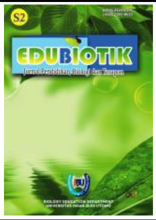
**Edubiotik : Jurnal Pendidikan, Biologi dan Terapan**
ISSN 2528-679X (print), ISSN 2597-9833 (online)
Vol. 9, No. 02, September 2024, pp. 108 – 116

Available online at:
https://ejurnal.uibu.ac.id/index.php/edubiotik



*Research Article*

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# Soil microorganism biodiversity in pule (*Alostonia scholarus* L.) and candlenut trees (*Aleurites moluccana* L.) at Wijaya Kusuma Campus Surabaya

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**Article Information**

**Article History:**
Submitted: 2024-12-07
Revision: 2024-12-26
Accepted: 2024-12-27
Published: 2024-12-30

**Keywords:**
Biodiversity;
isolation;
microorganisms;
rhizosphere;
Wijaya Kusuma Campus

**ABSTRACT**

A beautiful and fresh environment is visible with lush trees that illustrate the level of soil fertility and the diversity of microbes in it. Plant growth and development are influenced by the abundance of soil microbes, which reflect soil fertility and soil biological properties. Wijaya Kusuma Campus Surabaya is a campus whose slogan is Keluruhuran Jiwa Berbenah Lingkungan (Keji Beling) as a form of concern for the environment and the desire to create a healthy environment, it is necessary to explore the existence of potential microbes from the roots of trees in the campus environment. The purpose of this study was to determine the number and type of soil microbial isolates isolated from the rhizosphere of pule (*Alostonia scholarus* L.) and candlenut (*Aleurites moluccana* L.) trees on the Wijaya Kusuma Campus Surabaya. This research is exploratory descriptive qualitative research. Research methods include microbial isolation in the rhizosphere of pule and candlenut plants, macroscopic and microscopic characterization of microbes, KOH test, and catalase test. Data were analyzed descriptively describing the number and type of isolates. The results of the study concluded that the isolation results from the Pule tree obtained 12 colonies of fungi and the Candlenut tree obtained 6 colonies. The genus found were *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* sp., *Penicillium* sp. In addition, 27 bacterial colonies were found on the pule tree and 23 bacterial colonies on the candlenut tree, namely the genus *Bacillus* sp. The isolate results obtained can be used as microbial observation material in biology learning.

**Publisher**
Biology Education Department
Universitas Insan Budi Utomo,
Malang, Indonesia

**How to Cite**
Isrianto, P. L., Marmi, M., & Joeniarti, E. (2024). Soil microorganism biodiversity in pule (Alostonia scholarus L.) and candlenut trees (Aleurites moluccana L.) at Wijaya Kusuma campus Surabaya. *Edubiotik : Jurnal Pendidikan, Biologi dan Terapan*, *9*(02), 108–116. https://doi.org/10.33503/ebio.v9i02.7



## INTRODUCTION

Biology is a science related to all living things that are interrelated with everyday life. Biological material is important for students to master thoroughly so that biological science can be used as a provision in life. Not only mastering biology textually but can apply this knowledge in everyday life

(Aroyandini et al., 2020). Learning to characterize fungi, bacteria, and actinomycetes requires direct observation of objects to increase interest in material within the scope of microbiology and mycology. Direct observation and retrieval of objects can be done by utilizing the local potential that is owned in the campus environment. One of the local potentials of the campus of Wijaya Kusuma Campus Surabaya is one of the campuses that cares about the environment and has an environmental personality with many green plants that thrive in the campus environment so it is necessary to explore the study of the diversity of microorganisms from campus soil. One of the local potentials of the campus of Wijaya Kusuma Campus Surabaya is one of the campuses that cares about the environment and has an environmental personality with the many green plants that thrive in the campus environment so it is necessary to explore the study of the diversity of microorganisms from campus soil.

The key to a healthy soil ecosystem is the supply of organic substrate as an energy source for decomposer organisms such as bacteria, fungi, actinomycetes protozoa, insects, and others in the soil. Therefore, the abundance of soil biodiversity organisms is related to the soil's capacity to support plant growth and development and the sustainability of the earth as a biological factory for food and clothing. The sustainability of soil as a living system is highly dependent on the management of soil biota or soil ecology and the availability of organic matter including physical, chemical, and biological fertility (Simarmata, 2012). Plant growth cannot be separated from the soil conditions where the tree is. Plants in their growth require a medium, where plant roots can develop and get the necessary materials in the growth process. A plant that grows well can be an indicator of the level of soil fertility and the diversity of important microorganisms that live in the root area.

The biodiversity of microorganisms in soil is abundant in the rhizosphere. Exploration of new species in microbes is carried out by applying several techniques including cultivation, isolation, and microbes that cannot be cultivated using conventional media techniques to explore new functions of these microbes including secondary metabolites or antibiotics released (Panagan, 2011). The presence of microorganisms around plants plays an important role in maintaining the health of plant roots, maintaining nutrient cycles and soil formation processes, growth, influencing microbial activity including secondary metabolites or antibiotics, as well as biological control of root pathogens (Sulistiyani, 2011). Based on this background, it is necessary to conduct research on the isolation and characterization of soil microbes at Wijaya Kusuma Campus Surabaya, so that the purpose of the study is to determine the number and type of soil microbial isolates contained in the campus soil as a source of learning biology for the application of soil biology in agriculture and other developing fields, including the use of microbes as biofertilizers, biological agents to control pests and diseases biologically, and can produce various kinds of antibiotic drugs. The results of the actinomycetes isolates found can be used as microbial observations in the practicum of the microbial chapter of the mycology course.

The purpose of this study was to determine the number and type of soil microbial isolates isolated from the rhizosphere of pule trees (*Alostonia scholarus* L.) and candlenut (*Aleurites moluccana* L.) on the UWKS campus. The isolate results obtained can be used as microbial observation material in biology learning. The diversity of soil microorganisms in pule (*Alstonia scholaris* L.) and candlenut (*Aleurites moluccana* L.) plants at Wijaya Kusuma Surabaya Campus has not been widely explored in previous studies. Pule and candlenut plants, despite their important role in the ecosystem and pharmacological potential, have not been widely studied in the context of soil microorganism diversity, especially in unique locations such as Wijaya Kusuma Campus Surabaya. Most of the previous studies were conducted in areas with different environmental conditions (e.g., tropical forests, agricultural land, or large plantations). Research on soil microorganisms in semi-open urban environments such as Wijaya Kusuma Surabaya campus has not received attention, so the unique soil conditions in this area are still poorly understood. This research is expected to make a significant new contribution to science, particularly in understanding the relationship between plants, soil, and microorganisms in urban tropical environments.

## RESEARCH METHODS

This research is exploratory descriptive qualitative research. The results of microbial isolation from the rhizosphere of pule and candlenut trees can be used as learning in microbial observation of the number and type of microbial isolates as a learning resource. This research was conducted from March 2023 to October 2023. Research methods include microbial isolation in the rhizosphere of pule and candlenut plants, macroscopic and microscopic characterization of microbes, KOH test, and catalase test. Data were analyzed descriptively describing the number and type of isolates. The analysis was carried out at the  Biology Laboratory Wijaya Kusuma Campus Surabaya, and the UPN Faculty of Agriculture Protection Laboratory. The focus of the research was to determine the number and types of microbial isolates found from the rhizosphere of pule trees (*Alostonia scholarus* L.) and candlenut trees (*Aleurites moluccana* L.) on the UWKS campus as a source of learning biology. Bacteria are one group of soil microbes that are widely studied for their potential because they have high economic value, including as producers of antibiotics (Alwi & Lambui, 2021). The research stages are as follows (1) sampling was carried out using a purposive sampling method based on the environmental zone at the UWKS central campus, namely in the rhizosphere of tree A. pule tree and B. candlenut tree. In each location, soil samples at a depth of 20 cm from 4 holes, each soil sample were taken as much as 100 g and repeated 3 times. The soil samples were mixed evenly and put into sterile plastic as much as 1 kg in an ice box at 4°C and stored in the refrigerator for 18-24 hours before being analyzed. (2) Isolation of microbes from the rhizosphere of pule (*Alostonia scholaris* L.) and candlenut (*Aleurites moluccana* L.) trees in the UWKS campus environment by serial dilution method and spread on Potato Dextrose Agar (PDA) medium plus 50 mg/L streptomycin or Nutrient Agar (NA) medium plus 10 µg/L instant. A total of 10 grams of samples were diluted 10 times by adding diluent water to a final volume of 100 mL, and serial dilutions were carried out up to a dilution of 10-5. The results of the dilution were taken as much as 0.1 mL and spread on PDA and NA media. The inoculated PDA and NA media were then incubated for 5 days at 370C and room temperature, respectively. The growing microbial colonies were purified by scratching single colonies on new media and incubated for 48 hours at 37o C, to obtain pure cultures (Putra et al., 2020). Microbial population analysis using the dilution plate method. (3) Observation of isolation results in macroscopic and microscopic characteristics of microbes.

Morphological observations of microbial isolates that were successfully isolated have characteristics, namely cell morphology is very diverse and pleomorphic, irregular rod shape, branched filaments which are mycelial structures; non-motile; gram-positive; aerobic, facultative anaerobic or anaerobic and habitat. Macroscopic morphological characteristics include: colony shape, colony color, colony surface characteristics, colony growth, colony elevation, and colony edges.  After that, the microscopic characteristics test was continued, namely the staining test to determine the cell shape and gram culture to see conidia and gram staining hyphae, then continued with the KOH test, and the catalase test. Data analysis of microbial characterization, gram test, KOH test, and catalase test was done descriptively. The results of the isolation were inventoried as the local potential of microorganism diversity in the rhizosphere of pule and candlenut trees that can be implemented as a biology learning resource.

## FINDING AND DISCUSSION

A wide variety of microorganisms can grow well in soil. The complexity of nutrients for the growth of microorganisms contained in the soil causes microbes to grow very diverse. Samples in the form of rhizosphere sediments from pule and candlenut trees at the campus location of Wijaya Kusuma Campus Surabaya were extracted by soaking the soil in water so that all the nutrients needed for microbial growth are extracted properly. Rhizosphere microbes are microbes that grow in the root zone of plants. The number, diversity, and type are strongly influenced by exudates/organic compounds produced by plants. In soil that gets enough aeration, bacteria, and fungi will grow dominantly, but if the environment contains little or no oxygen, bacteria will play a role in almost all biological and chemical changes in the soil environment (Sulistiyani, 2011).

The isolated soil samples were observed macroscopically and microscopically. The macroscopic results in the rhizosphere of pule trees and candlenut trees are shown in Table 1. Each soil sample was repeated 3 times. From rhizosphere samples on pule trees, 12 fungal colonies were obtained, while for rhizosphere microbial samples on candlenut trees, there were 6 colonies with the description of macroscopic observations as follows Table. Microscopic Observation 1 can be seen in Figure 1. Microscopic Observation 2 can be seen in Figure 2.

**Table 1. Macroscopic Observation of Fungi**

| Sample | Macroscopic Result | Colony Count | Colony color | Genus/Species |
|---|---|---|---|---|
| Pule tree |  | a. 4<br>b. 1<br>c. 1<br>d. 1<br>e. 4 | a. White cotton (O)<br>b. Yellow-green (O)<br>c. Yellow white (O)<br>d. Black (O)<br>e. Dark green (O) | a. *Fusarium* sp.<br>b. *Aspergillus flavus*<br>c. *Aspergillus flavus*<br>d. *Aspergillus niger*<br>e. *Penicillium* sp. |
| Candlenut tree |  | a. 3<br>b. 1<br>c. 1<br>d. 1 | a. White cotton (O)<br>b. Black (O)<br>c. Dark green (O)<br>d. White green (O) | a. *Fusarium* sp.<br>b. *Aspergillus niger*<br>c. *Penicillium* sp.<br>d. *Aspergillus flavus* |

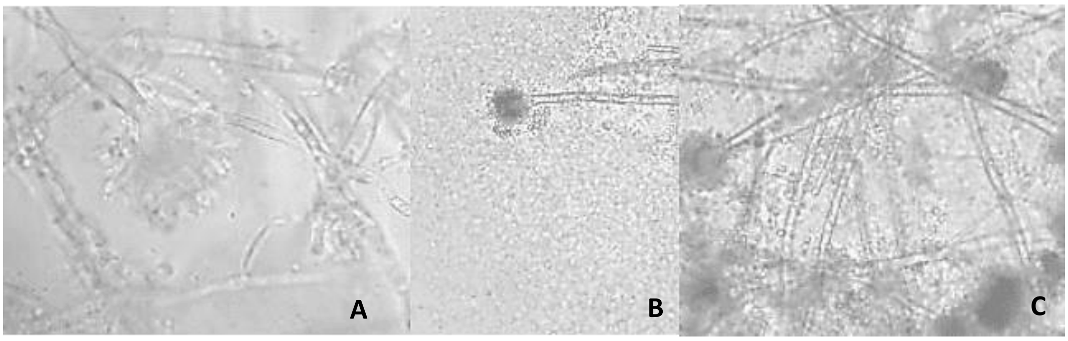



**Figure 1. Microscopic Observation 1: (A) *A. flavus* spora box; (B) *A. niger* spora box; (C) *A. Niger* spora box**

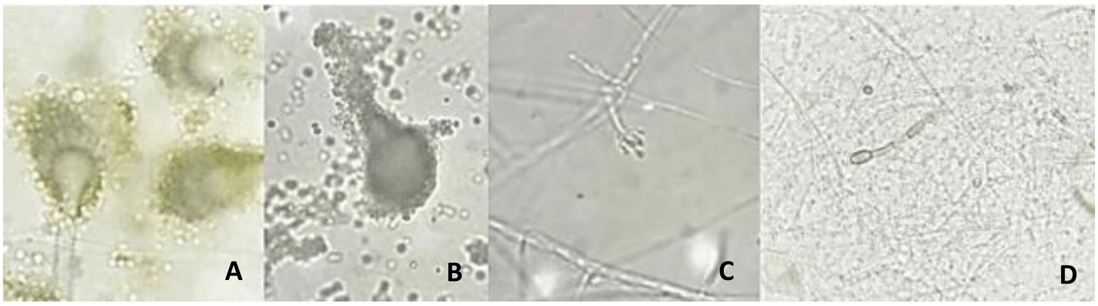



**Figure 2. Microscopic Observation 2: (A) *A. Flavus* spora box; (B) *A. Niger* spora box; (C) *Penicillium* spora box; (D) *Fusarium sp.* spora box**

Based on macroscopic observations on pule trees, *Fusarium sp., Aspergillus flavus, Aspergillus niger, and Penicillium sp.* On the macroscopic observation of candlenut trees, *Fusarium sp., Aspergillus niger, Penicillium sp., and Aspergillus flavus* were also found. The results of macroscopic *Fusarium sp.* show white colonies like cotton, and form sporodocium in large numbers, the colony will change from white to orange. In addition, the microscopic results (Figure 2) on *Fusarium sp*. conidium is formed on conidiophores that are monophialid, long and unbranched. The macroscopic results of *Aspergillus niger* show a black color, fungal colonies are round, granular, cottony with evenly distributed and slightly rough edges (Table 1) and for microscopic results, the microscopic results shown in Figure 1 show that the hyphae are intercepted, each conidiophora has one conidia are characterized by a round shape with long cylindrical conidiophores, and are colorless or hyaline (Wahdania et al., 2016). While Aspergillus flavus appears yellow green, yellow white, and white green (Table 1). The incubation period of the fungus is about 7 days on PDA (*Potato Dextrose Agar*) media, which initially has white colony edges (1-5 days) but at the age of 7 days changes color to black and has phialids that connect to the shoots that surround the entire surface (Rampa et al., 2022). As for *Penicillium*, the macroscopic results of dark green colonies appear dartmouth green with a little white at the edges (Table 1) and for the microscopic results of *Penicillium*, the walls of the conidia are smooth, the walls of the conidiophores are smooth, the conidiophores are branched, and have metulae and phialids, have skeletal and hyaline hyphae (Figure 2). In addition, *Penicillium* has round conidia, and is unicellular, and has a set of phialids (Ristiati et al., 2018).

Isolation of bacteria obtained in this study came from the rhizosphere of pule and candlenut trees. Soil samples were taken and then grown on *Nutrient Agar* (NA) media by diluting 10-1 to 10-5 to get pure isolates. The purpose of dilution is to minimize the number of bacteria to be isolated. The use of NA medium was chosen to isolate bacteria in general and adjusted to the nutritional needs of the bacteria to be isolated (Fitriani et al., 2016). The next step is to purify the bacteria and make observations both macroscopically and microscopically. Staining is done to distinguish cell morphology in positive and negative bacteria. which shows differences in cell wall structure. The results of bacterial isolation in the rhizosphere sample of pule tree obtained 27 bacterial colonies and 23 bacterial colonies in the rhizosphere of candlenut tree (Table 1). The results of the macroscopic description are circular in shape, convex and raised elevations, bacillus cell shape. The results of gram staining of both rhizosphere samples in pule and candlenut Trees showed a blue color, namely gram positive / + (Table 2). The results of macroscopic, microscopic observations and biochemical reaction tests (Table 3) obtained were then compared with the characteristics of antiotic-producing bacteria in the soil. The results obtained from the group of antiotic-producing bacteria from the soil are *Basillus sp.*

**Table 2 Bacterial Observations**

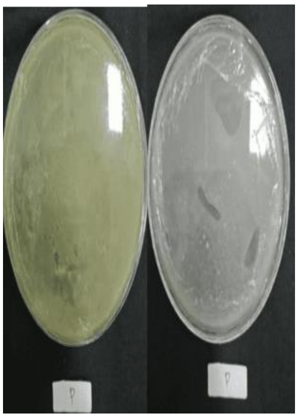
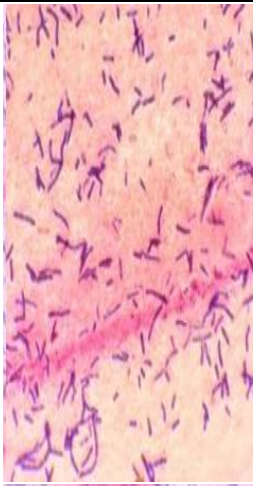
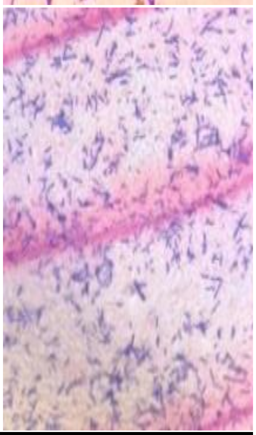
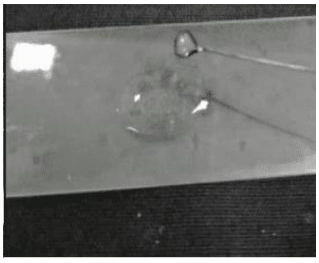
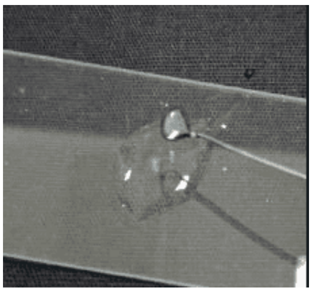
| Sample | Macroscopic Result | Colony shape | Elevation | Cell shape | Gram staining | Coloring Result |
|---|---|---|---|---|---|---|
| Pule Tree |  Colony Count 27 | Circular | Convex and raised | Basil | Blue/ + (positive) |  |
| Candlenut Tree |  Colony Count 23 | Circular | Convex and raised | Basil | Blue/ + (positive) |  |

**Table 3. KOH and Catalase Test Observations**

| Sample | KOH test | KOH Test Result | Catalase test | Catalase Test Results |
|---|---|---|---|---|
| Pule Tree | Not slimy result = + (positive) |  | Bubbly / + (positive) |  |
| Candlenut Tree | Not slimy result = + (positive) |  | Bubbly / + (positive) |  |

The results of the KOH test show that it is not slimy and the catalase test shows bubbly The results of the catalase test (Table 3) have been carried out on each isolate both for the rhizosphere of pule and candlenut trees, each of which was repeated 3 times producing bubbles after being dripped with $H_2O_2$

solution. This is also in line with Damayanti et al. (2020) which shows a positive reaction to form bubbles which means the formation of oxygen gas ($O_2$) as a breaker of $H_2O_2$ by the catalase enzyme produced by these bacteria. Catalase is an enzyme that can catalyze the decomposition of hydrogen peroxide ($H_2O_2$) into water and $O_2$. Based on observations, the colonies are round, flat elevation, shiny surfaces, wavy colony edges, and milky white color. The results of microscopic observations have a bacillus cell shape (rod), which is gram-positive. Based on Bergeys's Manual of Determinative Bacteriology 7th edition also that the genus *Bacillus* has rod-shaped characteristics, can be found in the soil, forms endospores, gram-positing, moves with erythric flagellum, is aerobic or facultatively anaerobic and is catalase positive. *Bacillus* bacteria can survive in all types of environmental conditions such as temperature, pH, and salinity levels (Ristiati et al., 2018). The pH value obtained is 6-6.5 which is a pH value that makes all nutrients in soluble conditions and will be easily absorbed by plants ranging from 5.5-6.5.

The total soil microbial population will produce soil microbial population levels in several classes. The higher the class level indicates the higher the soil microbial potential in supporting sustainable agriculture. Soil microbial activity plays a role in the biochemical processes in the soil as a whole which can be related to the chemical properties and physical properties of the soil where the growth of soil microbial populations is influenced by other soil properties (Solihin & Betty, 2017). Microbial community dynamics are a resource resources, which triggers rapid microbial responses (Bardgett & Putten, 2014). Plant roots are a good place for microbial growth. The interaction between bacteria and plant roots will increase the availability of nutrients for both. The thin thickness of the rhizosphere layer between each plant is different. Organic material released by the kara can be in the form of root exudates, root secretions, root lysates that are passively released during root cell autolysis, and mucilage, root secretion material, microbial cell residues, metabolite products, organic colloids, inorganic colloids (Sari, 2015).

The activity of soil microorganisms is influenced by factors such as food availability, soil moisture, soil temperature, moisture content, and human intervention (Kusumastuti, 2022). Based on the results found in the rhizosphere of pule and candlenut trees, namely *Aspergillus flavus, Aspergillus niger, Fussarium sp., Penicillium sp. Bacillus sp*. have the potential for the development of local biodiversity products. A*spergillus niger, Aspergillus flavus, and Penicillium* can have health benefits such as producing antibiotic (Zhang et al., 2023). *Aspergillus niger* plays a role in citric acid fermentation and can be utilized as a cosmetic ingredient for chitin glucan*. Penicillium* has a mechanism that can inhibit the growth of bacteria, besides that it can be used for the production of penicillin and cheese making (Hidayat et al, 2016). The *Fusarium* genus is one of the most important mycotoxigenic fungal genus in food and feed (Thrane, 2014). While *Bacillus sp*. bacteria are utilized as biofertilizers (Sugiyanta & Septianti, 2019) and as biological agents for sustainable agricultural uses and applications (Khan et al., 2022).

## CONCLUSION

The results showed that the microbes successfully isolated from the rhizosphere of the pule tree and candlenut tree, namely on the pule tree obtained 12 colonies of fungi, and the candlenut tree obtained 6 colonies. The genus found were *Aspergillus flavus*, *Aspergillus niger*, Fusarium sp, *Penicillium* sp. In addition, 27 bacterial colonies were found on the pule tree and 23 bacterial colonies on the candlenut tree, namely the genus *Bacillus* sp. The isolation results obtained can be inventoried as the local potential of microorganism diversity in the rhizosphere of pule and candlenut plants that can be implemented as a biology learning resource.

## ACKNOWLEDGMENT

We the research team would like to thank the Institute for Research and Community Service (LPPM) and the Dean of the Faculty of Teacher Training and Education UWKS for all the assistance that has been provided, both morally and materially, so that we can complete this activity well and smoothly.

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