CHARATERIZATION AND IDENTIFICATION OF INDOL ACETIC ACID PHYTOHORMONE-PRODUCING BACTERIA FROM CORN ROOT (*Zea mays* L.)

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ABSTRACT

Rhizobacteria are endophytic bacteria that inhabit plant tissues and the rhizosphere, producing secondary metabolites that support plant growth. This study is an exploratory descriptive investigation aimed at characterizing and identifying IAA- and phytohormone-producing endophytic rhizobacteria isolated from the roots of corn plants (*Zea mays* L.). The rhizobacteria isolates producing IAA phytohormones were obtained by culturing bacteria in Yeast Peptone Broth (YPB) medium supplemented with L-tryptophan. Bacterial cultures (1 mL) were treated with Salkowski reagent, and absorbance was measured spectrophotometrically at a wavelength of 530 nm. From 14 isolates, five were identified as producing the highest levels of indole-3-acetic acid (IAA), ranging from 17 to 50 ppm. Identification was conducted using the profile matching method to determine the genus of each rhizobacterium by comparing their characteristics with reference genera. Based on 62 tested traits, the five isolates exhibited diverse features, including Gram-positive and Gram-negative bacteria, coccus-shaped morphology, circular and irregular colony configurations, lobate and undulate margins, convex, umbonate, and raised elevations, and colony colors ranging from white to yellowish, along with varied physiological (biochemical) test results. The identification results revealed bacteria with a similarity index of ≥70%, suggesting proximity to the genus *Micrococcus* (77.4%, isolate N), *Rhizobium* (80.6%, isolate D), and *Shewanella* (80.6%, isolate B; 87.1%, isolate V; and 91.9%, isolate E). This research provides an important contribution to the development of biofertilizers aimed at enhancing the growth and productivity of corn plants in a sustainable manner.

Keywords: IAA-producing bacteria, Characterization Identification, IAA phytohormones, Corn plants, Rizobacteria

Introduction

Corn has great potential in the sustainable agriculture system in Indonesia. Sustainable maize development involves efficient use of inputs, wise resource management, and the implementation of agricultural practices that reduce negative impacts on the environment with the increasing awareness of the importance of eco-friendly agriculture. The use of *biofertilizers* and biological agents, such as bacteria that produce indol acetic acid (IAA), is also one of the strategies to increase corn yields while maintaining soil health (Glick, 2020). Corn production in Indonesia continues to face numerous challenges, including pests and diseases, land degradation, climate change, and reliance on chemical fertilizers. Efforts to increase the productivity and sustainability of corn need to be focused on the use of modern technology, effective pest and disease management, and the development of environmentally friendly agricultural systems. With the right strategy, corn can continue to contribute significantly to Indonesia's food security and economy (Kumar *et al*., 2021).

Climate change that causes unpredictable weather, such as long dry seasons and floods, also increases the risk of crop failure and lowers the quality of production. Other challenges include limited access for farmers to modern agricultural technology and superior seeds, as well as areas with sloping land conditions. The risk of soil erosion is also a serious problem that threatens the sustainability of maize cultivation (BRMP Gorontalo, 2025). The development of corn commodities contributes to the supply of food and industrial raw materials. The development of corn on a wider

scale with higher production has the potential to increase farmers' income and the regional economy. The corn commodity has a multipurpose function, namely for food, feed, fuel, and industrial raw materials. It is estimated that more than 58% of domestic corn needs are used for feed, while for food only about 30%, and the rest for other industrial needs and seeds (Panikkai *et al*., 2017).

The demand for corn as a raw material for animal feed continues to increase. The use of corn for feed is driven by its relatively affordable price, high calorie and protein content, with complete amino acid content, and is preferred by livestock compared to other feed raw materials. Efforts to replace corn with other grains seem to have not been successful so that corn remains the main feed raw material in the world (Kasryno *et al*., 2008). The challenge in the future is how to meet the needs of corn as a raw material for feed, food, and energy (Zakaria, 2016). In 2024, corn production is expected to continue to increase by 0.39% or reach 24.98 million tons. In 2022, Indonesia's population is around 277 million people, making Indonesia the country with the 4th largest population in the world. This is a challenge for food supply in the future (Prasetyo et al., 2024).

In the era of sustainable modern agriculture, the use of PGPR (*Plant Growth Promoting Rhizobacteria*) as a biofertilizer is one of the solutions to reduce dependence on chemical fertilizers. Endophytic microorganisms, including endophytic bacteria, are able to support plant growth through the production of secondary metabolites such as phytohormones. One of the important phytohormones produced is indol acetic acid (IAA), which is formed from the precursor L-tryptophan and is produced by various microorganisms in the plant rhizosphere. Indole acetic acid plays a key role in regulating plant physiological processes such as cell elongation, root formation, and response to environmental stress (Kumar *et al*., 2021).

The utilization of rhizobacteria capable of producing IAA provides a great opportunity to reduce the excessive use of chemical fertilizers, which often negatively impact the environment and soil quality. However, in order for these bacteria to be optimally utilized as a plant growth booster, a deeper understanding of their characteristics and potential is needed, especially in major food crops such as corn (*Zea mays* L.). Maize is one of the important agricultural commodities that requires optimal nutrition, and the use of IAA-producing endophytic bacteria can be an effective and environmentally friendly strategy to increase crop productivity (Yadav & Singh, 2022). This article the characterization, and identification of endophytic rhizobacteria from the roots of corn plants that are capable of producing IAA. Through biochemical assays, as well as macroscopic, microscopic, and colony morphology analysis, five bacterial isolates with high potential to produce IAA were identified. The results of this study not only broaden the horizon on the diversity of endophytic microorganisms in corn roots, but also provide a strong scientific foundation for the development of microorganism-based biofertilizers, which have the potential to support more sustainable agricultural practices (Bhattacharyya & Jha, 2012).

Methods

Isolate Endophytic Bacteria

Bacterial isolation is carried out by cutting the roots of the corn plant and washing them with running water, then drying them on filter paper. The root surface of the plant was sterilized with 70% alcohol (2 minutes) and 5.2% Na-hypochlorite solution (5 minutes), then rinsed with sterile aqueduct 2 times and dried on sterile filter paper. After drying, 1 g of the root is mashed in a sterile mortar, then put in an Erlenmeyer tube and 10 ml of sterile distilled water, after which the Erlenmeyer in a circular manner. Take 1 ml of the extract with a micropipette, then put it in a tube and add 9 ml of sterile water. Do until dilution 10-7. Then, 1 ml of the solution is grown in a petri dish with Nutrient Agar (NA) media. Bacterial growth is observed up to the 3rd day. The growing bacteria are separated and purified, then characterized based on their morphology (shape, edges, surface, and color) (Sondang *et al*., 2022).

Test the ability of bacteria to produce IAA

The test of bacteria's ability to produce IAA was carried out by means of a pure bacterial isolate grown on 5 ml *of Peptone Broth* (YPB) Yeast media in a sterile flask containing L-Tryptophan. YPB media was used in this study because it is superior in providing complete nutrients, such as Nitrogen. YPB media is easier to prepare and stable for IAA initial screening. One ml of bacterial suspension from each flask is centrifuged so that the pellets and supernatants are separated. One ml of the supernatant was taken and given 4 ml of Salkowski reagent (50 ml $HClO_4$ 35% + 1 ml $FeCl_3$ 0.5 M x $6H_2O$), then left for 20 minutes at room temperature. Positive reactions in producing IAA are characterized by the appearance of a pink to reddish color. The Standard Curve for the calculation of IAA levels is derived from the absorbance value at the 530 nm spectrum wavelength. The conversion formula uses regression data determined from the standard curve resulted in R^2 = 0.9825 from equation Y = 0.0111x + 0.015 with x = (absorbance value – 0.015)/ 0.0111

Data Collection Techniques

Observations of bacterial characteristics include surface color, shape, edge, elevation, and size of bacterial colonies. Characterization of bacterial cell morphology is carried out by Gram and endospore staining. Physiological (biochemical) identification of bacteria includes testing of oxygen requirements, catalase, H_2S production, indole production, motility, use of citrate as a carbon source, MR-VP, lysine decarboxylation, sugars, pH influence, and temperature influence.

Data Collection Techniques

Primary data were in the form of the results of the characteristics of each of the top 5 IAA-producing isolates out of a total of 14 bacterial isolates from corn roots (*Zea mays* L.).

Data Analysis Techniques

Bacterial identification was carried out by *the profile matching* method based on *Bergey's manual of Determinative Bacteriology* and relevant scientific journals. The dendrogram construction was carried out using *a Multivariate Statistical Package* (MVSP) with *a Cluster Analysis* test. The similarity index of each bacterium is obtained by *the Simple Matching Coefficient* (SSM). Then it was grouped with the *Unweighted Pair Group Method* (UPGMA) algorithm.

Results and Discussion

Characterization of the Morphology of Colonies and Bacterial Cells

Bacterial characterization was carried out by Gram and endospore staining. Staining is one of the most important parts in bacterial characterization which serves to help clarify the size and shape of bacteria using a microscope, bacterial staining can clarify the outer and inner structures of bacteria such as vacuoles, reveal the characteristics and chemistry typical of test bacteria with dyes, and increase the contrast of different microorganisms around them (Amin *et al*., 2023).

Table 1 Cell morphological characteristic data

Isolate Code	Properties of Grams	Cell Shape	Cell Arrangement	Endospore
B	Negatives	*Coccus*	*Streptococcus*	-
D	Negatives	*Coccus*	*Monococcus*	-
E	Negatives	*Coccus*	*Streptococcus*	-
N	Positive	*Coccus*	*Diplococcus*	-
V	Negatives	*Coccus*	*Monococcus*	-

Remarks: (-) no endospores found

Gram staining is a differential staining procedure that divides bacteria into two groups, namely gram-positive and gram-negative bacteria. After stained, the bacteria were observed under a microscope with a magnification of 400x. From the observation of 5 bacterial isolates there were 4 negative grams and 1 positive gram. Gram-negative is characterized by an isolate that is red when observed under a microscope, while gram-positive is characterized by an isolate that is purple when observed under a microscope.

Gram-negative bacteria have an efficient metabolic pathway because they have a diversity of characterized IAA biosynthesis pathways such as *indole-3-pyruvate* (IPyA) and *indole-3-acetamide* (IAM) as well as key enzymes such as indole-3-pyruvate *decarboxylase* (IPDC) which are commonly found in gram-negative bacteria (Li *et al*., 2018). This is appropriate because the highest IAA-producing bacteria found in the roots of corn plants have gram-negative characteristics. The production of IAA in rhizobacteria becomes more efficient according to the substrate in the environment because bacteria are often induced by the presence of aromatic amino acids such as tryptophan which is the main precursor to the formation of IAA. In addition, gram-negative bacteria contain porines and secretion systems that support the efficiency of the release of IAAs into the environment around the plant rhizosphere.

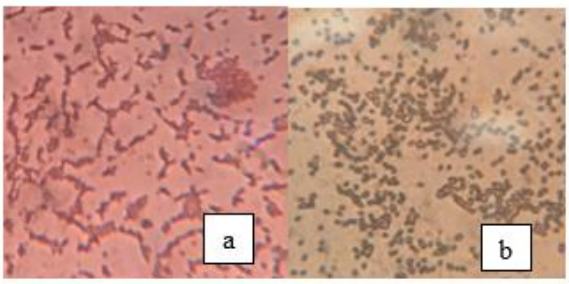

Figure 1. Gram characteristics and shape of *coccus bacterial cells* under a microscope with 400x magnification (a: gram negative, b: gram positive).

Data on the characteristics of bacterial colonies were obtained with irregular and circular configurations, lobate and undulate margins, umbonate, convex, and raised elevations, and white or yellowish white colony color. The results of gram-negative staining are marked with isolates that are red and the results of gram-positive staining are marked with purple (Atmanto *et al*., 2022).

Table 2 Characteristic data of colony morphology

No.	Isolation	Shape	Configuration	Margin	Elevation	Colony Color
1	B	Medium	*Irregular*	*São Paulo*	*Umbonate*	White
2	D	Small	*Circular*	*Undulate*	*Convex*	White
3	E	Small	*Circular*	*Undulate*	*Convex*	Yellowish
4	N	Medium	*Circular*	*Undulate*	*Raised*	Yellowish
5	V	Small	*Circular*	*Undulate*	*Convex*	Yellowish

The form of configuration that is widely found is a circular configuration with an undulate margin, a characteristic of this bacterium is thought to be related to the adaptation of bacteria to the rhizosphere environment. Bacterial colonies that form circulars with undulate margins can expand rhizometric contact with root exudates and nutrients in the rhizosphere, also facilitating the formation of biofilms by increasing colonization and interaction with plants (Arifiani & Lisdiana, 2021). An example of colony morphology results can be seen in Figure 2.

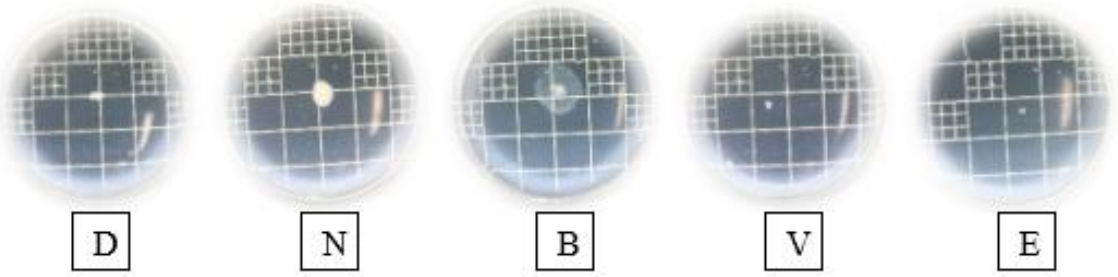

Figure 2. Morphological characteristics of colonies of 5 selected isolates of the highest IAA producers.

Physiological (Biochemical) Characterization of Bacteria

Physiological (biochemical) characterization is carried out with the aim of knowing cell activity and biochemical interactions that occur in bacteria. Biochemical tests are carried out to identify a pure culture of bacteria isolated through its physiological properties (Imtiyaz & Octavia, 2023).

Table 3. Oxygen requirement test results

Isolation Code	Growth Characteristics	
	Growth Type	Oxygen Requirements
B	Turbidity	Facultative anaerobic
D	Turbidity	Facultative anaerobic
E	Turbidity	Facultative anaerobic
N	Pellicle	Aerobic
V	Sediment	Anaerobic Tolerant

The characteristics of anaerobic bacteria are more commonly found in the highest IAA-producing bacteria in the plant rhizosphere, providing adaptive advantages. This ability allows the rhizobacteria located in the rhizosphere to maintain metabolic activity with consistent IAA production. The changing environment of the plant rhizosphere with low oxygen conditions causes bacteria that are able to live in this environment to have an anaerobic character, this shows the potential of rhizobacteria as biofertilizers (Bhat *et al*., 2023).

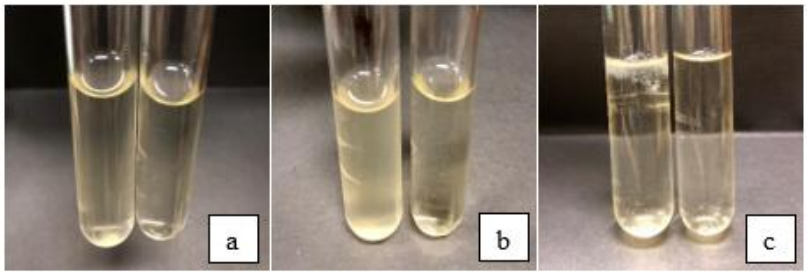

Figure 3. Oxygen requirement test results (a: aerotolerant anaerobic and control, b: facultative anaerobic and control, c: aerobic and control)

Data on the physiological and biochemical characteristics of the bacterium catalase test, indole production, H_2S production, motility, lysine decarboxylation, use of citrate as a carbon source, resistance to temperature and pH, MR-VP and carbohydrate fermentation tests can be seen in Table 4 and Table 5.

Table 4 Physiological (biochemical) test results

No.	Test Type	Bacterial Isolates				
		B	D	E	N	V
1.	Catalonia	+	+	+	-	+
2.	Indole	+	+	+	+	+
3.	H2S	+	-	+	-	-
4.	Motility	+	+	+	+	-
5.	MR	+	+	+	+	-
6.	VP	-	+	-	+	-
7.	Lysine	-	+	+	+	-
8.	Citrate	+	+	+	-	+
9.	pH 3	-	-	-	-	-
10.	pH 7	+	+	+	+	+
11.	pH 10	+	+	+	-	-
12.	Temperature <10°C	+	+	+	+	+
13.	Temperature 30°C	+	+	+	+	+
14.	Temperature 50°C	-	-	-	-	-

Description: (+): Positive Result, (-): Negative Results

Table 5. Results of carbohydrate fermentation tests

No.	Test Type	Bacterial Isolates				
		B	D	E	N	V
1.	Glucose	+	+	+	+	-
2.	Sucrose	-	+	-	+	-
3.	Maltose	-	+	-	+	-
4.	Lactose	-	+	-	-	-
5.	Fructose	+	+	-	-	+
6.	Mannitol	-	+	-	-	-
7.	Galactose	+	+	-	-	+

Description: (+) : Positive Result; (-) Negative Results

Identification of IAA-Producing Bacteria

The reference bacterial genera obtained are *Enterobacter*, *Micrococcus*, *Pseudomonas*, *Variovorax*, *Raoultella*, *Shewanella*, *Azotobacter*, and *Rhizobium*. The data in the form of the results of the bacterial isolate characterization test and the data on the characteristics of bacterial genera were obtained as the basis for determining bacterial kinship. The data was then analyzed and made a dendrogram using *the Multi-Variate Statistical Package* (MVSP) 3.22 application with the Cluster Analysis test. To obtain the similarity index of each bacterial isolate using the Simple Matching Coefficient (SSM). This grouping was carried out using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) algorithm.

The results of the dendrogram construction showed a kinship relationship between the bacterial isolates and the reference bacterial genera. The greater the similarity obtained, the closer the kinship relationship will be. The results of the dendrogram construction can be seen in Figure 4. Isolates with codes B, V, and E are bacterial isolates that have a high similarity index to *Shewanella* of 80.6%, 91.9%, and 87.1%. According to the concept of taxospecies, if the similarity index of a strain of microorganisms has a similarity index of ≥70%, it can be expressed as a single species (Sneath & Johnson, 1972). Meanwhile, D isolate has a closeness to the genus *Rhizobium* when viewed from the similarity of its dendrogram of 80.6%. Meanwhile, N isolate has a proximity to *Micrococcus* of 77.4%.

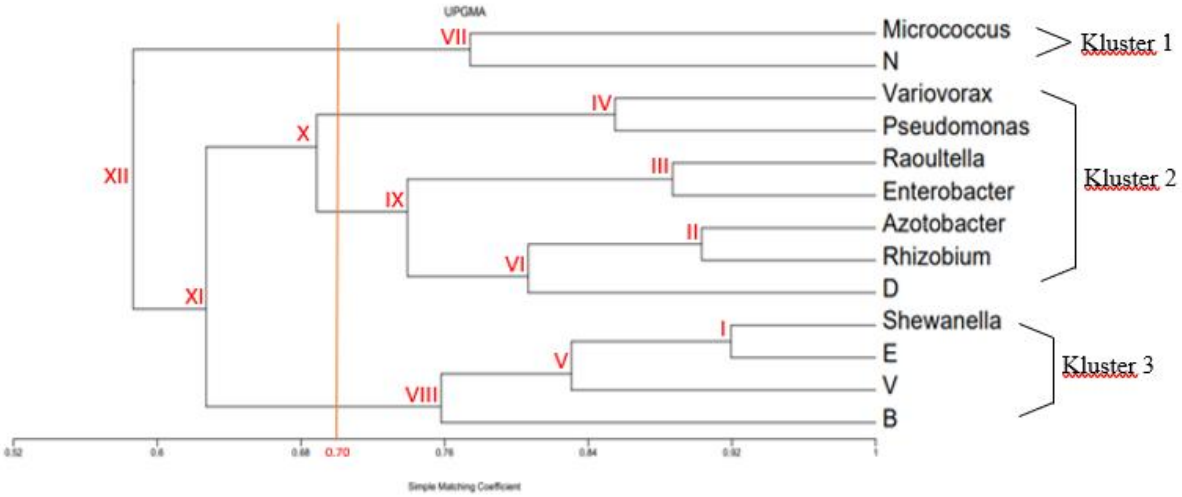

Figure 4. Construction results of dendrogram of bacterial isolates and genera of reference bacteria

Table 6. Levels of Similarity of Test Bacteria to Reference Genera

No.	Generate Mold	Similarity Matrix Index				
		B	D	E	N	V
1.	*Pseudomonas*	62.9%	61.3%	58.1%	54.8%	66.1%
2.	*Variovorax*	**71%**	62.9%	59.7%	56.5%	58.1%
3.	*Raoultella*	53.2%	**74.2%**	45.2%	54.8%	53.2%
4.	*Enterobacter*	61.3%	**82.3%**	53.2%	56.5%	54.8%
5.	*Stuart O'Neill*	**80.6%**	**72.6%**	**91.9%**	56.5%	**87.1%**
6.	*Azotobacter*	66.1%	**80.6%**	67.7%	67.7%	66.1%
7.	*Rhizobium*	66.1%	**80.6%**	67.7%	67.7%	69.4%
8.	*Stuart O'Neill*	53.2%	51.6%	64.5%	**77.4%**	62.9%

Isolates with codes B, V, and E are bacterial isolates that have a high similarity index to *Shewanella* of 80.6%, 91.9%, and 87.1%. According to the concept of taxospecies, if the similarity index of a strain of microorganisms has a similarity index of ≥70%, it can be expressed as a single species (Sneath & Johnson, 1972). Meanwhile, D isolate has a closeness to the genus *Rhizobium* when viewed from the similarity of its dendrogram of 80.6%. Meanwhile, N isolate has a proximity to *Micrococcus* of 77.4%.

Micrococcus is a widespread genus of bacteria and can be found in a variety of habitats, including in soil, water, air, and the skin of humans and animals. In soil environments, *Micrococcus* are often found in the rhizosphere zone or around plant roots, where they coexist with plants as rhizobacteria (Ahmad *et al*., 2020). *Micrococcus* has been shown to increase the germination rate of corn seeds, the length and dry weight of roots and shoots, and is able to produce IAA, bind nitrogen from the air, produce siderophores, and colonize corn roots. Under greenhouse conditions, treatment with these rhizobacteria significantly improves nutrient absorption, leaf chlorophyll content, net assimilation rate, and plant growth rate, suggesting that they have great potential to be developed as bio stimulants to support growth and increase corn yields (Maulina *et al.,* 2022).

Shewanella putrefaciens is a bacterium that has a flexible anaerobic respiration ability, which makes it colonize and adapt to the root rhizosphere environment that often experiences oxygen fluctuations. Based on research by Manjunatha *et al.* (2022), the application *of Shewanella putrefaciens* on *Pennisetum glaucum* with drought stress conditions can significantly increase the dry weight of shoots and roots, root architecture, relative water content, proline accumulation, and levels of ABA and IAA hormones. This suggests that *Shewanella* may act as a PGPR that supports plants especially against drought stress.

Rhizobium sp. is a plant that is widely known for its ability to form mutualism symbiosis with legume plants, which forms nodules for nitrogen fixation. In non-legume crops such as corn (*Zea mays* L.), this symbiosis does not occur. However, Rhizobium can still interact through colonization in the rhizosphere of *plants*. As in the research of Cavalcanti *et al.* (2020), Rhizobium colonization in corn plants is known to increase growth and crop yield. In the study of Lebrazi *et al.* (2020), found that *Rhizobium* increases plant growth as well as produces IAA optimally.

The bacteria's mechanism in increasing the content of IAA in plants is to use natural tryptophan produced and excreted by the roots and then used for the synthesis of IAA. IAA-producing bacteria are involved in several plant physiological processes by introducing the IAA they produce into the plant, so that the plant is more sensitive in changing the concentration of IAA it has. This condition is able to help the process of lateral, adventitious, and primary root extension formation. The involvement of bacteria capable of producing IAA will increase the number of root hairs and lateral roots of the plant (Istiqomah *et al*., 2017).

The ability of bacteria to produce IAA varies due to different pathways or mechanisms. Biosynthesis of IAA in prokaryotes can occur through two pathways, namely IAM and IPyA. The biosynthesis of IAA in *Enterobacter* through the IPyA pathway is influenced by the expression of the IPDC gene (Huda *et al.*, 2014). In this pathway, tryptophan is converted to IPyA by aminotransferase. Then the IPyA is decarboxylated into *indole-3-acetaldehyde (IAAId) by* the IPDC enzyme. The last phase of this biosynthesis pathway is the oxidation of IAAId into IAA (Rini *et al*., 2020).

The bacterial isolates studied are bacterial isolates that have the potential to become biological fertilizers because the five isolates are selected isolates that have the ability to produce IAA with the highest concentration. Further research is needed to determine the best activity of each isolate molecularly, as well as further tests in testing the ability of each isolate in its potential as PGPR in plants, especially corn plants (*Zea mays* L.).

Conclusion

The characterization of bacterial isolates consists of colony morphology, cell morphology, and diverse physiological (biochemical) characteristics. Based on the results of testing the morphological and physiological properties of 5 IAA-producing rhizobacteria, it is suspected that these isolates are closely related to members of the genus Micrococcus, Shewanella, and Rhizobium. Based on the dendrogram of phenotic diversity of IAA-producing bacterial isolates from corn roots (*Zea mays* L.), there are five isolates that have a similarity index of ≥70%, with the following degree of proximity: N isolates are suspected to have affinity with the genus Micrococcus at 77.4%, isolates D with the genus Rhizobium at 80.6%, and isolates B, V, and E with the genus Shewanella, respectively at 80.6%, 87.1%, and 91.9%. Based on the research that has been conducted, further research is needed on the effects of each rhizobacteria on the corn plant (*Zea mays* L.) specifically in fixing nitrogen, producing IAA, and dissolving phosphate in advanced field research.

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