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Exploring the biocontrol potential of soil bacteria isolated from North Sumatra, Indonesia, against pathogenic fungi

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Abstract. *Wahyuni S, Suryanto D, Nurtjahja K. 2025. Exploring the biocontrol potential of soil bacteria isolated from North Sumatra, Indonesia, against pathogenic fungi. Biodiversitas 26: 2039-2050.* The diversity of soil bacteria is very important in the ecosystem. Bacteria can function as biological control agents by exhibiting antagonistic activities and producing bioactive metabolite compounds that inhibit the growth of plant pathogens. This study aimed to determine the characteristics of various bacteria, test their pathogenicity, and inventory the production of metabolite compounds produced by these bacteria. Bacteria were isolated from soil sources of wood plant areas, bamboo, and agriculture in the Sibolangit area, North Sumatra, Indonesia. Various tests were carried out to obtain the morphological and biochemical characteristics of the isolates obtained, such as macroscopic, staining, triple sugar iron, catalase, starch, gelatin, and Gram. This research showed 20 isolates that could control pathogens with the highest inhibition value, namely isolate B6 with an inhibition value on *Fusarium* sp. (32.42 mm) and on *Colletotrichum* sp. (20.72 mm). Isolate SP1 showed an inhibition value on *Aspergillus niger* (28.32 mm), *Aspergillus flavus* (25.12 mm), *Fusarium* sp. (23.11 mm), and *Colletotrichum* sp. (20.23 mm). Identification of other metabolite compounds isolates B3, T4, and SP10 were able to dissolve phosphate, with inhibition zones of 15.2 mm, 14.3 mm, and 7.1 mm; chitinase activity was produced by species B6 with an inhibition zone of 10.3 mm, and IAA hormone activity was obtained with the highest concentration of 12.86 ppm and the lowest 2.89 ppm. Of the several bacterial isolates treated, species B6 and SP1 have good potential to be developed as biological control agents. Utilizing local natural resources is very important because local soil microorganisms have adapted to the environment and have a unique potential to fight pathogens.

Keywords: Chitinase activity, hormone activity, inhibition, metabolite compounds, phosphate solubilization

Abbreviations: IAA: Indole Acetic Acid; IP: Phosphate solubilization Index

INTRODUCTION

Global warming is characterized by climate change and extreme weather. Other changes can lead to rising sea levels and changes in soil salinity levels (Sembiring et al. 2020). Other impacts include reduced crop yields and degradation of agricultural land, such as severe droughts, high humidity levels, and an increased percentage of plant pests that can worsen the situation (Skendžić et al. 2021) and can also result in the development of more virulent pathogen strains, as well as detrimental impacts on crop productivity (Velásquez et al. 2018). Some pathogenic fungi are dangerous because they can attack plants from germination to mature plants. Like the ubiquitous *Aspergillus* species, these fungi can be found on various substrates, are distributed throughout the region, and can be associated with agricultural plant diseases and reported as plant pathogens. Another pathogen often found in agricultural fields is *Fusarium* sp., which can cause yield losses exceeding 50%. *Colletotrichum* sp. attack can also cause disease in around 80% to 100% of plants (Zakaria 2024).

Using chemical control to control pathogens has many negative effects, so biological control, or biocontrol using biological agents, is currently a promising alternative to disease control. Several antagonists are used in the field, including parasitoids, predators, nematodes, viruses, insects, and bacteria, and they are applied singly or in various combinations (Brodeur et al. 2017). Using natural antagonists, such as bacteria, as Biocontrol Agents (BCAs) has gained considerable attention recently as a sustainable approach to managing pests, pathogens, and diseases in agriculture (Lee et al. 2023). Bacteria, as biocontrol agents, have the potential to protect plants throughout their life cycle. Numerous strategies are employed by bacterial biocontrol agents to shield plants from pathogen infections. Through direct or indirect interactions with the pathogens, they may employ one or a combination of strategies to stop or lessen plant disease (Legein et al. 2020).

Some studies, such as those on rhizosphere bacteria of fluorescent *Pseudomonas* and *Bacillus* spp., are widely used as biological control agents for soil- and air-borne diseases. These microorganisms have several control mechanisms, including competition, hyperparasitism, producing microbial inhibitory compounds (antibiotics, lytic enzymes, and other physical or chemical interference), inducing plant resistance, and promoting plant growth (Ahemad and Kibret 2014; Comeau et al. 2021). As antagonists, bacteria can secrete various hydrolytic enzymes, including lipases, proteases, and chitinases, which lyse plant cell walls and prevent the proliferation of phytopathogens (Rahma et al. 2022). Furthermore, another role based on several studies (Liu et al. 2020) shows that bacteria on plants can produce metabolite compounds that play a role in plant development, stress response, and environmental adaptation. Through several processes, including nitrogen fixation, phosphate solubilization, phytohormone synthesis, biological control of pathogens, and increased nutrient uptake, bacteria support plant growth. Biological control of pathogens using biocontrol agents is a promising approach to protect crops, as it involves the production of nutrient-dissolving compounds and toxic substances and inducing plant resistance (Pandit et al. 2022). Farmers are increasingly interested in using biological agents due to their unique benefits, which include cost-effectiveness, environmental safety, and a balance of ecological stability.

These techniques that use local bacterial isolates are more likely to suppress pathogens in the area of origin and are more likely to survive than isolates transferred from other places (Larsson and Flach 2022). The development of antifungals that can be used as fungicides against certain fungi has taken center stage in the advancement of pest and disease control. Therefore, this study aimed to determine the characteristics of various bacteria, test their pathogenicity, and inventory the production of metabolite compounds produced by these bacteria. The novelty of this research lies in the local approach, identification of specific bacterial isolates that have strong biocontrol potential, and exploration of biochemical activities that support pathogen suppression and plant growth. This research makes a significant contribution in the field of biological control and sustainable agriculture, especially in the context of utilizing indigenous microbial resources. Local bacteria have significant potential to be developed as biological control agents; furthermore, previous studies did not emphasize the specific potential of local bacteria in the context of biological control and agricultural sustainability. In the study of Meza-Manzaneque et al. (2023), the research shows that unsustainable agricultural practices will be able to impact soil conditions and microbiological diversity. To restore the balance, environmentally friendly strategies will be an alternative. Research related to soil isolation will be able to provide hope for the application of soil bacteria in improving crop management and crop production.

MATERIALS AND METHODS

Study location

The samples were collected from Sibolangit Sub-district, Deli Serdang District, North Sumatra, Indonesia, which is located at coordinates 3° 16′ 46.69″ N, 98° 33′ 9.54″ E. This location is a highland with an altitude of 400-700 m above sea level, a 600-900 m slope, and an undulating hilly relief. The Sibolangit area has high plant diversity and composition; some plants include woody plants, bamboo, and agriculture. In addition, this area has a good soil structure that can be used to estimate rich nutrients and is suitable for good microbial ecology, often used for various purposes, such as research, education, and increasing awareness of environmental conservation. This location has become one of the destinations for studying the contribution and distribution of microorganisms in plant areas in this ecosystem because of the uniqueness of its unusual and extraordinary natural community. This is because the Sibolangit region of North Sumatra is an area whose natural conditions are still maintained, has a cool and humid climate, has a high diversity of vegetation, and has a variety of flora and fauna (Figure 1).

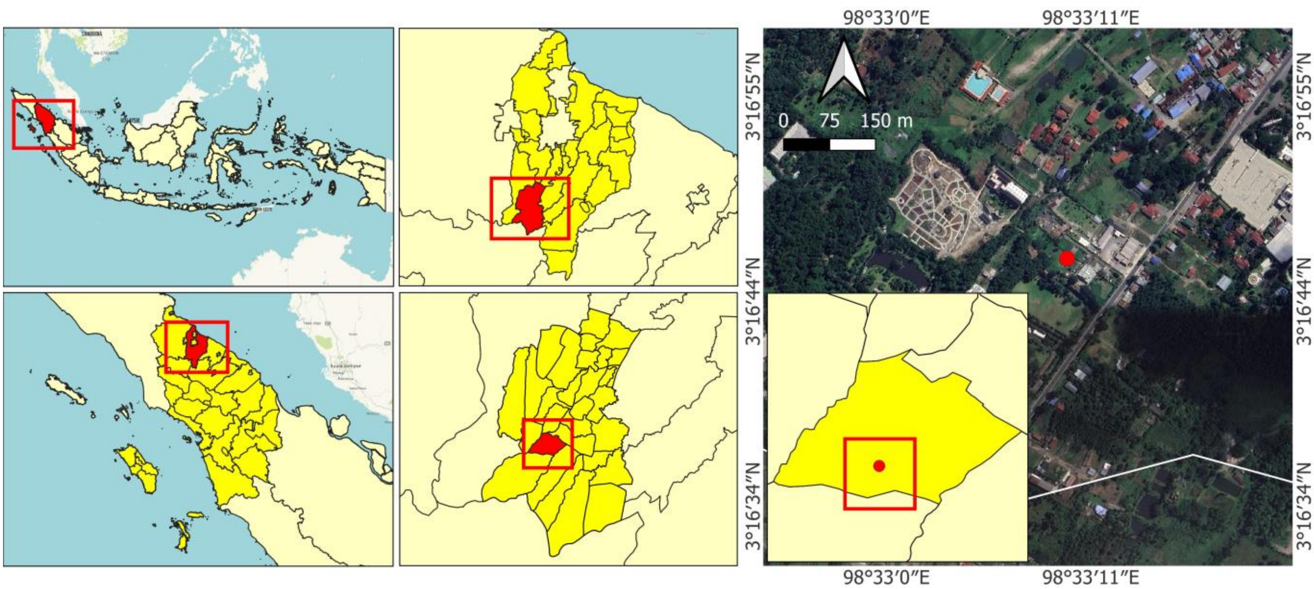


Figure 1. Location study in Sibolangit Sub-district, Deli Serdang District, North Sumatra, Indonesia

Procedures

Soil sample and isolation method

Soil sampling was conducted at three locations based on the production crop: agricultural crop, bamboo crop, and woody crop. Sampling was done randomly in three locations by taking soil in the area around plant roots with a depth of 0-20 cm, by digging in the area around the roots. Soil samples were taken in as much as 1 g of soil and replicated three times. The soil was diluted to a final dilution of 10^{-6} using the scattering method, then inoculated on Nutrient Agar and incubated for 24 hours at 37°C. After 24 hours, the isolated colonies were purified and separated using streaking. The isolation process aimed to separate and identify the diversity of a number of bacteria originating from the soil.

Characterization and identification

Staining is used to characterize and identify microbes and to differentiate between Gram-positive and Gram-negative bacteria. Staining shows the shape, edge, elevation and arrangement of bacteria. Biochemical tests such as catalase, Simon Citrate Agar (SCA), sugar fermentation (Triple Sugar Iron Agar)/TSIA, catalase, starch, and gelatin identify specific bacterial isolates. The SCA test was carried out by streaking one loop of pure isolate on citrate slant agar, then incubating at 37°C for 24-48 hours. The reaction is declared positive (+) if there is a change in the green color of the medium to blue. The reaction is declared negative (-) if there is no color change (Gebreyohans et al. 2024). Aseptically, the TSIA (Triple Sugar Iron Agar) test was carried out by inoculating bacteria into the TSIA medium. Then, it was incubated at 37°C for 24 hours. The reaction is declared positive if a yellow color and is declared negative if a red color (Saimin et al. 2020). The catalase test was done by making a smear on the object glass and then dripping 2 drops of 3% $H2O_2$. The reaction is positive if gas bubbles are formed due to H_2O_2 degradation due to catalase enzyme secretion (Khatoon et al. 2022). The starch test is carried out by inoculating bacteria into starch media at 37°C for 24 hours. After the bacteria grow, iodine/lugol solution is dropped around the bacterial colony; if a precise area is formed around the colony, there is starch hydrolysis due to amylase enzyme secretion (Algofar et al. 2021). The gelatin test is carried out by inoculating bacterial isolates on nutrient gelatin media at 37°C for 24 hours, then stored in an incubator at a temperature of 40°C for 30 minutes. Positive results indicate that gelatin remains liquid even though it is stored at 4°C (Nursyam et al. 2018).

Bacterial and fungal antagonist test

The dual culture method used in this study refers to Ramona (2021). Pathogenic fungal isolates *Aspergillus flavus*, cause of dry rot disease, *Aspergillus niger* cause of black mold disease, *Fusarium* sp. cause of *Fusarium* Wilt, and *Colletotrichum* sp. cause of anthracnose disease were isolated from infected chili plants. These pathogenic fungi in some studies can be pathogenic in chili plants because they have several mechanisms that can attack and damage plant tissues. Each bacterial isolates was tested using this test method to determine how antagonistic the bacterial isolate was to the growth of pathogenic fungi. Sabouraud Dextrose Agar (SDA) media was prepared, then the fungal sample was placed in the middle. With the point inoculation method, one loop of the intact bacterial culture was taken and put on a plate about 1 to 2 cm from the pathogen. To obtain the desired results, the culture was incubated for about 5 to 7 days at a temperature of 28°C.

Antibiosis compound production ability test

Chitinolytic bacteria were tested for their ability by inserting one dose of chitin-containing salt solution isolate with a media composition (CCA (Colloidal Chitin Agar) media consisting of (g/L): Na_2HPO_4 (6); KH_2PO_4 (3); NH_4Cl (1); NaCl (0.5); yeast extract (0.05); agar (15) and 0.5% (w/v) colloidal chitin). Then, the sample was incubated at 30°C for 48 to 72 hours. The chitinolytic bacterial isolates that grew would form colonies and form clear zones (halozones) around them (Gonfa et al. 2023).

The isolates were tested for proteolytic activity on the specific Skim Milk Agar (SMA) media. SMA media consists of 0.1% (w/v) peptone, 0.5% (w/v) NaCl, 2.0% (w/v) agar rods, and 10% (v/v) skim milk (Chu 2006). Isolates that had previously been inoculated on NB media, one ose was taken and inoculated on SMA media. The inoculum was then incubated at 37°C for ±24 hours (Muhiddin et al. 2024).

Qualitative metabolite production test

One ose of bacterial isolate was introduced into Pikovskaya medium (glucose 10 g; $Ca_3(PO_4)_2$ 5 g; $(NH_4)_2SO_4$ 0.5 g; NaCl 0.2 g; $MgSO_4.7H_2O$ 0.1 g; KCl 0.2 g; yeast extract 0.5 g; $MnSO_4.H_2O$ 0.002 g; $FeSO_4.7H_2O$ 0.002 g) to test bacterial phosphate solubilizing activity. The clear zone around the colony was observed, and the Phosphate solubilization Index (IP) was measured using an ose needle and incubated for 4 to 7 days at 37°C. The formula for calculating the Phosphate solubilization Index (IP) is:

$$IP = \frac{\text{clear zone diameter} - \text{colony diameter}}{\text{colony diameter}} \quad \text{...............(1)}$$

The culture separated from the soil sample is inoculated into the media. The plate is incubated for about five to seven days to obtain results (Prasanna et al. 2014). In Indole Acetic Acid (IAA) manufacture, bacterial cells are grown in broth media containing 100 g/mL tryptophan. To produce a strain capable of synthesizing auxin, the culture is incubated for two days at a temperature of 37°C. After 48 hours, centrifugation is carried out for 1 minute at a speed of 15000 rpm, and the supernatant is mixed into 2 mL of Salkowski reagent ($FeCl_3$ 1 mL, H_2SO_4 36 mL, Aquadest 63 mL). The mixture was left in the dark at room temperature for 30 minutes. Using a spectrophotometer at 530 nm, the absorbance of the resulting solution was compared with the control (Kamal et al. 2022).

The results of the analysis test if the pink color is visible; it shows positive IAA, while the yellow color shows negative results. The presence of IAA can be quantitatively tested by using a spectrophotometer (OD 530 nm). The IAA concentration of the sample was then calculated using a standard curve with IAA with the regression used is:

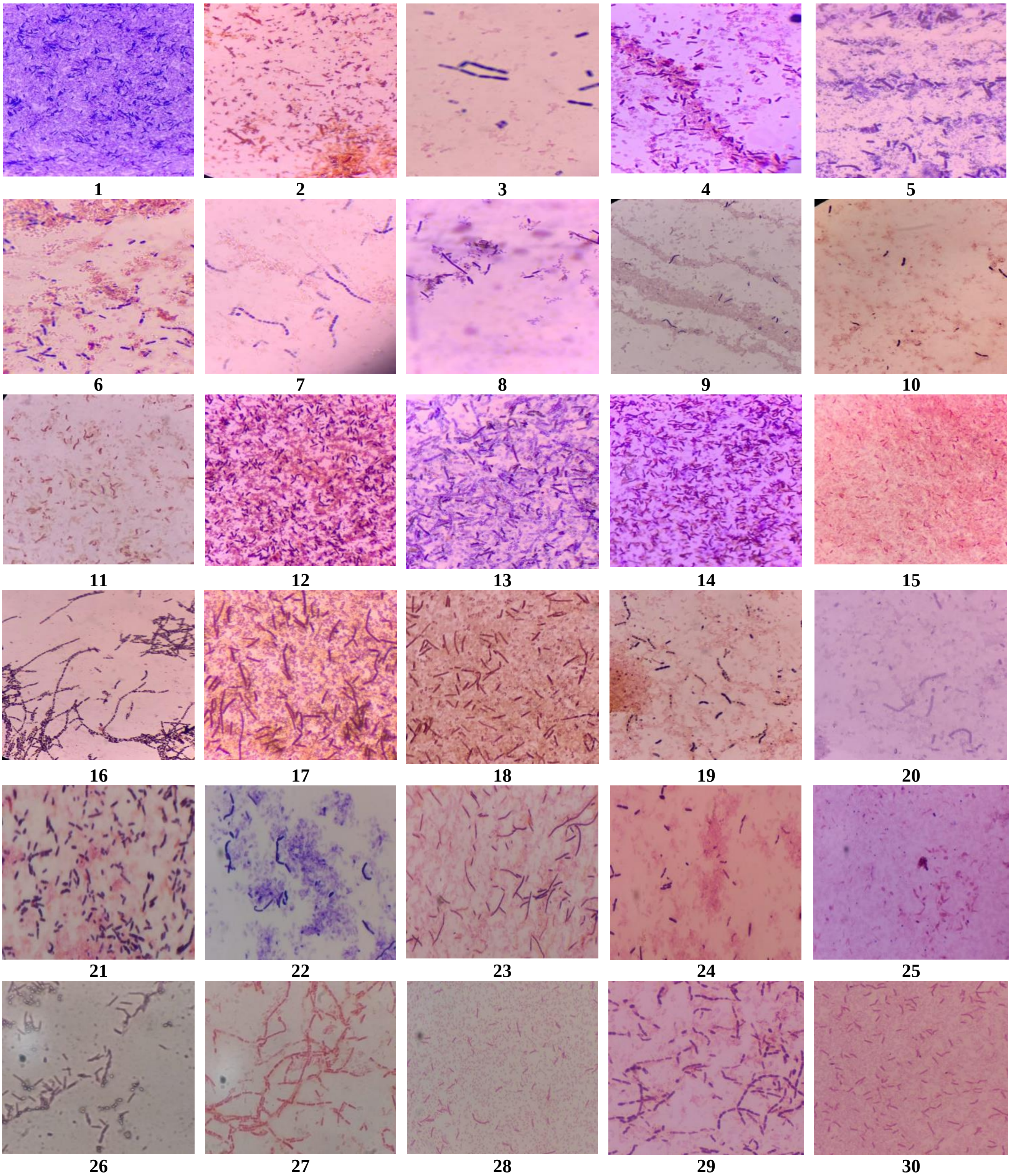
$$y = ax + b \quad \text{..(2)}$$

Where: y: Dependent variable (absorbance), x: Independent variable (concentration), a: Constant, b: Regression coefficient

RESULTS AND DISCUSSION

Characterization and identification

The study successfully isolated with diverse morphological characteristics and varying biochemical profiles. The bacterial colonies generally exhibited three primary shapes: circular, rhizoid, and irregular. The colony edges varied, including entire, curled, undulate, lobate, and filamentous. Elevation patterns observed were convex, umbunate, and raised. Colony colors ranged from yellowish white and creamy to transparent yellow, indicating a diversity of pigment production. Biochemical tests revealed that seven isolates tested positive in the SCIA test, suggesting the presence of specific bacterial activities. The catalase test was predominantly positive, indicating the bacteria's ability to break down hydrogen peroxide. All isolates showed positive results in starch and gelatin hydrolysis tests, confirming their ability to degrade complex organic substrates. Most of the isolates were identified as Gram-positive bacteria (Table 1, Figure 2).



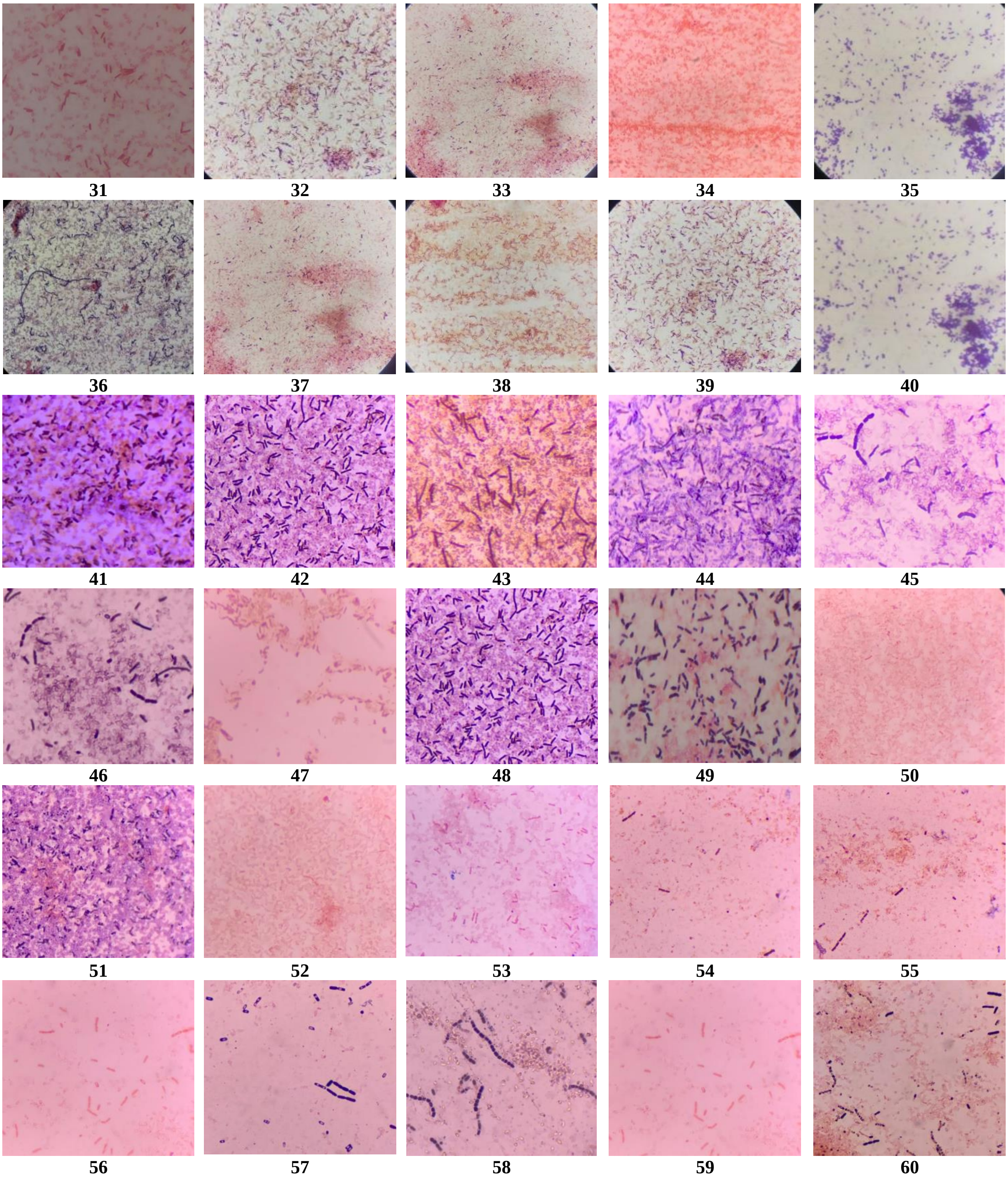


Figure 2. Morphological forms of soil bacteria by Gram staining. 1. B1; 2. B2; 3. B3; 4. B4; 5. B5; 6. B6; 7. B7; 8. B8; 9. B9; 10. B10; 11. B11; 12. B12; 13. B13; 14. B14; 15. B15; 16. B16; 17. B17; 18. B18; 19. B19; 20. B20; 21. T1; 22. T2; 23. T3; 24. T4; 25. T5; 26. T6; 27. T7; 28. T8; 29. T9; 30. T10; 31. T11; 32. T12; 33. T13; 34. T14; 35. T15; 36. T16; 37. T17; 38. T18; 39. T19; 40. T20; 41. T2; 42. T22; 43. T23; 44. T24; 45. SP1; 46. SP2; 47. SP3; 48. SP4; 49. SP5; 50. SP6; 51. SP7; 52. SP8; 53. SP9; 54. SP10; 55. SP11; 56. SP12; 57. SP13; 58. SP14; 59. SP15; 60. SP16

Table 1. Morphological and biochemical characterization of soil bacteria

Code	Shape	Edge	Elevation	Color	SCA	TSIA	Catalase	Starch	Gelatine	Gram
B1	Irregular	Undulate	Convex	Cream	-	M/K	-	+	+	+
B2	Irregular	Lobate	Convex	Cream	-	M/K	-	+	+	+
B3	Circular	Entire	Convex	Yellowish white	-	M/K	+	+	+	+
B4	Irregular	Undulate	Umbunate	Yellowish white	-	M/K	-	+	+	+
B5	Circular	Undulate	Umbonate	Creamy	-	M/K	-	+	+	+
B6	Circular	Entire	Convex	Transparent yellow	-	M/K	+	+	+	+
B7	Circular	Undulate	Umbonate	Creamy	-	M/K	-	+	+	+
B8	Circular	Entire	Umbonate	Creamy	+	M/K	+	+	+	+
B9	Circular	Curled	Convex	Creamy	-	M/K	-	+	+	+
B10	Irregular	Undulate	Umbunate	Creamy white	+	M/K	-	+	+	+
B11	Circular	Curled	Convex	Creamy	+	M/K	+	-	+	+
B12	Irregular	Undulate	Umbunate	White	-	M/K	-	+	+	+
B13	Circular	Undulate	Umbunate	Creamy	+	M/K	+	+	+	+
B14	Circular	Curled	Umbonate	Creamy	-	M/K	+	+	+	+
B15	Circular	Curled	Convex	White	+	M/K	+	-	+	+
B16	Irregular	Lobate	Umbonate	Creamy white	+	M/K	-	+	+	+
B17	Circular	Entire	Convex	Cream	+	M/K	-	+	+	+
B18	Filamentous	Filamentous	Umbonate	Creamy white	+	M/K	+	+	+	+
B19	Circular	Undulate	Convex	Creamy	+	M/K	-	+	+	+
B20	Circular	Undulate	Convex	Creamy	+	M/K	-	+	+	+
T1	Irregular	Undulate	Umbonate	Creamy	-	M/K	+	+	+	+
T2	Irregular	Undulate	Umbonate	Yellowish white	-	M/M/M/K	+	+	+	+
T3	Rhizoid	Lobate	Umbonate	Yellowish white	-		+	+	+	+
T4	Circular	Entire	Convex	Yellowish white	+	M/K	+	+	+	+
T5	Irregular	Undulate	Convex	Yellowish white	-	M/K				
T6	Irregular	Undulate	Raised	Yellowish white	+	K/K ((+) black sediment	-	+	+	+
T7	Filamentous	Filamentous	Umbonate	Yellowish white	+	M/K	-	-	+	-
T8	Irregular	Undulate	Convex	Creamy	+	K/K ((+) black sediment	+	+	+	-
T9	Circular	Entire	Convex	Yellowish white	-	M/K	+	+	+	-
T10	Irregular	Filamentous	Convex	Yellowish white	-	M/K	+	+	+	-
T11	Irregular	Lobate	Umbonate	White	-	M/K	+	+	-	-
T12	Irregular	Undulate	Convex	Creamy	+	M/K	+	+	+	+
T13	Irregular	Lobate	Umbunote	Yellowish white	-	M/K	+	+	+	+
T14	Rhizoid	Lobate	Umbonate	Creamy	-	M/K	-	+	-	+
T15	Irregular	Undulate	Convex	Creamy	-	M/K	+	+	+	+
T16	Irregular	Curled	Convex	Creamy	-	M/K	-	+	+	+
T17	Circular	Entire	Convex	Yellowish white	-	M/K	-	+	+	+
T18	Irregular	Undulate	Umbunate	Creamy	-	M/K	-	+	+	-
T19	Irregular	Lobate	Umbonate	Yellowish white	-	M/K	-	+	+	+
T20	Circular	Undulate	Umbunate	Creamy	-	M/K	-	+	+	+
T21	Circular	Entire	Convex	Yellowish white	-	M/K	+	+	+	+
T22	Circular	Entire	Convex	Yellowish white	-	M/K	+	+	+	+
T23	Irregular	Undulate	Umbonate	Yellowish white	-	M/K	+	+	+	+
T24	Biconvex	Undulate	Raised	Yellowish white	-	M/K	+	-	+	+
SP1	Irregular	Curled	Umbunate	Creamy	-	M/K	+	+	+	+
SP2	Circular	Entire	Convex	Yellowish white	-	M/K	+	+	+	+
SP3	Circular	Entire	Convex	Creamy	-	M/K	+	+	+	-
SP4	Irregular	Lobate	Umbunate	Yellowish white	-	M/K	+	+	+	+
SP5	Rhizoid	Filamentous	Umbunate	Yellowish white	-	M/K	+	+	+	+
SP6	Irregular	Undulate	Umbunate	Creamy	+	M/K	+	+	+	-
SP7	Filamentous	Filamentous	Umbonate	Yellowish white	-	M/K	+	+	+	+
SP8	Irregular	Lobate	Umbonate	Creamy	-	M/K	+	+	+	-
SP9	Irregular	Curled	Convex	Yellowish white	-	M/K	+	+	+	-
SP10	Irregular	Undulate	Convex	Yellowish white	-	M/K	+	+	+	+
SP11	Circular	Entire	Convex	White	-	M/K	+	+	+	+
SP12	Irregular	Lobate	Umbunate	Yellowish white	+	M/K	+	+	+	-
SP13	Circular	Entire	Convex	Yellowish white	-	M/K	-	+	+	+
SP14	Biconvex	Curled	Convex	Yellowish white	-	M/K	-	+	+	+
SP15	Circular	Entire	Convex	Creamy	-	M/K	+	+	+	-
SP16	Irregular	Lobate	Umbunate	Yellowish white	+	M/K	+	+	+	-

Note: M: Red; K: Yellow

Since isolates with higher percentages could break down starch and create a clear zone around the bacteria, they performed well in the starch test. The gelatin test for all bacterial species in isolate T18 showed negative starch findings; the TSIA (Triple Sugar Iron Agar) test showed that the slant area of the media turned yellow at the bottom; and isolate T6 showed black precipitate producing H2S gas. Gas bubbles were seen in isolates B3, B6, T2, T4, T21, T22, SP1, SP4, SP5, SP6, SP10, and SP11 according to positive catalase test results. This is similar to the study of Geraldi et al. (2024) which stated that bacterial colonies are usually rhizoidal, filamentous, round, and irregular. Common elevations include protruding, convex, flat, and oval craters. These elevations can be lobate, filiform, wavy, or curved, and have Gram-positive staining results.

The results of this study confirm that roots are home to various bacteria. Despite their small size and relative simplicity as prokaryotic cells, bacteria are key players in soil processes, participating in biogeochemical cycles. The ability of bacteria to thrive in various soil environments is due to their extraordinary metabolic flexibility. Plant exudates are rich in amino acids, carbohydrates, and other substances that support bacterial growth. As stated by Lahlali et al. (2022), bacterial isolates that produce the catalase enzyme are characterized by the appearance of air bubbles, which indicate positive results. When the catalase test was carried out after the addition of Hydrogen Peroxide (H_2O_2) to the bacterial isolate. This reaction will be favorable if catalyzed by the catalase enzyme, which decomposes H_2O_2 into water and oxygen (Gebicka and Krych-Madej 2019). Bacteria can release and produce the catalase enzyme, which aims to protect themselves from toxic H_2O_2 components (Murali and Patel 2017). The presence of the catalase enzyme in bacteria is significant and shows the ability of the isolate to manage environmental stress. Bacterial catalase enzymes are crucial in increasing the plant's Immune System Resistance (ISR) to pathogens (Saeed et al. 2023). Biotic and abiotic variables significantly impact the diversity of rhizosphere bacteria.

According to Schröter and Dersch (2019), one of the adaptive mechanisms that helps bacteria to adapt environmental stress is phenotypic diversity, which is indicated by the morphology of bacterial colonies. Growth time and medium composition are two factors that directly show the most significant influence on colony differentiation in a microorganism; therefore, colony type is one of the reliable indicators and techniques in assessing phenotypic transitions and indicating bacterial diversity in a sample. Color is another important component that can be concluded from studying bacterial morphological characteristics. The pigments produced by these bacteria can cause variations in the color of bacterial colonies. The color of bacterial colonies can be influenced by several pigments, such as actinorhodin, violacein, prodigiosin, pyocyanin, carotenoids, and zeaxanthin. Fitri et al. (2020) stated that medium, pH, and temperature are external variables that can affect colony color variations.

Three bacteria were categorized as negative spherical bacteria, and seventeen were classified as Gram-positive, rod-shaped bacteria out of twenty isolates on the roots in Gram staining. Gram staining is one of the most important steps in bacterial identification because it allows the correlation of several cell features, including a cell wall, which is the main building block for staining activity. Gram-positive bacteria have walls between 20 and 80 nm, while Gram-negative bacteria have relatively thin walls with a thickness of less than 10 nm. Gram-negative bacteria are characterized by layers with many holes and additional membrane layers. The main difference between these bacteria is the composition of their cell walls, which gives different characteristics to the cells, especially in response to external stressors, including heat, UV light, and antibiotic effects (Mai-Prochnow et al. 2016).

According to the Catalase test, there were 15 isolates with positive results and 5 isolates with negative results. Aerobic and facultative anaerobic metabolism can produce volatile products such as hydrogen peroxide. The presence of this catalase enzyme will help convert hydrogen peroxide into oxygen and water. This can be seen from the appearance of gas bubbles if the results obtained are positive (Bharti and Ibrahim 2020).

Bacterial and fungal antagonist test

The pathogenic fungi selected for this test are very dangerous in agriculture because they can grow quickly and develop on various substrates and are easily associated with plants, so they can cause significant attacks and damage to plants. Based on the observation results, only 20 isolates (Of the 60 bacterial isolates) showed good effectiveness in inhibiting the growth of pathogenic fungi pathogens (*Aspergillus flavus*, *A. niger*, *Fusarium* sp., and *Colletotrichum* sp.), as indicated by the formation of inhibition zones with inhibition values. In comparison, the other 40 isolates did not show good effectiveness in inhibiting the growth of pathogenic fungi. The most significant inhibition against fungi was shown by isolate B6, with inhibition values on *Fusarium* sp. of 32.42 mm and *Colletotrichum* sp. of 20.72 mm). The second largest inhibitory was isolate SP 1, with inhibition values on *A. niger* at 28.32 mm), on *A. flavus* at 25.12 mm, on *Fusarium* sp. at 23.11 mm, and on *Colletotrichum* sp. at 20.23 mm (Table 2, Figure 3).

Bacterial isolates can secrete certain compounds that can inhibit fungal growth; this can be seen from the presence of a clear zone between bacteria and fungi. This can be used as data in developing bacterial biocontrol against pathogenic fungi. The inhibition zones formed against different pathogenic fungi show various levels of inhibition. The mechanism of chemicals created and distributed by bacteria into the media to suppress fungal growth is the cause of inhibition by bacterial species. The variation in each one's inhibition diameter. The ability of bacterial isolates to create various inhibitory substances is regarded to be the cause of treatment. Variations in the kind and quantity of inhibitory secondary metabolites that bacteria create can in variations in the inhibitory rate that the bacteria produce. Variations in each isolate's production of inhibitory secondary metabolites in terms of both type and quantity by every individual isolation (Ouchari et al. 2019).

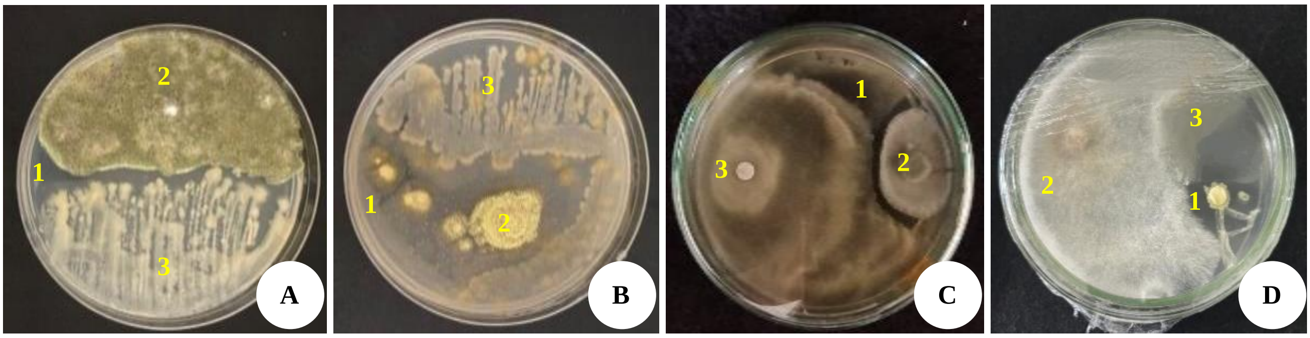


Figure 3. Inhibition of: A. Bacterial and *A. niger*; B. Bacterial and *A. flavus*; C. Bacterial and *Colletotrichum* sp.; D. Bacterial and *Fusarium* sp. Note: 1. Inhibition; 2. Fungi; 3. Bacterial

The ability of bacteria to create antagonistic substances, such as antibiotics, antifungals, or other secondary metabolite compounds, is one of the mechanisms that occur. These substances have an inhibitory effect on the development of proteins or nucleic acids. These substances can also cause changes in permeability by creating pores in the target cell membrane. Backer et al. (2018) stated that the formation of secondary metabolites is highly dependent on bacterial strains, with different strains producing different products and types of metabolites, thus potentially producing different effects on target pathogens. Due to the non-static environmental impact, although isolates belong to the same species or genus, they are not always the same strain. Thus, strain selection plays an important role in forming bacterial strains. In addition, according to Singh et al. (2020), differences in the rate of compound diffusion and the mechanism of compounds/extracts against pathogenic bacteria can also cause variations in the inhibition zone.

Antibiosis compound production ability test

Chitinase test

The test showed that the bacteria could produce chitinase, an enzyme that controls fungi, based on the growth of the isolate on media containing chitin and the results of the chitinase activity test. The clear zone surrounding the former colony proves that the B6 bacteria can break down chitin. If the bacterial colony that grows on chitin media produces a clear zone around it, this indicates chitinase activity. A clear zone is formed around the bacterial colony at a certain incubation period, and the bacteria can produce chitinase (Figure 4).

The formation of a clear zone around the bacterial colony can be a sign that extracellular enzymes are being produced. Enzymes made in cells and released into the growth medium are known as extracellular enzymes. The chitinase enzyme can hydrolyze chitin to produce chitin derivatives and has several technological uses, including a biocontrol gene fund for biological control. Isolated from soil, *Serratia plymuthica* C48 can be useful as a biological control agent, inhibiting the growth of *Verticillium dahliae*. Based on the chitinolytic index, B6-positive bacterial isolates were categorized as having a high chitinolytic index (Gonfa et al. 2023). The hydrolytic enzyme chitinase can break down the β-1,4-glycosidic bonds found in chitin. The main cuticle component of fungal cell walls, crustacean skeletons, and peritrophic membranes—the protective layer covering the guts of many insects is chitin. Because this substance is non-toxic, inelastic, and biodegradable, the capacity of bacterial species to defend themselves against pathogen attacks is essential for defense and self-defense mechanisms (Montoya 2022).

Protease test

Protease activity was detected in isolates B6 and SP1 according to the protease activity test. By cultivating bacterial isolates on skim milk agar media and then measuring the resulting clear zone, the study showed that the activity of the isolate protease enzyme was observed qualitatively (Figure 5). Casein, a milk protein impermeable to the isolated membrane, is present in the media as a protein. So, isolated microbes with external protease capabilities can hydrolyze casein into amino acids. A clear zone formed around the isolate in the media indicates the hydrolysis reaction of casein by extracellular proteases. On the other hand, the absence of a clear zone in the media suggests that the protease does not hydrolyze (Indrayani et al. 2022).

Table 2. Antagonist test of pathogenic bacteria against pathogenic fungi

Code	*Aspergillus niger* (mm)	*Aspergillus flavus* (mm)	*Fusarium* sp. (mm)	*Colletotrichum* sp. (mm)
B3	1.82	1.35	1.33	1.54
B6	30.25	31.22	32.42	20.72
B9	0.23	0.33	3.23	0
B10	0.12	0.11	1.22	0
B13	20.12	14.44	17.45	18.45
B19	0.78	0.33	0.45	0
T2	10.23	11.22	15.06	12.33
T4	0.76	0.22	0	0
T6	0.56	0	0	0
T18	0.88	0	0	0
T20	0.78	0	0	0
T21	0.91	0	0	0
T22	0.67	0	0	0
SP1	28.32	25.12	23.11	20.23
SP4	15.73	16.56	20.11	15.65
SP5	5.21	0.43	0	0
SP6	2.38	0	0	0
SP10	2.12	0	0	0
SP11	1.36	0.88	0	0
SP12	2.19	0	0	0

Qualitative metabolite production test

Phosphate capability test

In addition to acting as an antagonist against pathogenic fungi, the bacteria from this study can produce other metabolite components, namely, the ability of these bacteria to produce phosphate. Bacterial phosphate is one of the advantages that has currently been developed because it plays a role as a provider of macronutrients, which is the most important part for plant growth and development. The use of phosphate-solubilizing microbes has several advantages, including saving energy and not polluting the environment (Sharon et al. 2016). The ability of bacteria as phosphate producers can be shown in Figure 6.

Phosphate (Phosphate Solubilizing Bacteria, PSB) significantly contributes to providing plant nutrients in agriculture and plays a vital role in providing soluble phosphorus to plants (Paul and Sinha 2016). The role of phosphate-producing bacteria in biocontrol in plants is to provide resistance and nutrients so that plants can grow well and so they are not easily attacked by pathogens. Phosphatase levels can be determined by measuring bacteria that dissolve phosphate, such as SP 10 (7.1 mm), T4 (14.33 mm), and B6 (15.2 mm) bacteria. The macroscopic observations of colonies on selected Piskovskaya media served as the basis for this work's observations of phosphate-solubilizing bacteria. The features of morphological observations are crucial for aiding in the identification and type of bacteria, according to research done by Pan and Cai (2023). The qualities of morphological observations are essential in assisting in identifying bacteria. Bacteria breaking down accessible phosphate elements can form colonies with clear zones when grown on selective Pikovskaya media.

Bacteria develop colonies when their excreted organic acids bind to Ca ions from Ca_3 (PO_4) in selective Piskovkaya media and release H_2PO_4-bonds, as a plant defense mechanism against pathogen management. Three phosphate-solubilizing bacterial isolates with different solubility indices were discovered based on the measurement findings of the tested bacterial isolates. According to Amri et al. (2023), an index value falling within the 2.6-3.0 group of the phosphate solubility index is considered very high. The clear zone index category indicates that the bacteria can utilize organic acids and the enzyme phosphatase.

IAA test

The regression equation can be used to examine the results of measuring the absorbance of the bacterial sample as a y value. Regarding qualitative value, the more pink the IAA content, the higher the IAA content will be. The IAA standard curve can find the amount of IAA in the bacterial supernatant. $Y = 0.007x + 0.219$ is the regression equation derived from the spectrophotometry data. The bacterial isolates of the research results were calculated by comparing the absorbance measurement results of the supernatant of the highest bacterial isolate of the two isolate samples with the notation y in the equation standard curve. This allowed for the determination of the content or concentration of IAA in the supernatant. The IAA concentration of the bacterial isolate supernatant is determined by the x value that is acquired from the measurement findings of the IAA standard curve (Figure 7).

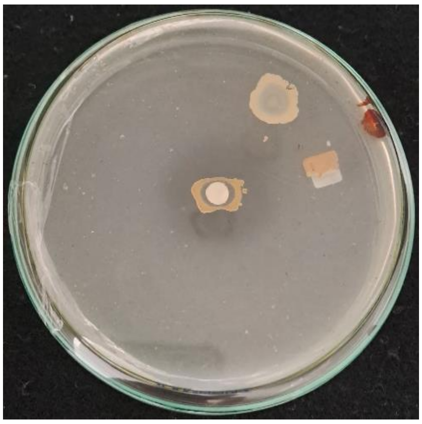


Figure 4. Chitinase activity was secreted by bacteria

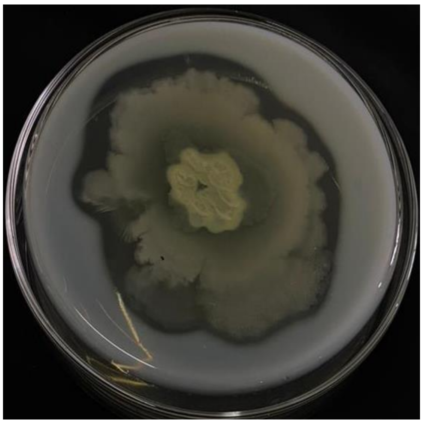


Figure 5. Protease activity was secreted by bacteria

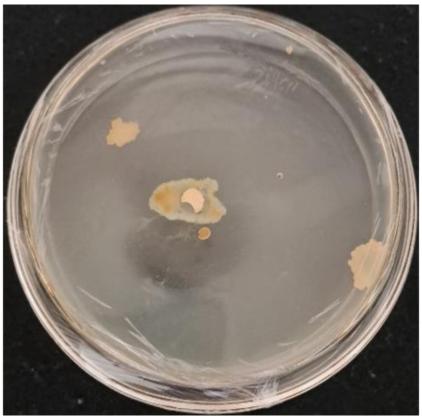


Figure 6. Activity of phosphate bacteria secreting the enzyme posfatase into the media

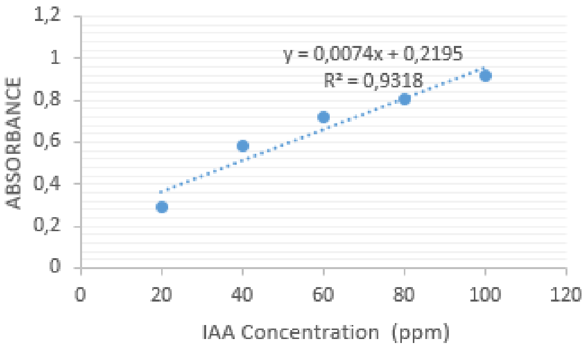


Figure 7. IAA standard curve concentration measurement

Table 3. Absorbance measurement of Indole Acetic Acid-producing bacteria

Isolate	Absorbance	Concentration of IAA (ppm)
B3	0.212	6.288378
B6	0.432	12.81405
B9	0.231	6.851959
B10	0.221	6.555338
B13	0.245	7.26723
B19	0.339	10.05547
T2	0.311	9.224932
T4	0.121	3.589122
T6	0.098	2.906892
T18	0.087	2.580608
T20	0.071	2.106014
T21	0.123	3.648446
T22	0.211	6.258716
SP1	0.342	10.14446
SP4	0.411	12.19115
SP5	0.121	3.589122
SP6	0.073	2.165338
SP10	0.078	2.313649
SP11	0.043	1.275473
SP12	0.054	1.601757

A qualitative examination of several isolates showed that the bacteria can produce IAA. When Salkowski reagent is added to bacteria that produce IAA, the bacteria turned pink. This is because IAA and Fe bind to form a complex molecule called $Fe_2(OH)_2(IA)_4$, where IAA stands for indole-3-acetate. Interaction occurs if the environment is acidic (Tirta 2016). Based on the test results, isolate B6 produced the most IAA (12.81 ppm), so the color changed to pink. Isolation SP11 produced the least IAA (1.275 ppm), which was not too concentrated and only experienced a slight color change. A more concentrated, deep pink color can indicate increased IAA production by bacteria. A spectrophotometric investigation revealed that B6 had the highest IAA production capacity after 24 hours. After 24 hours, the isolate could move into the stationary phase, where the nutrient medium began to decrease, and the isolate began to produce relatively high levels of secondary metabolites (Table 3).

Numerically and qualitatively, the two isolates showed differences. One thing that can affect IAA synthesis is tryptophan. Tryptophan is an amino acid component composed of amino acids with protein components; it facilitates the use of microbes. When tryptophan, an amino acid precursor, is available or when bacterial growth circumstances are less than ideal, IAA hormone is generated as a secondary metabolite. By adding L-tryptophan as a precursor to the bacterial growth medium, it is possible to increase the production of IAA by bacteria. It has been demonstrated that tryptophan is a physiological precursor in the manufacture of auxins in both microbes and plants Nonhebel (2015) and Li et al. (2018). It is clear from this situation that the development of bacteria that reach the stationary stage is correlated with IAA synthesis. Several variables, including growth rate, non-plant nitrogen sources, amino acid availability, and environmental conditions, may contribute to some of these conditions. The tryptophan metabolic pathway, which uses tryptophan as the primary precursor for IAA biosynthesis, may also be necessary for bacteria that produce IAA hormones. Bacteria that produce IAA have the potential to interact significantly with plants. Plant growth can be modulated by the ideal IAA concentration (Fitri et al. 2020).

In conclusion, this research showed 20 isolates that could control pathogens with the highest inhibition value, namely isolate B6 with an inhibition value on *Fusarium* sp. (32.42 mm) and on *Colletotrichum* sp. (20.72 mm). Isolate SP 1 showed an inhibition value on *A. niger* (28.32 mm), *A. flavus* (25.12 mm), *Fusarium* sp. (23.11 mm), and *Colletotrichum* sp. (20.23 mm). Identification of other metabolite compounds isolates B3, T4, and SP10 were able to dissolve phosphate, with inhibition zones of 15.2 mm, 14.3 mm, and 7.1 mm; chitinase activity was produced by species B6 with an inhibition zone of 10.3 mm, and IAA hormone activity was obtained with the concentration of 12.86 ppm and the lowest 2.89 ppm. Of the several bacterial isolates treated, species B6 and SP1 have good potential to be developed as biological control agents. Currently, the species obtained are still identified based on observations of morphological characteristics, biochemical testing, and testing of several metabolite compounds produced to determine more definitive identification using molecular and genetic tests that will be tested next. Utilizing local natural resources is very important because local soil microorganisms have adapted to the environment and have a unique potential to fight pathogens.

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