JAPSI (Journal of Agriprecision and Social Impact)
Volume 3 Issue 3 November 2026
e-ISSN: 3032-7849; p-ISSN: 3046-5397, Page 468-489
DOI: https://doi.org/ 10.62793/japsi.v3i3.135
Available online at: https://journal.kdp.co.id/index.php/japsi

Identification of Microorganisms with Potential as Biological Control Agents for Grecek Kepok Banana Plants (*Musa Paradisiaca* L)

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Abstract. *Isolation and Identification of Fungi and Bacteria in the Rhizosphere of Musa Paradisiaca L. Grecek Banana Plants, Kaliorang Village. Banana plants are one of the horticultural crop commodities with significant economic value for the Indonesian people. The Grecek Kepok banana is a premier variety native to East Kalimantan, particularly East Kutai Regency, which is in high demand by foreign markets. The Grecek Kepok banana differs from the common Kepok banana which fruit is brighter yellow that firm flesh and a sweeter taste. The rhizosphere is the soil surrounding plants that supports microbial activity essential for maintaining plant development and growth. The rhizosphere contains various types of microbes, including fungi and bacteria. This research aims to isolate and identify fungi and bacteria in the rhizosphere of the Grecek Kepok banana plant. The research method involves taking soil samples from around healthy, diseased and recovering banana plants. Identification is carried out by observing fungi macroscopically and microscopically, while bacteria were identified based on several biochemical tests such as gram staining, the KOH test, the hypovirulence test, the fluorescent pigment test, and the phosphate test. The results of this research identified the following fungi : Trichoderma sp, Aspergillus niger, Penicillium sp, Fusarium oxysporum, Colletotrichum sp, Aspergillus flavus, Mucor sp were found. As for bacteria, there were 32 bacterial isolates observed from macroscopic observers and by biochemical test.*

Keywords: *Grecek kepok banana, Rhizosphere, Fungi, Bacteria*

Received: September 26, 2026 ; Accepted: October 29, 2026 ; Published: November 20, 2026
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INTRODUCTION

The banana plant is the horticultural crop commodity with significant economic value for the Indonesian people. Banana plants are commonly found in various regions of Indonesia and have become one of the most popular fruits. Bananas can be consumed fresh or firstly processed. The uses of the banana plant are extensive and diverse, ranging from the trunk, leaves and the fruit itself. Bananas are fruits that grow in humid tropical regions.

The “Grecek Kepok” banana is a premier commodity native of East Kalimantan, particularly East Kutai Regency, which is in high demand in foreign markets. This “Grecek Kepok” banana variety sells exceptionally well in the domestic market and has been exported to various countries, such as Malaysia and Singapore. The Central Statistics Agency (BPS) reports that banana production in Indonesia reached 9.60 million metric tons in 2022. This figure represents a 9.79% increase compared to the previous year’s total of 8.74 million metric tons. Based on current data, banana production in East Kalimantan has already reached 113,000 metric tons.

According to the East Kalimantan Food, Food Crops, and Horticulture Agency (DPTPH), the Grecek Kepok banana market in East Kutai has secured a two-year contract to export Kepok bananas worth Rp 37.6 billion to Singapore. The area of Grecek Kepok banana plantations continues to expand. The DPTPH records that in 2021, the total area under Grecek Kepok banana cultivation was 1,700 hectares, with a monthly production of 1,340 metric tons. In 2022, the cultivated area increased to 1,820 hectares, with a monthly production of 1,650 metric tons. Marketing of Grecek Kepok bananas in both the domestic and export markets has also been quite stable. Sales of Grecek Kepok bananas in Sangatta reached 90 metric tons per month, in Samarinda 760 metric tons per month, in Bontang 120 metric tons per month, and in Balikpapan 350 metric tons per month.

Grecek Kepok bananas are also shipped to several regions outside East Kalimantan. These include Banjarmasin (124 metric tons per month), Bandung (60 metric tons per month), and Surabaya (72 metric tons per month). Export data for 2022 also shows a steady increase in Grecek Kepok banana sales. From March through June 2022, a total of 340 metric tons of Grecek Kepok bananas were exported to Malaysia. One of the places where the Grecek Kepok banana thrives is in Kaliorang Village, Kaliorang Subdistrict,

East Kutai Regency. The plant's vitality is influenced by several factors, one of the factor is the microorganisms found in the rhizosphere, the area surrounding the roots, which play a vital role in maintaining the roots.

The rhizosphere is the portion of soil surrounding a plant that supports microbial activity in sustaining the plant's development and growth. The rhizosphere contains carbon and amino acids, which are essential for the survival of microorganisms. Rhizosphere microbes play a role in various processes, such as nutrient cycling, soil formation, plant growth, and other microbial activities. The high banana production in Indonesia prompted this research to identify the fungi and bacteria microbe present in the rhizosphere of Grecek Kepok banana plants. Research on the identification of fungi and bacteria in the rhizosphere has been extensively conducted on various plants; based on the above description, this research will isolate and identify the fungi and bacteria in the rhizosphere of Grecek Kepok banana plants.

LITERATURE REVIEW

A. Banana Plant

Indonesia is one of the primary centers of banana diversity, including fresh, processed, and wild bananas. There are more than 200 types of bananas in Indonesia. The high diversity of bananas in Indonesia offers benefits and allows for the selection of commercial banana varieties needed by consumers (Mudita, 2012). Bananas are a commodity with promising prospects, as they are favored by the majority of the world's population. Indonesia's banana production in 2022 totaled 9.60 metric tons, an increase of 9.79% compared to the previous year's production of 8.74 million metric tons. Based on current data, banana production in East Kalimantan has already reached 113 thousand metric tons (Badan Pusat Statistik [BPS], 2022).The banana plant is also known as a giant perennial herb; it is called this because the offshoots that grow from the corm (rhizome) will eventually replace the parent plant, these offshoots grow without having to wait for the parent plant to die first, eventually forming a clump of banana plants (Mudita, 2012). Bananas are a type of climacteric fruit, meaning they experience a rapid increase in respiratory rate after being harvested or picked from the tree. This increase in respiratory rate is marked by various physical and chemical changes, including changes in color, texture, carbohydrate content, total sugar content, and total acidity. An increase in the

respiratory rate of climacteric fruits indicates the onset of the ripening process (Mudita, 2012).

The kepok banana is a somewhat flat and square-shaped banana, because of its flat shape, some people call it the “flat banana.” The fruit is small, measuring 10–12 cm in length and weighing 80–120 g. Its peel is very thick, greenish-yellow in color, and sometimes has brown spots. The kepok banana has high commercial value as a processed banana. There are two well-known varieties of kepok bananas in Indonesia: the yellow kepok banana and the white kepok banana. They differ in color and flesh. The flesh of the yellow kepok banana is yellowish, while that of the white kepok banana is paler. The yellow kepok has a sweeter taste, while the white kepok is more tart. The industry cultivates kepok bananas for flour, chips, vinegar, beer, and puree (Rumpis, 2011).

B. Microbes in the plant rhizosphere

Soil is home to a wide variety of organisms, ranging from microscopic to macroscopic in size. These microorganisms play a vital role in the cycles that occur within the soil. In addition, soil serves as a growing medium for plants; thus, the relationship between plants and soil organisms is crucial to the plants’ survival. Consequently, the population of soil organisms is determined by the quality of the vegetation above it (Sylvia et al., 2005).The rhizosphere is the soil within the root zone that is still influenced by root activity and serves as a favorable habitat for the growth of soil microorganisms (Astiriani et al., 2014). The term “rhizosphere” is used to refer to the portion of the soil that influences plant roots, characterized by a higher level of microbiological activity compared to soil areas farther from the root zone (Sumarsih, 2003).

Living organisms that are very small in size are called microbes, microorganisms, or microscopic organisms. These microscopic organisms are referred to as microbes not only because of their small size, which makes them invisible to the naked eye, but also because their way of life and adaptations are simpler compared to those of higher-order organisms. The human eye cannot see organisms measuring 0.1 mm because they are so small. The size of microbes is generally expressed in microns (μ), where 1 micron is equivalent to 0.001 mm. To observe such tiny microbes, one must use a magnifying device or a microscope (Ilyas, 2001). Fungi are a group of microorganisms that have been shown to induce plant resistance to various diseases, including both soil-borne and airborne diseases (Hyakumachi & Kubota, 2003). Fungi that inhabit the plant rhizosphere

and live on plants as symbionts are known as endomycorrhizal and ectomycorrhizal fungi. Almost every plant species has different types of endophytic fungi, resulting in a high range of biodiversity (Tanaka et al., 1999). Fungi generally form mutualistic symbiotic relationships with their host plants. These fungi provide benefits to their host plants, including increased growth rates and resistance to pests, diseases, and drought. Among soil fungal species, some are beneficial to plants, while others act as plant pathogens (Worosuryani et al., 2006).

Some types of soil bacteria found around plants include Rhizobium sp., Bradyrrhizobium japonicum, Frankia, and cyanobacteria. Through this symbiotic association, the bacteria obtain a better source of energy and a more favorable growing environment, while the plants receive nitrogen (N) and growth factors; thus, both parties benefit from this association. Rhizobium and Bradyrrhizobium japonicum have low tolerance for stress caused by low pH in highly weathered soils but adapt quite well (Khaeruni et al., 2010). There have been numerous studies on the identification of microbes in the rhizosphere of plants. In a study conducted in Pendem Village, Penden Subdistrict, Jembrana Regency, at a banana plantation, Streptomyces bacteria were identified as a means to control bacterial wilt in banana plants. The study identified and isolated nine Streptomyces strains: Streptomyces sp. 1, Streptomyces sp. 2, Streptomyces sp. 3, Streptomyces sp. 4, Streptomyces sp.5, Streptomyces sp.6, Streptomyces sp.7, Streptomyces sp.8, and Streptomyces sp.9 in the rhizosphere of healthy banana plants (Kawuri, 2016). Additionally, a study was conducted on the isolation and identification of fungi in banana plant soil, yielding 12 rhizosphere fungal isolates. The rhizosphere fungi successfully identified macroscopically and microscopically belong to the genera Penicillium sp., Trichoderma sp., and Aspergillus sp. Previous studies served as references for this research on the identification of microorganisms in the rhizosphere of Kepok Grecek banana plants (Anggraeni & Usman, 2015).

RESEARCH METHODS

This study was conducted from August 2023 to February 2024. Samples were collected in Bangun Jaya Village, Kaliorang Subdistrict (coordinates: Lat 0.868650, Long 117.886548), and the research was conducted at the Laboratory of Plant Pests and Diseases, Faculty of Agriculture, Mulawarman University.

The equipment used in this research included a microscope, Petri dishes, Erlenmeyer flasks, glass cover, glass object, microscope slides, ose needles, an inoculating loop, a measuring cylinder, a Bunsen burner, an autoclave, a dissecting knife, plastic cling wrap, an Opti-Lab, cotton swabs, scissors, labels, a UV lamp, test tubes, writing utensils, a camera, Whatman paper, aluminum foil, and a vortex mixer. The materials used in this research included Potato Dextrose Agar (PDA) medium, Nutrient Agar (NA), King's B medium, Pikovskaya medium, 70% alcohol, distilled water, denatured alcohol, cucumber seeds, agar powder, 2% hypochlorite solution, 3% KOH solution, and Gram stain solution.

Soil samples were taken from the rhizosphere surrounding healthy plants, diseased plants, and recovering plants. The samples were then taken to the laboratory for dilution and isolation on PDA medium for fungi and NA medium for bacteria. The fungi isolated from the samples were then purified for identification based on macroscopic and microscopic characteristics. Bacterial identification was performed by observing macroscopic and microscopic forms and conducting several biochemical tests, including Gram staining, the 3% KOH test, the hypovirulence test, the fluorescent pigment test, and the phosphate test.

RESULTS AND DISCUSSION

The isolation results of 30 rhizosphere soil samples using PDA medium yielded 6 genera of fungi, namely Trichoderma sp., Penicillium sp., Fusarium oxysporum, Colletotrichum sp., Mucor sp., and Aspergillus sp. (A. flavus and A. niger). The isolation of samples using NA medium yielded 32 bacterial isolates.

Table 1. Diversity of Fungal Genera and Species Isolated from the Rhizosphere of Grecek Kepok Banana Plants at Different Health Conditions

Sample	Fungi Genus	Fungi Species
Healthy Plants	Trichoderma sp	-
	Aspergillus sp	Aspergillus niger
	Penicillium sp	-
Diseased Plants	Trichoderma sp	-
	Fusarium sp	Fusarium oxysporum
	Colletotrichum sp	-
	Aspergillus sp	Aspergillus flavus
Recovering Plants	Trichoderma sp	-
	Aspergillus sp	Aspergillus niger
	Penicillium sp	-
	Mucor sp	-
	Colletotrichum sp	-
	Aspergillus sp	Aspergillus flavus

A. *Penicilium sp.*

The research results revealed a greenish-yellow *Penicillium sp.* fungus. According to Singh and Mathur, the macroscopic characteristics of *Penicillium sp*. colonies include rapid growth, a flat, filamentous appearance, and a texture resembling velvet, wool, or cotton. The colonies are initially white and turn bluish-green, grayish-green, olive gray, and occasionally yellow or reddish. When the culture in the Petri dish is inverted, it is usually pale yellow. Meanwhile, the microscopic characteristics of *Penicillium sp.* include hyaline hyphae, round, unicellular conidia, and a cluster of phialides. This is consistent with the findings of Singh et al., who noted that *Penicillium sp*. has the following microscopic characteristics, those are : hyaline (translucent) hyphae; conidiophores that emerge erect from the mycelium and branch near their tips; and a cluster of phialides at the tip of each conidiophore. *Penicillium sp.* also produces round, unicellular conidia.

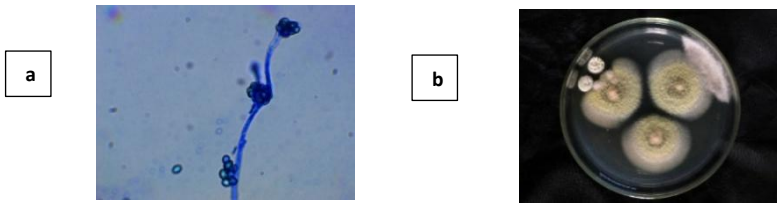


Figure 1. (a) Microscopic characteristics of Penicilium sp., (b) Macroscopic characteristics of Penicilium sp.

B. *Aspergillus sp*

1. *Aspergillus flavus*

Aspergillus flavus has colonies that range in color from green to yellowish-green, with a granular and compact shape. Young colonies are white and turn yellowish-green after forming conidia. Examination of A. flavus reveals vesicles that are round to oval in shape. The conidia are round and 3–6 µm in diameter, and the conidiophores are long and cylindrical.

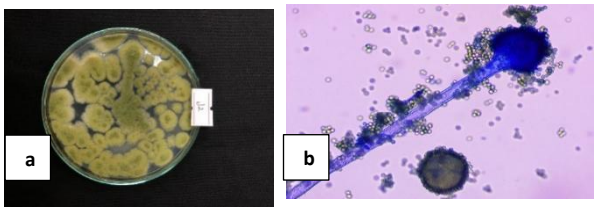


Figure 2. (a) Microscopic characteristics of *Aspergillus flavus*., (b) Macroscopic characteristics of *Aspergillus flavus*

2. *Aspergillus niger*

Aspergillus niger is characterized by blackish-white spores, and the color intensity increases as the culture ages. The colony surface is raised with a smooth texture on PDA medium. A. niger is characterized microscopically by round vesicles with diameters ranging from 17.52 to 23.4 µm. On the surface of the vesicles are sterigmata followed by phialides, which contain the conidia. The conidia are spherical with diameters ranging from 3.5 to 4.5 µm. The conidiophores are long, cylindrical, and colorless (hyaline).

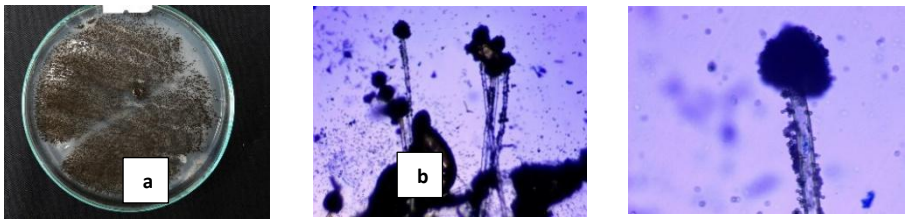


Figure 3. (a) Microscopic characteristics of *Aspergillus niger* (b) Macroscopic characteristics of *Aspergillus niger*

C. *Fusarium sp*

Macroscopic observations show that *Fusarium sp.* forms white colonies. In isolates that produce large numbers of sporodochia, *Fusarium sp.* colonies form conidia on monofialid conidiophores that are long and unbranched, or on monofialid conidiophores that are branched.

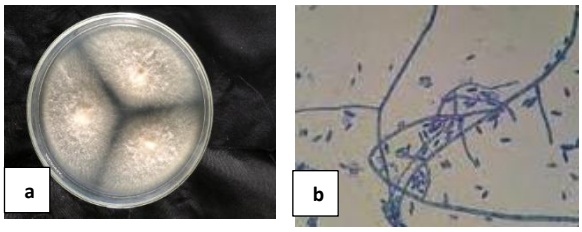


Figure 4. (a) Microscopic characteristics of *Fusarium sp.*, (b) Macroscopic characteristics of *Fusarium sp*

D. *Colletotrichum sp.*

Colletotrichum sp has grayish-white mycelium. On PDA medium, mature colonies exhibit black spots on their surface. The fungus Colletrotrichum sp. has the following microscopic features: cylindrical conidia measuring 7–14 µm in length and 3–5 µm in width; the spores are hyaline and non-septate. The hyphae of this fungus are non-septate.

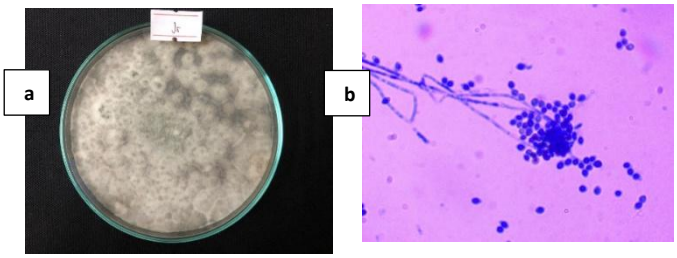


Figure 5. (a) Microscopic characteristics of *Colletotrichum sp*., (b) Macroscopic characteristics of *Colletotrichum sp*

E. *Trichoderma sp.*

The results of the research showed that the colonies were green in color and had highly branched hyphae that formed right angles at the main branches; identification results indicated that the isolates exhibited characteristics typical of Trichoderma sp. colonies. The color of *Trichoderma sp.* colonies can range from yellowish to yellow, white, and green. These colonies are typically compact or flocculent, a trait related to the growth of their conidiophore structures. Some colonies form a distinct, characteristic ring-like zone.

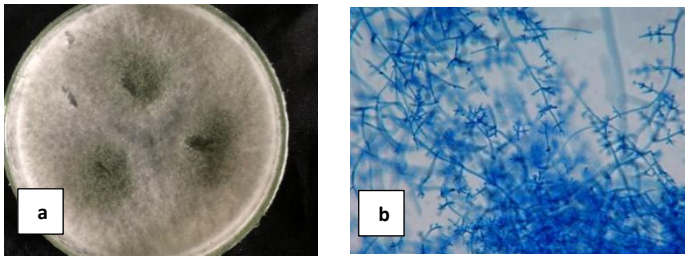


Figure 6. (a) Microscopic characteristics of *Trichoderma sp*., (b) Macroscopic characteristics of *Trichoderma sp*

The phialides are mostly bottle-shaped. In culture, Trichoderma sp. colonies grow rapidly. The mycelium is hyaline, highly branched, and forms right angles at the main branches, with soft-walled hyphae. Trichoderma sp. is a soil fungus that acts as an antagonist against plant-pathogenic fungi. This fungus can be found in nearly all soil types and in various habitats. Additionally, it can grow rapidly in the root zone.

F. *Mucor sp*

This genus grows relatively quick on PDA medium, as evidenced by the numerous and long aerial hyphae of the Mucor isolate, which fill the Petri dish and extend all the way to the bottom of the petri dish. Several species of the genus Mucor can sporulate at temperatures ranging from 5° to 20° to 30°C. This is consistent with the results of this research, in which the genus Mucor sporulated rapidly when grown on PDA medium incubated at room temperature. After purification on PDA medium, Mucor sp. by the 6th day, the Petri dish was filled with numerous, dense aerial hyphae; the colony surface was initially white with black aerial hyphae tips, then turned gray with black aerial hyphae tips. Microscopic observations revealed that the colonies had non-septate, bluish-hyaline hyphae; thick-walled sporangiophores with a columella; and spores that were round, oval, elliptical, ovoid, subround, and obovoid.

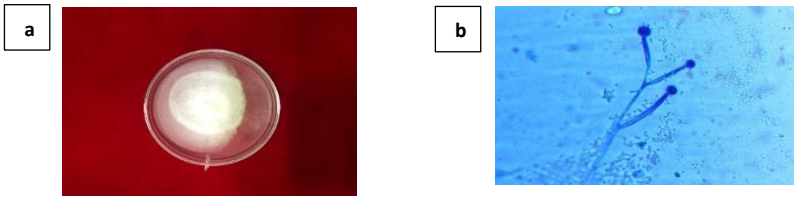


Figure 7. (a) Microscopic characteristics of Trichoderma sp., (b) Macroscopic characteristics of Trichoderma sp

G. Characterization of Bacterial Isolates

The results of macroscopic observations of 32 bacterial isolates from Grecek Kepok banana

Table 2. Macroscopic Characteristics of Bacterial Isolates from the Rhizosphere of Grecek Kepok Banana Plants

No.	Sample Code	Elevation	Shape	Color	Margin	Size
1	TH1a	Convex	Point	Yellowish	Entire	Small
2	TH1b	Raised	Point	White	Entire	Small
3	TH2a	Flat	Irregular	White	Entire	Large
4	TH2b	Flat	Root-like	White	Root-like	Small
5	TH4c	Convex	Point	Yellowish	Entire	Small
6	TH5a	Convex	Round	White	Entire	Small
7	TH6a	Raised	Round	White	Entire	Small
8	TH9a	Raised	Round	White	Entire	Small
9	TH10b	Raised	Point	White	Filamentous	Small
10	TSK1b	Flat	Coiled	White	Undulate	Large
11	TSK3b	Flat	Round	Yellowish	Filamentous	Small
12	TSK4a	Flat	Round	White	Serrated	Small
13	TSK2c	Raised	Round	White	Undulate	Small
14	TSK2d	Convex	Irregular	Yellowish	Entire	Small
15	TSK2a	Convex	Irregular	Yellowish	Undulate	Small
16	TSK4c	Raised	Point	White	Entire	Small
17	TSK5a	Flat	Coiled	White	Entire	Large
18	TSK5c	Raised	Point	White	Entire	Small
19	TSK6a	Flat	Point	White	Entire	Large
20	TSK6b	Flat	Coiled	Yellowish	Entire	Small
21	TSK7a	Raised	Irregular	Yellowish	Entire	Medium
22	TSK9a	Flat	Coiled	White	Entire	Large
23	TSK9b	Raised	Irregular	Yellowish	Filamentous	Large
24	TSK10b	Raised	Round	Yellowish	Filamentous	Small
25	TP1b	Convex	Irregular	Yellowish	Filamentous	Medium
26	TP2a	Flat	Irregular	Yellowish	Filamentous	Large
27	TP2b	Raised	Round	White	Entire	Medium

No.	Sample Code	Elevation	Shape	Color	Margin	Size
28	TP5a	Raised	Coiled	White	Entire	Medium
29	TP5b	Flat	Coiled	Yellowish	Wavy	Medium
30	TP8a	Flat	Coiled	White	Entire	Medium
31	TP8b	Flat	Coiled	White	Wavy	Medium
32	TP9a	Raised	Irregular	White	Entire	Large

Description : TH = Healthy Plant, TSK = Diseased Plant, TP = Recovering Plant

The microscopic observations and biochemical tests were conducted to further characterize the 32 bacterial isolates obtained from the rhizosphere of Grecek Kepok banana plants. The identification process included cell morphology observations, Gram staining, the 3% KOH test, and fluorescent pigment testing to determine the basic characteristics of each bacterial isolate. The results of these observations and tests are presented in Table 3.

Table 3. Microscopic Characteristics and Biochemical Test Results of Bacterial Isolates from the Rhizosphere of Grecek Kepok Banana Plants

Sample Code	Cell Shape	Gram Test	KOH Test	Pigmen Flourescent Test
TH1a	Basil	-	-	+
TH1b	Basil	-	+	-
TH2a	Basil	-	-	+
TH2b	Basil	-	-	-
TH4c	Cocus	-	+	-
TH5a	Basil	-	-	-
TH6a	Basil	-	+	+
TH9a	Basil	-	-	-
TH10b	Cocus	+	+	-
TSK1b	Basil	+	-	-
TSK3b	Basil	+	-	+
TSK4a	Cocus	+	+	-
TSK2c	Basil	-	+	-
TSK2d	Cocus	+	-	-
TSK2a	Cocua	-	+	+
TSK4c	Cocus	+	+	+
TSK5a	Basil	-	-	-
TSK5c	Cocus	+	-	-
TSK6a	Basil	-	-	-
TSK6b	Basil	-	+	+
TSK7a	Cocus	+	-	-
TSK9a	Basil	-	+	-
TSK9b	Cocus	+	+	+
TSK10b	Cocus	-	-	+
TP1b	Cocus	-	+	-
TP2a	Cocus	+	+	-
TP2b	Basil	-	-	-
TP5a	Basil	-	+	-
TP5b	Cocus	-	+	+
TP8a	Basil	-	-	-
TP8b	Cocus	-	+	+
TP9a	Cocus	-	-	-

1. Cell Shape

Microscopic observations revealed that the bacterial isolates were cocci and bacilli. There were 15 isolates with cocci-shaped cells and 17 isolates with bacilli-shaped cells. Cocci-shaped bacterial cells are round, while bacilli-shaped cells are rod-shaped or capsule-shaped.

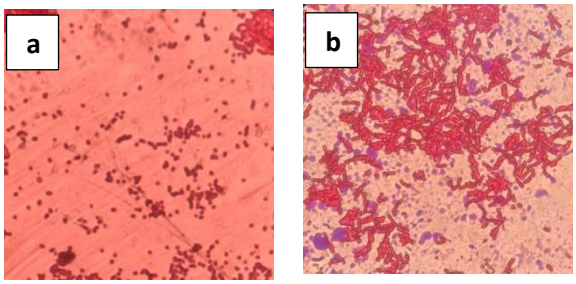
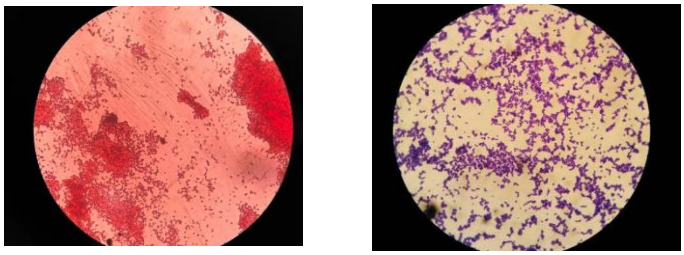


Figure 8. (a) Cocci-shaped bacterial cells, (b) Bacilli-shaped cells

2. Gram Staining

Gram staining performed on 32 bacterial isolates revealed blue or purple and red colors, indicating that these 32 isolates included both Gram-positive and Gram-negative bacteria. Gram-negative bacterial isolates were found in 22 isolates, including TH1a, TH1b, TH2a, TH2b, TH4c, TH5a, TH6a, TH9a, TSK2c, TSK2a, TSK5a, TSK6a, TSK6b, TSK9a, TSK10b, TP1b, TP2b, TP5a, TP5b, TP8a, TP8b, and TP9a. Gram-positive bacteria were found in 10 isolates, including TH10b, TSK1b, TSK3b, TSK4a, TSK2d, TSK4c, TSK5c, TSK7a, TSK9b, and TP2a.

The blue or purple color observed in Gram-positive bacteria that retain the stain is due to crystal violet, while the reddish color in Gram-negative bacteria is due to their absorption of the safranin solution. Gram-positive bacteria are those that retain the methyl violet stain during the Gram staining process. These bacteria appear blue or purple under a microscope. Meanwhile, Gram-negative bacteria appear red or pink because they do not retain the methyl violet dye during the Gram staining process. The difference in classification between these two types of bacteria is primarily based on differences in the structure of their cell walls. Gram-positive bacteria have cell walls composed largely of peptidoglycan. Gram-negative bacteria, on the other hand, contain less peptidoglycan but have a more complex structure, with their outermost layer composed of lipopolysaccharides (chains of carbohydrates and lipids). The outer membrane of Gram-negative bacteria protects them against cell damage.



a b

Figure 9. (a) gram-negative test result , (b) gram-positive test result

3. KOH3% Test

Bacterial testing using a 3% KOH solution yielded 16 negative isolates, which were characterized by the formation of slime, whereas the positive isolates did not form slime. Isolates that formed slime or negative were found in TH1a, TH2a, TH2b, TH5a,TH9a, TSK1b, TSK3b,TSK2d, TSK5a, TSK5c, TSK6a, TSK7a, TSK10b, TP2b, TP8a, dan TP9a. The 3% KOH test may yield different results from the Gram stain; this occurs because applying too much or too little KOH solution can affect the visibility of mucus on the isolate.

Gram-positive bacteria have thick cell walls and a thin lipid layer, while Gram-negative bacteria have a thick lipid layer and thin cell walls; consequently, KOH penetrates the lipid layer and causes the Gram-negative bacteria to rupture. The rupture of the cell is caused by the release of genetic material, very long DNA molecules that form sticky strings (resembling mucus).

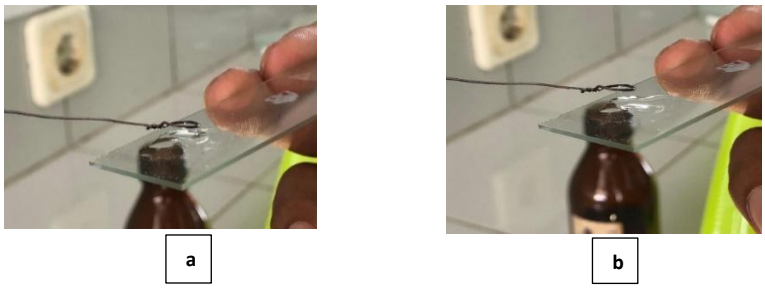


Figure 10. (a) Negative KOH test result , (b) Positive KOH test result

4. Hipovirulence Test

Table 4. Virulence Assessment of Bacterial Isolates

Sample Code	DSI Value (Disease Severity Index)	Description
TH1a	0,25	Hipovirulence
TH1b	0,19	Hipovirulence
TH2a	1,22	Moderate
TH2b	0	Hipovirulence
TH4c	0,57	Low Virulence
TH5a	0,56	Low Virulence
TH6a	0	Hipovirulence
TH9a	0	Hipovirulence
TH10b	0,44	Hipovirulence
TSK1b	0,44	Hipovirulence
TSK3b	1,12	Moderate
TSK4a	0,62	Low Virulence
TSK2c	0,19	Hipovirulence
TSK2d	0	Hipovirulence
TSK2a	0,25	Hipovirulence
TSK4c	0,44	Hipovirulence
TSK5a	0,19	Hipovirulence
TSK5c	0	Hipovirulence
TSK6a	0,13	Hipovirulence
TSK6b	0,44	Hipovirulence
TSK7a	0,44	Hipovirulence
TSK9a	0,56	Low Virulence
TSK9b	0,72	Low Virulence
TSK10b	0	Hipovirulence
TP1b	0,25	Hipovirulence
TP2a	1,24	Moderate
TP2b	0	Hipovirulence
TP5a	0,44	Hipovirulence
TP5b	0,13	Hipovirulence
TP8a	0,44	Hipovirulence
TP8b	0,25	Hipovirulence
TP9a	0	Hipovirulence

Description : TH = Healthy Plants, TSK = Diseased Plants, TP = Recovering Plants; DSI 0-0,4 = Hipovirulence, DSI 0,5-0,9 = Low Virulence, DSI 1-1,9 = Moderate, DSI 2-2,9 = Virulence, DSI 3-4 = Highly Virulence

Virulence testing of 32 bacterial isolates revealed that 24 isolates were hypovirulent, 5 were low-virulence, and 3 were moderate-virulence. The DSI values ranged from 0 to 1.24. The lowest DSI values were observed in the hypovirulent isolates TH2b, TH6a, TH9a, TSK2d, TSK5c, TSK10b, TP2b, and TP9a. The highest DSI values were observed in the moderately virulent isolates TH2a, TSK3b, and TP2a.

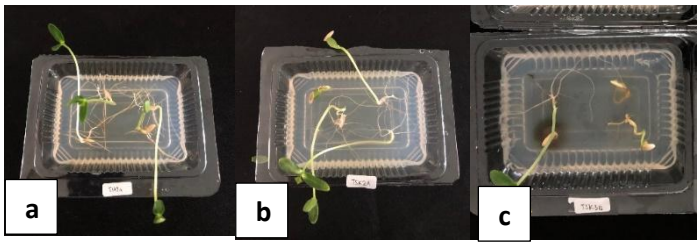


Figure 11. (a) Hypovirulence test results showing hypovirulence in isolate TH9a; (b) Hypovirulence test results showing low virulence in isolate TSK2a; (c) Hypovirulence test results showing moderate virulence in isolate TSK3b

5. Fluorecent Pigment Test

A fluorescent pigment test of 32 bacterial isolates revealed that 11 isolates produced bacteria that fluoresced (positive) and 21 isolates did not fluoresce (negative) when viewed under UV light. A positive reaction in bacteria that fluoresce under UV light indicates that these bacteria belong to the genus Pseudomonas. In a research by Schaad et al. (2001), the presence of fluorescent pigments after the bacteria were grown on King's B medium, as indicated by fluorescence under UV light, showed that the bacteria belonged to the genus Pseudomonas. Positive results, or fluorescence, from the fluorescent pigment test on King's B medium were observed in the isolates TH1b, TH2b, TH4c, TH5a, TH9a, TH10b, TSK1b, TSK4a, TSK2c, TSK2d, TSK5a, TSK5c, TSK6a, TSK7a, TSK9a, TP1b, TP2a, Tp2b, TP5a, TP8a, and TP9a.

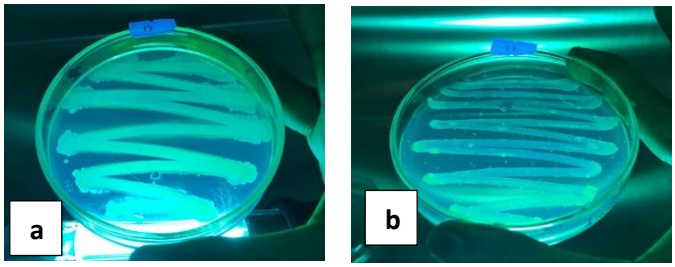


Figure 12. (a) Results of the fluorescent pigment test on Kings B medium for isolate TSK5a, which yielded a positive result; (b) Results of the fluorescent pigment test on Kings B medium for isolate TSK9a, which yielded a negative result.

6. Phosphate Test

Table 5. Phosphate Assay Results for 32 Phosphate-Solubilizing Bacterial Isolates from the Rhizosphere of Grecek Kepok Bananas

Sample Code	DZB (mm)	DK (mm)	IKF
TH1a	3	0,5	6,4
TH1b	0	0,3	1
TH2a	1,8	0,5	4,4
TH2b	1,8	0,7	3,3
TH4c	1,1	0,3	4,1
TH5a	0,9	0,5	2,7
TH6a	2,1	0,6	4,5
TH9a	0	0,4	1
TH10b	0,5	1,7	1,3
TSK1b	1,5	0,6	3,5
TSK3b	1,2	0,4	4
TSK4a	0,7	0,4	2,8
TSK2c	1,2	0,3	4,2
TSK2d	0,7	2,1	1,3
TSK2a	2	0,6	4,2
TSK4c	1	0,2	5
TSK5a	1,3	0,7	2,7
TSK5c	1,4	0,8	2,6
TSK6a	0,6	0,7	1,8
TSK6b	0,9	0,3	4,1
TSK7a	0	0,3	1
TSK9a	1,5	0,4	4,8
TSK9b	2,2	0,4	5,8
TSK10b	1,1	0,4	3,5
TP1b	0	0,4	1
TP2a	0,5	0,7	1,6
TP2b	0,3	3	1,1
TP5a	0,8	0,5	2,6
TP5b	0,9	0,4	3,3
TP8a	1,3	0,5	3,6
TP8b	0	0,3	1
TP9a	0,6	0,3	2,7

Description : TH = Healthy Plants, TSK = Diseased Plants, TP = Recovering Plants, DK = Colony Diameter, DZB = Clear Zone Diameter, IKF = Phosphat Solubility Index

The phosphate test conducted on 32 bacterial isolates revealed that they possess the ability to dissolve phosphate. Bacteria capable of dissolving phosphate are characterized by the appearance of a halozone, a clear zone surrounding the bacterial colony. The PhS Index (Phosphate Solubility Index) values for the bacterial isolates ranged from 1 to 6.4. The highest PhS Index was observed in isolate TH1a, while the lowest values were found in isolates TH9a, TSK7a, TP1b, and TP8b. According to Situmorang et al., the higher the enzymatic activity produced by phosphate-solubilizing bacteria, the larger the clear zone formed. The clear zone forms as a result of insoluble phosphate being converted into a

soluble form by phosphate-solubilizing bacteria. This occurs because these bacteria produce phosphatase enzymes. Phosphatase enzymes are a group of enzymes that catalyze enzymatic hydrolytic mineralization reactions, releasing insoluble phosphate into a soluble form.

Results of the phosphate test on isolates TSK2d, TSK6a, TP8a, TSK10b, and TSK9b that formed a halozone or clear zone.

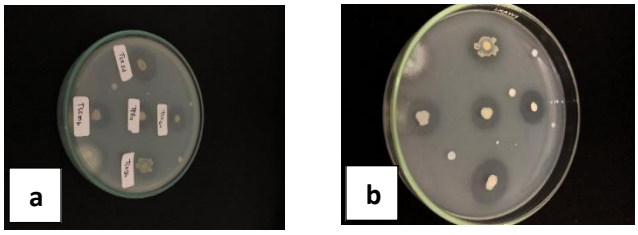


Figure 13. Phosphate Solubilization Activity of Bacterial Isolates Showing Clear Zone (Halozone) Formation on Pikovskaya Medium

7. Plant Growth Promoting Rhizobacteria Test (PGPR)

Table 6. Effects of Bacterial Isolates on Seedling Growth in the PGPR Test

Sample Code	Heading Length	Roots Length	Heading and Roots Length
KONTROL	5,22 b	2,92 b	7,68 b
TH1a	6,83 a	3,58 a	10,41 a
TH1b	0,42 g	0,42 f	1,33 fgh
TH2a	0,39 g	0.39 f	0,79 h
TH2b	1,14 efg	1,08 cd	2,16 efg
TH4c	0,28 g	0,59 ef	0,86 h
TH5a	0,67 fg	0,60 ef	1,26 fgh
TH6a	1,70 cde	1,16 cd	2,29 cde
TH9a	2,02 cde	1,29 c	3,3 cd
TH10b	2,13 cd	1,21 c	3,35 cd
TSK1b	7,02 a	3,17 ab	9,79 a
TSK3b	6,31 a	3,46 a	9,78 a
TSK4a	1,76 cde	1,07 cd	2,90 cde
TSK2c	0,68 fg	0,48 ef	1,16 gh
TSK2d	1,52 def	0,79 def	2,31 def
TSK2a	1,50 def	0,87 cde	2,33 def
TSK4c	2,40 c	1,15 cd	3,55 c
TSK5a	0,86 ef	1,54 c	3,40 d
TSK5c	6,61 b	1,02 d	1,82 efg
TSK6a	6,58 b	3, 66 a	10,15 a
TSK6b	0,94 ef	3,42 a	9,90 a
TSK7a	7,81 a	0,92 d	1,86 efg
TSK9a	0,37 f	3,78 a	11,59 a
TSK9b	0,56 ef	0,41 ef	0,78 fg
TSK10b	0,45 ef	0,65 def	1,13 efg
TP1b	0,32 f	0,60 def	1,06 efg
TP2a	0,50 ef	0,37 f	0,68 g
TP2b	0,27 f	0,60 def	1,11 efg
TP5a	0,66 ef	0,38 f	0,62 g
TP5b	1,10 ef	0,63 def	1,30 efg
TP8a	0,31 f	0,93 d	1,99 ef

Sample Code	Heading Length	Roots Length	Heading and Roots Length
TP8b	1,35 e	0,60 def	0,88 fg
TP9a	2,40 d	0,88 de	2,23 de

Based on the results of the analysis of 32 bacterial isolates in the PGPR test, TSK9a and TH1b showed significant differences compared to the control treatment of TH1b, TH2a, TH2b, TH4c, TH5a, TH6a,TH9A, TH10b, TSK4a, TSK2c, TSK2d, TSK2a, TSK4c, TSK5a, TSK5c, TSK6a, TSK6b, TSK7aTSK9b, TSK10b, TP1b, TP2a, TP2b, TP5a, TP5b, TP8a, TP8b, and TP9a, however, there is no significant difference compared to TSK1b, and TSK3b. To TH9a, TH10b, TSK4c, TSK5a showed significant differences compared to the control treatment of TH1b, TH2a, TH2b, TH4c, TH5a, TSK2c, TSK5c, TSK7a, TSK9b, TSK10b, TP1b, TP2a, TP2b, TP5a, TP5b, TP8a, TP8b, however there is no significant difference compared to TH6a, TSK4a, and TP9a. To TSK2c and TSK2a showed significant differences compared to the control treatment of TH1b,TH2a, TH5a, TSK2c, however, there is no significant differences compared to TH2b, TSK5c, TSK7a, TSK9b, TSK10b, TP1b, TP2a, TP2b, TP5a, TP5b, TP8b. The results of the PGPR test showed that isolates TH1a, TSK6a, TSK6b, and TSK7a exhibited good growth, while isolates TH2a and TH4c exhibited low growth rates.



Figure 14. (a) The Result of PGPR Test in Control (b) The Results of PGPR Test that isolates TSK6b (c) The Result of PGPR that isolates TH2a

CONCLUSION

1. Isolation and identification of fungi in the rhizosphere of *Grecek Kepok* banana plants revealed 8 fungal genera, including *Trichoderma sp., Aspergillus niger, Penicillium sp., Fusarium oxysporum, Colletotrichum sp., Aspergillus flavus,* and *Mucor sp*.
2. Isolation and identification of bacteria in the rhizosphere of *Grecek Kepok* banana plants revealed 32 bacterial isolates. Several biochemical tests were conducted,

including Gram staining, which identified 22 Gram-negative and 10 Gram-positive isolates; a 3% KOH test, which identified 16 negative and 16 positive isolates; the hypovirulence test revealed that 24 isolates were hypovirulent, 5 were virulent, and 3 were moderately virulent. The *fluorescent pigment* test showed 11 positive isolates and 21 negative isolates, while the phosphate test indicated that all 32 bacteria were capable of dissolving phosphate and producing a clear zone or *halozone*.

ACKNOWLEDGEMENTS

1. The Faculty of Agriculture, Mulawarman University, for financial support provided through the 2026 Fiscal Year Research Grant scheme.
2. The author would like to express gratitude and appreciation to all those who assisted with this research, including the advisor, colleagues, and laboratory technicians at the Plant Pests and Diseases Laboratory, who provided guidance and assistance in conducting this research.

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