum, Rizophus oryzae*
Ulin bunches	*Aspergillus niger , Aspergillus penicilloides, Aspergillus oryzae, Penicillium citrinum, Penicillium chrysogenum, Penicillium expansum, Fusarium chlamydosporum*
Mas bunches	*Aspergillus niger , Aspergillus oryzae, Penicillium chrysogenum, Penicillium expansum, Rizophus nigricans, Mucor piriformis*

decaying plants, soil and food. The results of the isolation and identification of *Aspergillus oryzae* were found in all banana bunches. *Penicillium* is widespread in the natural environment and causes damage to vegetables, fruits and cereals. *Penicillium chrysogenum* is a species of molds that lives in various habitats [7]. Based on the results of isolation, this mold was found in

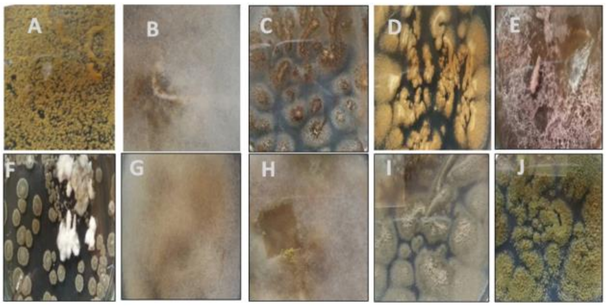

Figure 1. Macroscopic observations, A. *P expansum*, B. *R oryzae*, C.*A niger*, D. *P citrinum*, E. *F chlamydosporum*, F. *P chrysogenum*, G. *M piriformis*, H. *R nigricans*, I. *A penicilloides*, J.*A oryzae*

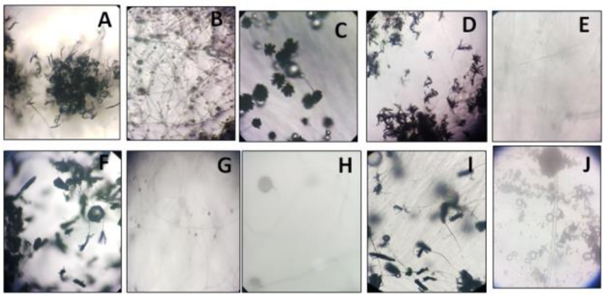

Figure 2. Microscopic observations , A. *P expansum*, B.*A oryzae*, C. *A niger*, D. *P citrinum*, E. *F chlamydosporum*, F. *P chrysogenum*, G. *M piriformis*, H. *R nigricans*, I. *A penicilloides*

Table 2. Results of Mold Population Calculation in Banana Bunches

Molds	Population of mold (CFU/ml)
Aspergillus niger	6.2×10^7
Aspergillus penicilloides	5.4×10^7
Aspergillus oryzae	4.9×10^7
Rhizopus nigricans	4.2×10^7
Rhizopus oryzae	3.6×10^7
Mucor piriformis	4.1×10^7
Fusarium chlamydosporum	4.1×10^7
Penicillium citrinum	3.3×10^7
Penicillium chrysogenum	3.8×10^7
Penicillium expansum	4.1×10^7

7 banana bunches. The genus *Rhizopus* and *Mucor* are also included in saprophytic molds. *Mucor* was only found in Lavendis and Mas banana bunches. The results of macroscopic observation of isolation and identification of molds from banana bunches are presented in Figure 1.

The result of microscopic observations of isolation and identification of molds from banana bunches are presented in Figure 2.

The results of the calculation of the number of mold colonies from the isolation and identification of banana bunches showed that the highest mold population of *A. niger* is 6.2 x 10^7 CFU / ml and the lowest *P. citrinum* is 3.3 x 10^7CFU / ml. The results of the mold population calculation are presented in full in Table 2.

Based on Table 2, namely that the population of all molds is high, this shows that all molds from isolation and identification have rapid growth. Molds are generally saprophytic and act as decomposers of organic matter [8]. The rapid growth and saprophytic properties and decomposition of molds have the potential to be a bioniculan decomposition of organic waste.

4. CONCLUSION

From the results of isolation and identification of 8 banana bunches, it was obtained 10 types of fungi, namely *Aspergillus niger, Aspergillus penicilloides, Aspergillus oryzae, Rhizopus nigricans, Rhizopus oryzae, Mucor piriformis, Fusarium chlamydosporium, Penicillium citrinum, Penicilliun chrysogenum, Penicillium expansum*. From the results of TPC calculations, the number of colonies of all molds is high which means that it has fast growth so that it has the potential as a bioinoculant to accelerate the organic waste decomposition.

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