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Characterization of the Existence of Morphology Endophyte Bacteria of Garlic (*Allium sativum* L.) with Plant Growth Promoting Rhizobacteria (PGPR) in the Highland Areas

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ABSTRACT

Utilization of plant growth promoting rhizobacteria (PGPR) is a strategy to increase the growth and yield of local garlic varieties. Bacteria contained in PGPR can act as a plant growth hormone and as a biological fertilizer. This research aimed to determine the characteristics and presence of bacteria on the roots of garlic plants after PGPR application and to evaluate the growth and yield of the best garlic varieties in the highlands. Isolation of garlic root endophytic bacteria was taken from PGPR applied in garlic cultivation in the highlands. The experiment used a randomized block design with six garlic varieties (Lumbu Putih, Lumbu Kuning, Lumbu Hijau, and Jangkiriah Adro) treated with 12.5 ml.L^{-1} of PGPR. Observations included plant height, bulb weight, number of colonies, and macroscopic form of bacteria. The results showed that the highest number of endophytic bacterial colonies (225) was found in the root isolates of Lumbu Putih variety, non-treated with PGPR. After PGPR treatment, the highest number of bacteria colonies (200) was found in Lumbu Kuning root isolates. The morphological characteristics of the bacterial colonies in the six isolates were round, wavy, convex, and raised, jagged edges, raised and flat, and the color was yellowish white. Jangkiriah Adro variety had higher values in plant height and bulb weight per plant compared to Lumbu Putih, Lumbu Kuning, and Lumbu Hijau varieties.

INTRODUCTION

Indonesia's natural wealth has an abundance of diverse flora and microbes. One of them is that various types of garlic are available and widely used in medicine and food. Each garlic variety has a suitable growing environment. Garlic's ability to survive can be achieved with the help of microbes in the tissue and environment around where it grows, where the microbes are beneficial for photostimulators, which have a direct effect on increasing growth and producing hormones (Cahyaty et al., 2017).

The existence of microbes in the form of bacteria in plants and the planting environment is still unknown. Identifying the types of endophytic bacteria in plant roots by looking at their characteristics, number of colonies, and potential still needs to be done. Plants have a close relationship with endophytic bacteria

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because the chemical compounds produced by bacteria have various types of bioactivity. The similarity of the bioactive compounds of endophytic bacteria with the host plant occurs due to genetic transfer from the plant. Endophytic bacteria are useful in producing nutrients resulting from cell metabolic processes and resistance to pathogen attacks on host plants (Yulianti, 2012; Susilowati et al., 2018).

Endophytic bacteria colonize the internal parts without damaging the plant tissue. Secondary metabolic compounds produced by endophytic bacteria can be produced by isolating the host (Tan et al., 2001; Radji, 2005; Ryan et al, 2007)characterization of bacteria by looking at the morphology of colonies and cells in isolates through selection. The aim of bacterial characterization is to observe colony morphology and bacterial cell morphology in the selected bacterial isolates (Cappuccino and Sherman, 2001). Endophytic bacteria live in the roots, stems, leaves, and fruit tissues of all types of plants. The addition of several bacterial strains in the plant growth phase can help plants minimize pathogen attacks, absorb nutrients, and withstand extreme environments.

The group of bacteria used to increase growth and yield in garlic is also called Plant Growth Promoting Rhizobacteria (PGPR). PGPR colonizes plant roots and supplies growth-stimulating hormones, biocontrol agents, and biopesticides (Hayat et al., 2018; Mohamed et al., 2019; Hafez et al., 2021). Several rhizosphere bacteria live freely in root nodules and plant root surfaces, such as *Rhizobium, Pseudomonas,* and *Bacillus* (Ristiana *et al*., 2021).

Research regarding the isolation, characterization, and identification of the presence of endophytic bacteria that colonize the roots of garlic plants after PGPR application needs to be carried out. This study aims to visually characterize endophytic bacteria in garlic roots (*Allium sativum* L.) after being given PGPR and to see the growth and yield of garlic given PGPR in the highlands.

MATERIALS AND METHOD

This research was carried out in Alahan Panjang, Solok Regency. West Sumatra from November 2023 to May 2024. The research was carried out in 2 stages, designed using a Randomized Group Arrangement (RAK). The first stage uses treatment with four varieties of garlic, namely, Lumbu Putih (BP1), Lumbu Kuning (BP2), Lumbu Hijau (BP3), and Jangkiriah Adro (BP4). Bacteria Consortium PGPR (contains *Rhizobium* sp., *Bacillus polymixa,* and *Pseudomonas fluorescens*) with a concentration of 12.5 $ml.L^{-1}$ (Iswati, 2012; Purniawati et al., 2021). The second stage, isolation of plant root bacteria, counts all bacterial cell colonies still active or inactive in a medium (Madigan et al., 2012). Macroscopic observations consist of elements that can be seen with the naked eye, namely the shape, edges, height, and surface of bacterial colonies (Cappuccino dan Sherman, 1987).

Planting Garlic Varieties in the Highlands

Garlic plants were cultivated in Alahan Panjang, Solok Regency, West Sumatra, at an altitude of 1,450 m above sea level and a temperature range of 14^0C -21^0C, with an average rainfall of 1 978.50 mm per month per year (BPS, 2023). Garlic plants were cultivated according to a predetermined plan until harvest.

Root Sampling

Garlic roots were taken after the plants were harvested according to the samples specified in the design. Then, the roots are cut and stored in plastic to keep the roots in fresh condition.

a. Isolation of Rhizosphere Bacteria

The tools that will be used for isolation are cleaned and sterilized first. Plant roots were taken from the bulb's base to the root's tip, cleaned of dirt with running water, and sterilized by soaking in 70% alcohol for 5 minutes. Ten grams of sterilized roots were ground, and 1 ml of extract was taken and then put into a test tube containing 9 ml of 0.85% NaCl solution. Isolation was carried out by making a dilution series (10^{-1} – 10^{-7}). The following isolation process is by pipetting 1 ml of the sample solution at a dilution of 10^7, placing it in a petri dish filled with still liquid Nutrient Agar (NA) media using a pour plate, and incubating at a temperature of 28°C.

Bacterial growth was observed every day; observations were made for 2 days. The number of bacterial populations that grow on NA media is calculated in colony-forming units (CFU). The estimated number of colonies is calculated based on a formula (Klement et al., 2015):

Colonies per ml = Number of colonies per plate x 1/dilution factor ($CFU.mL^{-1}$).

b. Purification Process

The bacterial purification process uses scratch by taking all colonies with different shapes and then purifying them on NA media. Purification aims to obtain pure isolates. The isolate obtained was then streaked repeatedly until a single isolate was obtained. Next, incubation was carried out at 28 °C and growth was observed for 2 days.

c. Macroscopic Morphology Identification

All single isolates that had been grown on NA media were observed for their morphology by making direct observations. The morphology of the bacteria is seen in the colony shape, elevation, edge, and color.

Observation Variables

The observation variables carried out in the field were plant height (cm) and plant bulb weight (grams). Observations in the laboratory include the number of colonies, colony shape, agar surface height, and colony edge (degree of bacterial growth).

Data analysis

Field observation data was analyzed using analysis of variance (ANOVA), and the mean comparison was performed by the honestly significance difference (HSD) at the α=0.05 level. Laboratory observation data, namely, the morphological characteristics of endophytic bacteria, are presented in macroscopic and descriptive visual form.

RESULTS AND DISCUSSION

Field Observation Results

Plant height and bulb weight per plant of garlic varieties were significantly different from those given PGPR. The variety Jangkiriah Adro (BP4) showed the best growth in plant height at 70 HST and bulb weight per plant compared to Lumbu Putih (PB1), Lumbu Kuning (BP2), and Lumbu Hijau (BP3). The varieties Lumbu Kuning (BP2) and Lumbu Hijau (BP3) showed almost the same growth in plant height and bulb weight per plant but were significantly different from Lumpu Putih (BP1). Plant height and bulb weight per plant can be seen in Table 1.

The growth of plant height and bulb weight of the Jangkiriah Adro variety is still good even though it is planted outside the area of origin of the variety namely Kerinci, also with plant height and bulb weight of the Lumbu Hijau and Lumbu Kuning varieties. This is because the three varieties grow well in the highlands, where the climatic conditions are the same as the location of this research. The vegetative and generative growth of Jangkiriah Adro, Lumbu Hijau, and Lumbu Kuning is influenced by the availability of nutrients that the plants can absorb and the favorable conditions in which they grow.

This is also influenced by the plant's ability to adapt to the growing environment with the help of the PGPR strain provided. The bacteria contained in PGPR work together with bacteria in plant roots, helping the Jangkiriah Adro, Lumbu Putih, Lumbuh Hijau, and Lumbuh Kuning varieties against environmental stress. According to Koza et al. (2022), bacteria that develop in plants can increase growth and help plants adapt to less favorable growing environments. The mechanism of PGPR's role is through hormonal regulation, nutritional balance, dissolution of nutrients, facilitating plant absorption, and increasing resistance to pathogen attacks (Vejan et al., 2016)

The bacteria that make PGPR in the form of a mixed inoculum of *Rhizobium* sp., *Bacillus polymixa,* and *Pseudomonas fluorescens*, which are applied to garlic varieties, can play

Table 1. Plant height and tuber weight of garlic plant treated with PGPR

Variety	Plant height (cm)	Plant bulb weight (g)
Lumbu Putih	26.03a ± 5,44	2.76a ± 0,43
Lumbu Kuning	36.03b ± 2,44	5.39b ± 1,99
Lumbu Hijau	40.57b ± 5,73	5.33b ± 1,36
Jangkiriah Adro	48.72c ± 4,10	10.78c ± 2,05

Note: numbers followed by different letters in the same column show significant differences at the 5%

an essential role in helping maintain the stable growth of Jangkiriah Adro, Lumbu Hijau, Lumbu Kuning, and Lumbu Putih through various mechanisms, such as producing phytohormin which functions to stimulate root growth so that they can absorb more nutrients. The role of *Pseudomonas* bacteria is to dissolve and release nutrients bound in the soil to make them available to plants. *Bacillus* bacteria can produce phytohormone compounds. San-Lang et al. (2008) state that the phytohormone compounds produced by *Bacillus* include auxin, cytokinin, ethylene, gibberellin, and abscisic acid. *Bacillus* has endospores that respond to extreme environments. Meanwhile, Rhizobium bacteria can carry out their activities with the help of compounds produced by plant roots.

Lumbu Putih experienced significant differences in plant height growth and bulb weight per plant compared to other varieties. Genetic and environmental factors influence height growth and lower bulb weight. Lumbu Putih is an adaptive variety in the lowlands; the growth and weight of the bulbs per plant will reach the optimal weight in a suitable environment. Plants that can grow and produce in an unsuitable environment are a form of positive response to the growing area, which can be seen from the characteristics of the plant's growth. According to (Simon et al., 2003; Volk et al., 2003; Allard, 2005), garlic is very responsive to the environment so that a clone will vary depending on the planting location.

Providing PGPR can trigger the ability of Lumbu Putih to survive and minimize the impact of environmental factors on the development of plant cell metabolism. The applied PGPR can increase growth through random metabolite production (Tang et al., 2020). PGPR can also increase the availability of other nutrients such as phosphate, sulfur, iron, and copper.

Results of Isolation of Rhizosphere Bacteria

a. Number of Root Endophyte Bacterial Colonies

Observations of the number of bacterial colonies of Lumbu Putih (PB1), Lumbu Kuning (BP2), Lumbu Hijau (BP3), and Jangkiriah Adro (BP4), which were developed on NA media for 2 days at a temperature of 28°C, can be seen in Figure 1.

Figure 1 shows bacterial colonies incubated for 2 days on garlic varieties. Based on the number of garlic root endophytic bacterial colonies calculated in 8 petri dishes with a retail of 10^{-7}(Table 2).

The number of endophytic bacterial colonies of garlic roots without PGPR treatment on Lumbu Putih, which is as many as 225 colonies (Table 2). This is thought to be caused by the endophytic bacteria in Lumbu Putih tissue suppressing the development of exophytic bacteria that enter the host's body. Meanwhile, Lumbu Kuning, Lumbu Hijau, and Jangkirah Adro had fewer colonies. Lumbu Putih is a garlic variety that is adaptive to high and medium temperature conditions so that the development of plant cell metabolism is more stable. The addition of the PGPR strain during growth did not affect the presence of bacteria on the roots. Afzal et al. (2019) state that bacteria are related to bacterial strains, species, cultivars, genotypes, and plant-growing environmental factors.

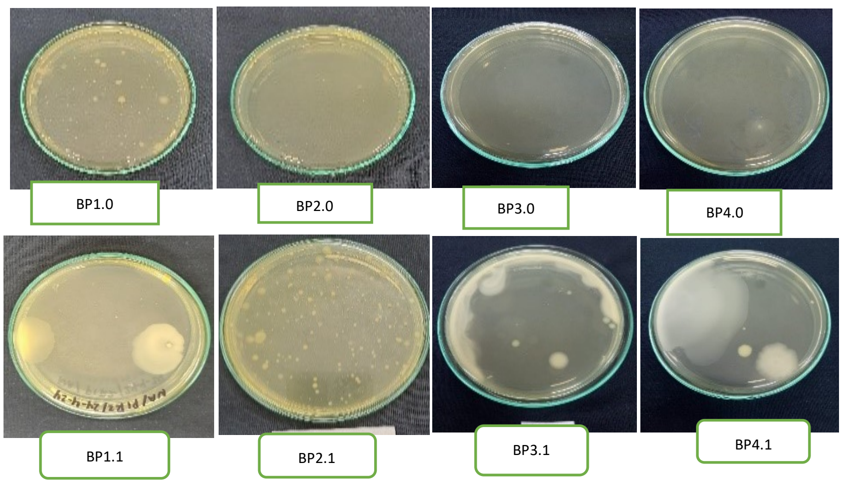

Figure 1. Isolation of root endophytic bacteria of garlic varieties, Lumbu Putih (BP1.0 and BP1.1), Lumbu Kuning (BP2.0 and BP2.1), Lumbu Hijau (BP3.0 and BP3.1), and Jangkiriah Adro (BP4.0 and BP4.1)

The number of garlic bacterial colonies after PGPR application for the Lumbu Kuning variety

Table 2. Number of root endophytic bacterial colonies of garlic varieties treated with PGPR

Isolate code	∑ Bacterial colony	Bacterial cell density
BP1.0	225	CPU 2,33 x 10^9
BP1.1	3	CPU 3,0 x 10^7
BP2.0	14	CPU 4,1 x 10^8
BP2.1	200	CPU 2,0 x 10^9
BP3.0	2	CPU 2,7 x 10^8
BP3.1	15	CPU 1,5 x 10^8
BP4.0	2	CPU 2,0 x 10^7
BP4.1	9	CPU 9,0 x 10^7

Note: Lumbu Putih (PB1), Lumbu Kuning (BP2), Lumbu Hijau (BP3), Jangkiriah Adro (BP4), dilution factor (CFU.mL^{-1})

reached 200 colonies, while for Lumbu Putih, Lumbu Hijau, and Jangkiriah Adro, the number of colonies was less. This could be caused by the application of the inolum that makes up PGPR during garlic growth, which can influence the development of bacteria that previously existed on the roots. The resistance factors of plant tissue can also affect the development of bacteria on plant roots in responding to microbes in the soil. Plant roots can also release bioactive compounds such as flavonoids, carotenoids, vitamins, and minerals. According to Vacheron et al. (2013); Husseiny et al. (2021), endophytic bacteria produce metabolites similar to their hosts, and plants secrete exudates through the roots, which influence the expression of bacterial genes so that plants can utilize them. Produce metabolites similar to those of their hosts.

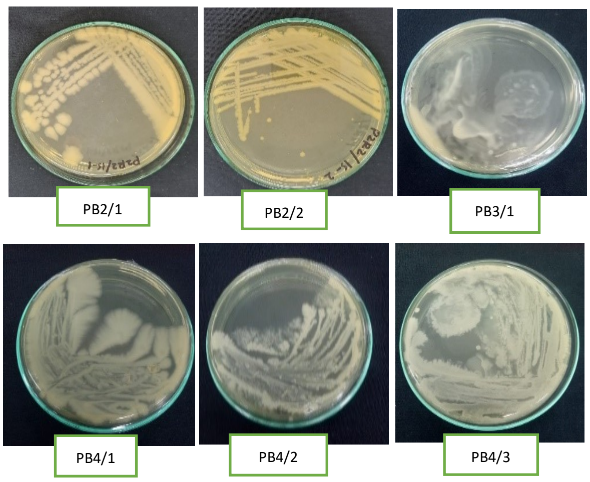

Figure 2. Single isolate resulting from purification of garlic root endophytic bacteria, Lumbu Kuning (BP2/1, BP2/2), Lumbu Hijau (BP3/1), dan Jangkiriah Adro (BP4/1, BP4/2, BP4/3)

The bacteria that make up PGPR have a role according to the type of bacteria contained therein. The group of microbes from the phytomicrobiome can not only increase plant production but also increase the ability of plants to resist biotic and abiotic stresses (Backer et al., 2018; Lyu et al., 2020; Sindhu et al., 2020).

b. Macroscopic Observation of Root Endophytic Bacteria

The results of inoculation of bacteria that develop on the roots of garlic plants of the Lumbu Putih (PB1), Lumbu Kuning (PB2), Lumbu Hijau (BP3) and Jangkiriah Adro (BP4) varieties are in Figure 2.

The number of endophytic bacterial isolates found in Lumbu Kuning (PB2) was 2 isolates, Lumbu Hijau (PB3) 1 isolate, and Jangkiriah Adro (BP4) produced 3 isolates, each of which showed various morphological characteristics in each isolate petri dishes in Figure 2 and Table 3. The macroscopic observations of 6 isolates showed the morphological characteristics of bacterial colonies with a round shape, wavy filaments like roots, convex and raised elevations, jagged and flat edges, white and yellowish white.

Differences in the types of bacteria present in the roots of Lumbu Putih, Lumbu Hijau, Lumbu Kuning and Jangkiriah Adro cause the various shapes, edges, elevations and colors of endophytic bacteria. Other causes are influenced by environmental factors such as climate, soil, and where the host grows. Huang (2019) states that the genus and species of bacteria affect the character of endophytic bacteria in plants. Meanwhile, the color that appears in bacterial isolates is due to the presence of pigments.

Bacteria synthesize pigments for secondary metabolites which are useful as protection from UV rays, oxidants, temperature and dryness (Wada et al., 2013; Kramar and Kostic, 2022). Bacteria isolated on solid media are in the form of pointed, round, and circular colonies. The surface of endophytic bacterial colonies is flat, raised, curved, forming hills. The edges of the colony are intact, split, or thready (lobate), stringy, and curly (Mohamed et al., 2019).

Table 3. Morphological characteristics of garlic root endophyte bacterial isolates

Isolate code	Colony macroscopic characteristics			
	Form	Edge	Elevation	Color
PB2/1	Round	Serrated edge	Arise	White
PB2/2	Like filament	Regular edges	Arise	Yellowish white
PB3/1	Round	Irregular	Flat	Yellowish white
PB4/1	Like roots	Serrated edge	Arise	Yellowish white
PB4/2	Like roots	Serrated edge	Arise	Yellowish white
PB4.3	Like roots	Irregular	Arise	Yellowish white

Note: PB = isolate code, 1, 2, 3 = isolate code number

CONCLUSION

The Jangkiriah Adro variety showed the best growth in plant height and bulb weight per plant compared to the Lumbu Putih, Lumbu Kuning, and Lumbu Hijau varieties. The number of bacterial colonies in the root isolate of the Lumbu Putih variety without PGPR was 225 colonies. After administering PGPR, the highest number of colonies was found in the Lumbu Kuning variety isolate, namely 200 colonies. Purification isolation was carried out for 6 isolates. Almost all isolates have similarities in colony color and shape but differ in the colony's surface. The morphological characteristics of the bacterial colonies in these isolates are round, wavy, convex, raised elevations, jagged edges, raised and flat, and yellowish-white in color.

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