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Endophytic Bacteria in Highland and its Ability to Enhance Plant Growth Environment

Siti Maryam Yasin[1,4], Elkawakib Syam'un[2], Burhanuddin Rasyid[3] and Amir Yassi[2]

[1]Postgraduate Program, Agriculture Study Program, Hasanuddin University, Makassar, South Sulawesi, Indonesia; [2]Department of Agrotechnology, Faculty of Agriculture, Hasanuddin University, Makassar, South Sulawesi, Indonesia; [3]Department of Soil Science, Hasanuddin University, Makassar, South Sulawesi, Indonesia; [4]Agrotechnology Study Program, Andi Djemma University, Palopo City, South Sulawesi, Indonesia
*Corresponding author's e-mail: sittimaryamyasin@gmail.com

The objective of this study was to isolate and characterize endophytic bacteria from the rhizosphere of local highland North Luwu rice, which exhibits the ability to promote plant development and function as a biofertilizer. The research was conducted in the Pest Science Laboratory, Department of Pests and Diseases, Hasanuddin University, South Sulawesi. The present study involved conducting morphological and physiological characterization tests, índole-3-acetic acid producing bacteria ability test, and phosphate dissolution test seed growth test in vitro and identified using the 16S rRNA sequencing method. This study successfully isolated 22 bacterial isolates from soil samples and rhizospheres of local rice varieties. Morphological characterization showed different results in color, size, and shape. Testing the ability of índole-3-acetic acid bacteria from isolates of local rice rhizosphere bacteria of the Bandarata rice variety produced the brightest pink hue (3.39 milligrams per liter). The local tarone hoyane rice variety can dissolve phosphate with a dissolution index value of 2.64. The results of the 16S rRNA gene sequence using the BLAST-N program showed that bacterial isolates from local North Luwu rice produced Pseudomonas bacteria. sp strain, Pseudomonas fluoresces bacteria, Pseudomonas montel strain, and Stenotrophonas sp strain. This research produced isolates of endophytic bacteria from local highland rice which can increase plant growth.
Keywords: Endophytic bacteria; rice; rhizobacteria; phosphate solubilization, IAA.

INTRODUCTION

One of the agricultural crops that is essential to life on Earth is rice (*Oryza sativa*) (Ibrahim *et al.*, 2024). Half of the global population consumes rice, which serves as a fundamental source of sustenance. However, pests and plant diseases are two of the many barriers that contribute to the fall in rice production. Rice plant diseases, specifically blast disease, midrib blight, and bacterial leaf blight, are widely recognized as the primary challenges confronting rice agriculture on a global scale (Kumar *et al.*, 2020). The utilisation of pesticides or chemical agents to manage pests and plant diseases continues to be the predominant approach preferred by agricultural practitioners. This method is carried out intensively, causing residues in foodstuffs, toxic contamination of pesticides in the environment, as well as the emergence of pests and pathogens that are resistant to a pesticide, contamination of the environment, and degrading of the soil (Ayilara *et al.*, 2023). Further, control of plant diseases by utilizing biological agents is one of the appropriate control technology options and needs to be developed, because the negative effects on the environment are relatively low and more sustainable. A diversity of microorganisms is currently used and utilized in sustainable agriculture. The plant microbiome contains several microorganisms in plant tissues and organs including as roots, stems, leaves, flowers, fruits and seeds. Microorganisms found in plant tissues and organs as endophytes which improve plant conditions. Endophytic bacteria play a critical role in the host plant through the plant growth physiology and processes, development, and fertility and have benefits for plants such as nitrogen fixation, minerals, and phytohormones (Singh *et al.*, 2023). The endophytic bacteria can participate in providing benefits for host plants by the release of antimicrobial metabolites and siderophores, as well as by fighting against infections for dietary nutrients, from a variety of biotic and abiotic stressors (Deepika *et al.*, 2023). Endophytic microbes from plants can be utilized as both

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biocontrol agents to manage phytopathogens and as agents that promote plant development. This microbial community has a pivotal role in fertilizing plants and increasing crop yields. Endophytic bacteria can increase growth and improve plant production. Thus, the endophytic bacteria can be produced as a substitute for synthetic fertilizers, and biological control agents, and as a superior endophytic bacteria from various types of agriculture plants (Medison, 2022). Seko sub-district, North Luwu, Indonesia is an isolated area with a hilly and mountainous topography with an area of 2,109.19 kilometers (km) surrounded by unspoiled forests and different regional conditions at an altitude of 1,500 above sea level. This area includes low-temperature highlands, not all rice varieties can grow well and produce and most use local rice varieties such as tarone, bath, and dambo which are naturally cultivated. This condition makes it necessary to analyze microorganisms in the soil and local plants. The number of bacteria present in the soil is affected by various conditions of its growth, such as temperature, humidity, aeration, and energy sources. Farmers in Seko District cultivate local rice naturally. The decline in rice production is due to the lack of fertilizer use and due to uncontrollable pests and diseases of rice plants. Endophytic bacteria are bacteria that live and colonize plant tissues without causing disease symptoms. Endophilic bacteria have a beneficial effect on plant growth and development, producing phytohormones and other compounds that can cope with plant stress thereby helping plants be more tolerant of extreme environments. However, research needs to be carried out on the isolation and characterization of endophytic bacteria in local North Luwu rice cultivated in the highlands. Research on the characteristics of endophytic bacteria in local North Luwu rice in the highlands is still lacking. The presence of a wide range of endophytic bacteria in these rice varieties facilitates the identification and characterization of these bacteria, which may have a positive impact on plant development and yield. This study aims to isolate, characterize, and discover different types of endophytic bacteria collected from the local North Luwu rice rhizosphere that have the potential to act as plant promoters and biofertilizers. This research was carried out in North Luwu rice plants, North Luwu Regency, South Sulawesi in 2022.

MATERIALS AND METHODS

***Research location*:** This study was performed from August to October 2022 at the Plant Pests and Diseases Laboratory, Faculty of Agriculture, Hasanuddin University, Makassar, Indonesia. The source of bacteria was taken from the soil and root samples of local paddy fields at North Luwu rice plants, North Luwu Regency, South Sulawesi. The sampling location is a highland area, namely, the Seko sub-district and the Ronkong sub-district. Seko sub-district altitude 1,485 above sea level, with a yellowish red regosol soil type with the potential of hydrogen (pH) 5 – 7 percent (%) and rainfall 2301 millimeter (mm). At an elevation of 900 meters above sea level, the Rongkong subdistrict has an average temperature of 20 – 25 degrees Celsius (ºC), a yellowish-red regosol soil type with pH 5 - 7%, and 2301 mm of rainfall. Two varieties of rice that can only grow in the Seko sub-district area are tarone and tarone hoyane, while the varieties from the Rongkong sub-district consist of bandarata and banjara. These varieties are organically cultivated by farmers in the Seko area. All samples were taken by removing healthy rice plants up to the fibrous roots, including a little soil attached to the plant. Sampling at the site was done randomly by taking three samples of rice plants. Furthermore, soil and plant samples were obtained and placed in paper envelopes identified with their location and local rice kinds. Plant samples were transported in a cool box to the lab for quick analysis.

***Endophytic bacteria isolation*:** A number of 5 gram (g) of plant samples for endophyte isolation, namely, the roots of rice plants of tarone, tarone hoyane, bandarata, and banjara varieties from the cool box were weight. The root samples were sterilized by washing the plant roots in running water and then dried on a petric dish covered with filter paper. The surface sterilization process was performed by washing the roots using sterile water for 2 minutes followed by soaking in 70% alcohol for 1 minute then rinsing with aquadest 3 times for 2 minutes. After that, the root samples were dried using sterile tissue (Gupta *et al*., 2024). All procedures were carried out under sterile method. Finally, the dried plant roots were cut into small pieces up to 1 centimeter (cm) in size. The soil and plant samples were diluted by finely crushing 1 gram of sampel in a mortar, then adding 10 milliliters (mL) of distilled water and agitating until a homogeneous solution was formed. By extracting 1 milliliters (mL) of the liquid with a pipette, stratified dilutions in the range of 10^{-1} to 10^{-6} were also generated. Dilution is done up to several times to reduce the number of microbes suspended with water. The solution was put into a preliminary test vessel containing 9 mL of sterile water and stirred vigorously using a vortex for 30 minutes to obtain a homogeneous mixture. This process is repeated until a concentration of 10-6 is reached, resulting in a total of 6 test tubes. This dilution is then applied to a petri dish containing nutrient agar medium (NA) with a triangular spreading rod. The cups were then incubated for 24 hours at a room temperature of 28 0c. After the formation of solitary colonies on the growth medium, the colonies are cultivated by scratching on NA media before undergoing three stages of purification. The morphological characteristics of pure colonies that exhibit different appearances are analyzed.

***Morphological and biochemical characterization*:** The procedures to identify and characterize endophytic bacterial isolates were based on their morphology. The observation of the morphological characteristics of endophytic bacteria was performed macroscopically and microscopically. The microscopic observations were based on their shape, cell wall arrangement in bacteria, and Gram staining. The Gram staining was done by inoculating the isolate in a glass object that had been dripped with distilled water. The number of 10 microliter (µL) of 3% Potassium Hydroxide (KOH) was added and stirred repeatedly. The Gram-negative classification was indicated by the mixture of bacterial and KOH suspension becoming very thick or forming a gel within 5 to 60 seconds. Meanwhile, a positive reaction was shown by the isolated bacterial colonies appearing slimy, and sticky like a thread when lifted from the top of the suspension. If there is no slimy gel formation, and when lifted above the suspension is released, the isolate is classified as Gram-positive. Meanwhile, the catalase test is useful for identifying groups of bacteria or microbes to produce the catalase enzyme. The catalase test was performed by taking a bacterial isolate and then dipped in a test tube containing hydrogen peroxide (H_2O_2). The positive reaction was characterized by air bubbles, while the negative reaction was indicated with no air bubbles.

Growth-promoting test analysis

***Indole-3-acetic acid (IAA) production*:** A test was conducted to assess the rhizobacteria's capacity to generate the indole-3-acetic acid (IAA). The production of IAA was now assessed using a spectrophotometer. In summary, microorganisms were cultivated on NA medium with a concentration of 200 parts per million (ppm) of tryptophan. After that, a 10 mL aliquot of the bacterial isolate was put into a 15 mL tube and centrifuged for 10 minutes at an 8000 revolutions per minute. Isolated supernatant in a 2 mL amount was put into a test tube. To the test tube were then added 4 milliliters of Salkowski's reagent and 2 drops of orthophosphoric acid (Ganesh *et al*., 2024). Additionally, the solution was placed in a dark room and left undisturbed for a period of 24 hours. A change in color to pink indicates that bacteria may produce auxin. The hormone and Salkowski's reagent interact chemically to provide the pink color. The optical density was determined using an Ultra Violet-Visible (UV-VIS) spectrophotometer at 535 nanometers (nm) wavelength (He *et al*., 2021). In the meantime, the catalase test was examined using the procedure outlined with adjustments. This experiment was undertaken to assess the microbial capacity for hydrogen peroxide degradation. A single colony culture was put on a slide that had been previously treated with two drops of a 3% H_2O_2 solution, completing one complete cycle. A positive reaction is distinguished by the presence of gaseous bubbles consisting of unbound oxygen.

***Phosphate solubility test*:** The ability to test bacteria in phosphate dissolution using Pikovskaya agar media with spot inoculation method in petric dishes was done for three days. The formation of a clear zone around the colony indicates a positive result, namely, the presence of phosphate dissolution which can be observed and calculated the phosphate dissolution index. The endophytic bacteria were able to dissolve phosphate tested qualitatively and quantitatively. The qualitative characteristic of dissolved phosphate is the appearance of a transparent zone encircling the colony when purified isolates are inoculated onto solid Pikovskaya media which was added with Bromophenol blue 0.01 gram per liter (g/L).

***In vitro Growth Test*:** In vitro, study of rice seed growth spurt using the best bacterial isolate from IAA and phosphate dissolution. The 10 best isolates that synergistically used as a conservatory of bacterial isolates. The in vitro culture medium used was a half-concentration MS liquid medium at pH 5,7. Isolate cultured on 24-hour-old NA media is used to suspend on sterile water. Bacterial isolate is rejuvenated by growing in 100% TSA medium and incubating for 48 hours. Next, the isolate is propagated in the Petri dish and 10 ml of equates is added to the petrik branch then mixed with equates as a suspension of bacteria. Rice seeds are soaked in a suspension of bacterial isolate and incubated for 6 hours. Then the seeds are planted on the branch medium with as many as 3 seeds per isolate. Observations were made, namely, the germination power of rice seeds. The best isolates are the results of Sequencing analysis and 16s rRNA analysis.

***Molecular identification*:** The endophytic bacteria that were chosen for isolation were identified using molecular techniques. Respectively, nucleic acids were isolated from bacterial samples and their purity was assessed using 1.0% agarose gel electrophoresis. Segments of 16 Svedberg (16S) ribosomal ribonucleic acid (rRNA) gene sequences were amplified utilizing 16S rRNA-Forward and 16S rRNA-Reverse primers. The polymerase chain reaction (PCR) product, consisting of a 1500 base pair template, was subjected to purification. The Aligner Software was used to align the forward and reverse sequencing data in order to build a consensus genome of the 16S rRNA gene. The Basic Local Alignment Search Tool (Blastn) program was used to compare the obtained sequences with 16S rRNA sequences accessible on the National Centre for Biotechnology Information (NCBI) website in order to identify the bacterial strains.

RESULT DISCUSSION

***Endophytic Bacterial Isolate*:** There were twenty-four samples isolated from the rhizosphere of local organic rice cultivated in the North Luwu Regency area of the highlands of two locations with different altitudes. From the Seko sub-district area with an altitude of 1,485 above sea level, there were 11 isolates were found, while from the Rongkong sub-district area with an altitude of 900, therefore 11 isolates were identified. Torene rice varieties consist of 4 isolates, tarone hoyane rice 7 isolates, and in the Rongkong area, 2 different varieties were found. The bandarata rice variety consists of 4 isolates and the banjara rice variety consists of 7 isolates.

***Morphological and biochemical characterization*:** Endophytic bacterial isolates from the rhizosphere of local organic rice with different highlands showed different morphological characterization. The morphological characterization of bacterial isolates based on Table, shows 4 of the tarone variety samples, and 7 of the tarone hoyane variety of local rice from Seko District. In the area of Sedangkang from Rongkong District, there were 4 samples of bandarata varieties and 7 banjara varieties were determined. The endophytic bacterial isolates collected exhibited distinct morphological traits. When colonies were categorized according to their size, there were 15 colonies classified as small and 7 colonies classified as medium. Grouping based on colony shape was dominated by 20 round colonies, while there were 2 irregular shapes. The periphery of the colony exhibits a range of morphologies, including flat, undulating, curving, and serrated. The macroscopic characterisation findings revealed 20 colonies having a flat elevation and 2 colonies with a convex elevation. The color of bacterial colonies varies greatly, namely: 5 yellow, 14 beige, 2 clear, and 1 white Table 1.

The variation in colony color is attributed to the existence of internal pigments produced by bacteria (Chauhan and Singh, 2024). Gram test results showed 17 bacterial isolates of Gram-negative and 5 isolates of Gram-positive bacteria. The Gram test method using KOH provides more accurate and practical analysis results compared to the Gram staining procedure (Sohel *et al*., 2016). The results of endophytic bacteria from local organic rice rhizospheres with different plateaus produced catalase tests that were dominated by positive catalase (17 isolates), while catalase negative was only 5 isolates. After adding H_2O_2 solution bubbles indicating that the sample is positive for the catalase enzyme, while the remaining 5 isolates including catalase-negative bacteria do not have a catalase enzyme that can break down H_2O_2 into water and oxygen (O_2). The H_2O_2 is s hazardous to the metabolic system of bacteria when they are unable to break down the molecule into less harmful substances. However, this can also be achieved when the catalase enzyme is available without bacteria.

Growth-promoting test analysis indole-3- acetic acid (IAA) production: The production of the IAA by bacterial isolates is influenced by the specific kind of local rice rhizosphere isolates and the altitude of the sampling location. The findings showed that all isolates possess the ability to generate the IAA. The qualitative test results (Figure 1) demonstrated a range of suspension color changes to pink during the synthesis of IAA in all isolates of local rice nitrogen-fixing bacteria after the application of Salcowski reagent, as compared to the control. The outcomes of qualitative analysis exhibit a direct correlation with the results of the quantitative test.

Table 1. Morphological and biochemical characteristics of bacteria isolated from the rhizosphere of local rice in the highlands of North Luwu Regency, South Sulawesi, Indonesia.

No.	Sample Code	Colony Morphology					Gram	Catalase
		Size	Colony Shape	Colony Edge	Altitude	Color		
1	PTHKN01	Small	Round	Whole	High	Yellow	-	+
2	PTHKN02	Small	Round	Whole	Low	Beige	+	+
3	PTHKN03	Small	Round	Whole	High	Beige	+	+
4	PTHKR02	Small	Round	Whole	High	Beige	+	+
5	PTAKN01	Small	Round	Whole	Low	Beige	-	-
6	PTAKN02	Medium	Round	Whole	Low	Transparent	-	-
7	PTAKN03	Medium	Irregular	Wavy	Low	Yellow	-	+
8	PTAKN04	Small	Round	Whole	High	Beige	-	+
9	PTAKR01	Small	Round	Whole	Low	Beige	-	+
10	PTAKR02	Small	Round	Whole	Low	Yellow	-	+
11	PTAKR03	Medium	Round	Whole	Low	Beige	-	+
12	PBU101	Small	Round	Whole	Low	Yellow	-	+
13	PBU102	Medium	Round	Whole	Low	Beige	-	+
14	PBU103	Medium	Round	Whole	High	Beige	-	+
15	PBU104	Medium	Round	Whole	Low	Transparent	+	+
16	PBU201	Medium	Round	Whole	Low	Beige	+	+
17	PBU202	Small	Irregular	Wavy	Low	Beige	-	+
18	PBU203	Small	Round	Whole	Low	White	-	+
19	PBU204	Small	Round	Whole	Low	Beige	-	-
20	PBU205	Small	Round	Whole	Low	Beige	-	-
21	PBU206	Small	Round	Whole	Low	Yellow	-	+
22	PBU207	Small	Round	Whole	Low	Beige	-	-

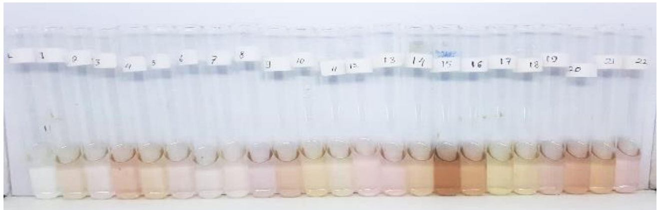

Figure 1. IAA synthesis by bacterial isolates: a qualitative study using Salkowski reagent. Pink color change was examined in the supernatant.

The production of IAA by endophytic bacterial isolates exhibits significant variation depending on the specific rhizosphere isolates of local rice varieties and the sample locations at different elevations. The endophytic bacterial isolates were quantitatively examined for their ability to generate IAA, with findings ranging from 0.28 milligrams per liter to 3.39 (Figure 2). The bacterial isolates found in the rhizosphere of local rice plants in Rongkong, specifically the banda rata rice variety, produced a stronger pink color and had the highest concentration of IAA at 3.39 milligrams per liter. This was followed by the local rice isolates of the banjara variety from the Rongkong sub-district, which had an IAA concentration of 2.33 milligrams per liter. The local rice rhizosphere bacterial isolates of the tarone hoyane variety from the Seko sub-district had the lowest IAA concentration at 2.08. The bacterial isolate obtained from local rice varieties in the Seko sub-district has an IAA concentration of 1.14 milligrams per liter. The variation in type is believed to arise from differences in plant sampling locations and the bacterial species involved. Differences in species and strains within the same genus might result in different levels of IAA production, which can be influenced by environmental conditions, the rate of growth, and the presence of substrates such as amino acids and other nitrogen sources (De Souza *et al.*, 2015; Susilowati *et al.*, 2018)

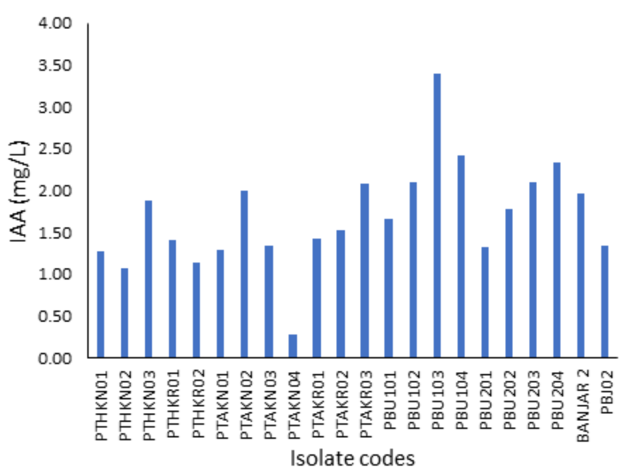

Figure 2. Hormone IAA-producing ability of bacterial isolates from local rice rhizosphere.

Phosphate solubility test: Endophytic bacterial isolates that have the ability to dissolve phosphate can be known qualitatively by the formation of a halo zone around bacterial isolates grown on Pikovskaya agar media modified by the administration of Bromophenol blue about 0.01 gram per liter (g/L). The results revealed the existence of 22 endophytic bacteria isolates capable of creating separate clear zones surrounding bacterial colonies on a solid Pikovskaya medium. These zones exhibited varying colony and zone sizes. The capacity of endophytic bacteria to solubilize phosphate is distinguished by the development of transparent regions surrounding colonies that thrive on solid Pikovskaya media. The ability of endophytic bacteria to solubilize phosphate is contingent upon the growth and formation of a distinct zone by bacteria (Cherchali *et al.*, 2015). The present study also found that the solubilization index of phosphate dissolution varied from 1.86 to 2.64 Table 2. Isolate code PTKNO4 was the highest in phosphate solubilization ability with a solubilization index (IP) value of 2.64 and the lowest isolate was isolate code PBU101. Phosphate available in nature can be in the form of organic phosphate and inorganic phosphate. The availability of organic phosphate comes from components of living things, such as plants, animals, and microorganisms in the form of phospholipids and phytin.

Table 2. Qualitative analysis of phosphate solubilization ability of endophytic bacterial isolates from the rhizosphere of local North Luwu rice.

	solubilization index	Halo zone diameter (mm)	Colony diameter (mm)
PTHKN01	2.16	1.20	0.91
PTHKN02	2.26	1.13	1.01
PTHKN03	2.47	1.00	0.91
PTHKR01	2.28	1.16	0.86
PTHKR02	2.52	1.32	1.10
PTAKN01	2.60	1.01	0.73
PTAKN02	2.36	1.00	0.70
PTAKN03	2.13	1.26	0.98
PTAKN04	2.64	0.75	0.71
PTAKR01	2.29	1.06	0.91
PTAKR02	2.39	1.43	1.16
PTAKR03	2.51	1.36	1.18
PBU101	1.86	1.15	0.75
PBU102	2.37	0.86	0.86
PBU103	2.32	0.88	0.63
PBU104	2.47	0.68	0.65
PBU201	2.51	1.03	0.76
PBU202	2.24	1.35	1.06
PBU203	1.86	0.93	0.88
PBU204	2.17	1.26	0.86
PBU205	2.25	1.06	0.91
PBU206	2.27	1.35	1.06

***In vitro Growth Test*:** In vitro study of rice seed growth spurt using the best bacterial isolate from IAA and phosphate dissolution. Of the 22 bacterial isolates found, 10 of the best isolates were selected for phosphate solvent test (PTAKN04, PTAKN03, PTHKR02, PBU201, PTAKR03, PTHKN03, PBU104, PTAKR02, PTAKN02, PBU102). 10 isolates from the indole three acetic acid (IAA) test (PBU103, PBU104, PB204, PBU203, PBU102, PTAKR03, PTAKN03, PBU205, PTHKN03, PBU202). Synergistic bacterial isolates are used as a consortium of bacterial isolates. The in vitro culture medium used was MS liquid medium with half concentration at Ph 5.7 The results of observation of sprout growth power after one week showed that the consortium B1 (PTHKN04), B20 (PB204), B10 (PBU103). After the growth test, there were 3 isolates, the best of which were the results of sequencing analysis and 16s rRNA analysis.

***Molecular identification*:** The results of 16S rRNA gene sequencing of local rice rhizosphere bacteria isolates of North Luwu Highland produced the three best isolates to be analyzed. Three superior isolates have physiological characters are thermotolerant non-pathogenic that have the ability to boost the growth of rice plants. The isolation of Deoxyribonucleic Acid (DNA) and 16S rRNA gene application with PCR technique obtained DNA fragments with a length of 1,500 base pair (bp). The results of the 16S rRNA gene sequence using the Blastn program showed that bacterial isolate B1 has a percentage similarity of 99.22% (*Pseudomonas* species strain), B20 was 99.57% (*Pseudomonas fluoresces* and *Pseudomonas montel* strain), while bacterial isolate B10 was 99.93% (*Stenotrophonas* species strain bacteria).

DISCUSSION

The present research was conducted to isolate endophytic bacteria in the rhizosphere of local rice at high altitudes in North Luwu Regency, Sulawesi province, Indonesia. This activity was carried out to characterize four local rice varieties with different sea level altitudes. The morphological characteristics, biochemistry, molecular identification, and plant growth-promoting analysis were performed to determine the potential of endophytic bacteria as plant growth and biofertilizers. The morphological characteristics test of colonies which include size, shape, color and edges on endophytic bacterial isolates showed several differences. Grouping based on colony size resulted in small size, and medium size, namely 15 small size, and 7 medium size respectively. Grouping based on colony shape was dominated by 20 round/round colonies, while there were 2 irregular shapes. The edges of the colonies look varied shapes ranging from flat, wavy, curved, and jagged. Colony elevation of macroscopic characterization results showed 20 colonies with flat elevation and 2 convex colonies. The color of bacterial colonies varies greatly, namely, 5 yellow, 14 beige, 2 clear, and 1 white. Morphological characterization of bacterial isolates based on Table, shows 4 of the tarone variety samples, 7 of the tarone variety hoyane local rice from Seko District, while from Rongkong District there are 4 samples from bandarata varieties and 7 from banjara varieties. The diversity of endophytes in different plants has been reported and it has also been noted that in one host plant, endophytic bacteria are not limited to a single species but have several different genera and species living in it (Kuźniar *et al.*, 2020). The present study is in line with (David *et al.*, 2016), who noted that changes in sample sites, rainfall, and cultivation conditions influence the variety of endophytic fungal characteristics. According to (Kumar *et al.*, 2020) that Endophytes are ubiquitous in almost all plant species growing worldwide and these microbes are known to provide direct benefits to the host plant by increasing nutrient uptake by the plant and controlling plant growth through phytoharmon synthesis. The presence of endophytic bacteria in highland plants greatly contributes to the growth and development of plants in highlands such as local rice in North Luwu. Gram test using 3% KOH exhibited 17 bacterial isolates of Gram-negative and 5 isolates of Gram-positive bacteria. This is because the cell wall structure of bacterial isolates for the Gram-positive test is composed of peptidoglycan and Gram-negative cell wall structure consists of lipids. Gram-positive bacteria occur protein denaturation due to alcohol washing so that the protein becomes hard and frozen, the pores shrink and the permeability of the cell wall decreases so that the colored violet crystals can be retained, while Gram-negative bacteria in the cell wall dissolve during alcohol washing so that the pores and cell walls are large so that the violet crystals are absorbed (Alfred, 2015; Sianipar *et al.*, 2020). Identification of bacteria and production isolates through physiologic properties is a way of biochemical physiology test. Catalase test is done to identify bacteria that produce catalase enzyme. Endophytic catalase enzyme is known when using 3% hydrogen peroxide solution as a substrate. The results of the catalase test giving 2 drops of 3% H_2O_2 resulted in 17 isolates reacting positively with the appearance of gas bubbles while 4 isolates reacted negatively with no gas bubbles. A positive reaction on local rice endophytic bacterial isolates is able to produce catalase enzyme. Catalase enzyme in bacteria is an enzyme that functions to break down H_2O_2 formed from the aerobic respiration process of bacteria, into water (H_2O) and O_2. Accumulating H_2O_2 within bacterial cells can impair the metabolic function of the bacteria and potentially result in mortality (Deepika *et al.*, 2023). Based on the results of 16S rRNA sequence analysis in this study, it shows that all isolates analyzed have 98% similarity so the isolates can be used for species identification. The results of the 16S rRNA gene sequence using the Blastn program showed that bacterial isolate B1 has a percentage similarity of 99.22% with the description of *Pseudomonas* species strain and B20 has a percentage identity of 99.57% with the description of

Pseudomonas fluorences, *Pseudomonas montel* strain. Bacterial isolate B10 has a percentage identity of 99.93% with the description of *Stenotrophonas* species strain. Pseudomonas is a major producer of salicylic acid, HCN, and IAA that promotes plant growth and growth and improves environmental conditions. Pseudomonas fluorescens is composed of environmental opportunistic pathogenic bacteria that are beneficial to plants known as biocontrols and growth promoters (Raio, 2024). The capacity of endophytic bacterial isolates to generate the IAA varied substantially depending on the kind of rhizosphere isolates from local rice types and sampling locations at various elevations. Endophytic bacterial isolates evaluated quantitatively to generate IAA yielded findings ranging from 0.28 milligrams per liter to 3.39 milligrams per liter Figure 2. Auxin, the phytohormone most commonly released by endophytic bacteria in plants, stimulates the formation of adventitious roots, lateral roots, and root filaments by providing nutrients to the roots in order to promote root growth and development (Hilbert *et al*., 2012; Sahu, 2019). Endophytic bacteria that colonize in plant tissues can act as biocontrol agents and also plant growth-promoting factors (Petrović *et al*., 2024). Endophytic bacteria has the capability to solubilize phosphate via the production of clear zones surrounding their colonies, resulting in increased colony diameters. The findings demonstrated that all endophytic bacterial isolates had the capacity to solubilize phosphate, as shown by varying colony sizes and clear zone diameters observed. The ability of endophytic bacteria to solubilize phosphate is contingent upon the growth and formation of a distinct zone by bacteria (Cherchali *et al*., 2019). The findings indicated that the solubization index of phosphate dissolution ranged from 1.86 to 2.64, as shown in Tabel 2. Phosphate is a crucial macronutrient for the growth and development of plants. The soil contains a significant amount of phosphorus, both in organic and inorganic forms. However, the majority of it is insoluble (around 95-99%), making it inaccessible for plant absorption. To address this issue, the use of phosphate-solubilizing endophytic bacteria is necessary to supply plants with the required phosphate nutrients (Wiratno *et al*., 2019). Phosphate solubilization is a significant characteristic in which bacteria enhance plant development by increasing the availability and absorption of phosphate. The PGPR employs an insoluble form of phosphate and transforms it into a soluble form that may be readily used by plants MENDELEY CITATION PLACEHOLDER 15. A total of 22 bacterial isolates were discovered in the root systems of two distinct rice plant kinds in the Seko region, as well as two other rice plant varieties in the Rongkong area, which vary in height. The isolates possess the capability to synthesize IAA and solubilize phosphate. The findings of this investigation demonstrated a shift in the supernatant's color to pink and variations in the concentrations of IAA present in each endophytic bacteria isolate. Likewise, the presence of distinct areas devoid of growth surrounding the bacterial colonies on solid Pikovskaya medium demonstrated the capacity of the endophytic bacterial isolates to break down phosphate. The code PTKNO4 isolate had the best phosphate dissolving ability, with an Solubilization index of phosphat (IP) value of 2.64. On the other hand, the PBU101 isolate had the lowest ability, with an Solubilization index of phosphat (IP) value of 1.86. The variation in type is due to the location of plant sample and the types of bacterium.

Conclusion: A number 22 isolates of rhizobacteria has been found in the sampling location. Morphological characterization showed high diversity and differences in isolates in terms of color, size, and shape. The 16S rRNA gene sequence results found that bacterial isolate B1 the description of Pseudomonas sp strain and B20 the description of Pseudomonas fluorences, Pseudomonas montel strain. Meanwhile, bacterial isolate B10 the description of Stenotrophonas species strain bacteria. The IAA bacterial ability test revealed that the IAA-producing local rice rhizosphere bacterial isolates from Rongkong, specifically the bandarata rice varieties showed a more vivid pink tone and had the greatest concentration of IAA (3.39 mg/L). Following closely were the local rice isolates of banjara varieties from Rongkong district (2.23 mg/L) and the local rice rhizosphere bacterial isolates of tarone hoyane varieties from Seko District (2.08 mg/L). In contrast, the isolate of local rice bacteria varieties tarone from Seko sub-district had a lower value of (1.14 mg/L). The isolate ability test of rice rhizosphere varieties revealed that tarone hoyane, a local variety, possesses the capacity to solubilize phosphate with an IP value of 2.64. Isolate PBU101, which originates from the rhizosphere bandarata in the Rongkong district, has the lowest IP value (1.86), followed by rice varieties banjara. The results of this study are intended to be continued to plants because the bacteria produced can be used as growth spurs and as biocontrols.

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SDG's addressed: Zero Hunger, Responsible Consumption and Production.

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