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Diversity analysis of soil microbial communities in Inpari 32 rice fields following indigenous endophytic bacteria applications

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Abstract. *Ridwansyah R, Prihatiningsih N, Lestari P, Kharisun, Djatmiko HA, Irwandhi. 2025. Diversity analysis of soil microbial communities in Inpari 32 rice fields following indigenous endophytic bacteria applications. Asian J Agric 9: 245-254.* The challenge of rice farming in Indonesia is exacerbated by land degradation resulting from the use of chemical fertilizers and pesticides, which is further intensified by climate change. The use of endophytic bacteria in rice farming is an environmentally friendly and sustainable approach that increases microbial diversity and soil productivity. This study investigates the effects of indigenous endophytic bacterial applications on soil microbial diversity in Inpari 32 rice (*Oryza sativa* L.) fields. The study involved the application of indigenous endophytic bacteria to rice plants, the isolation and characterization of soil bacteria following application, and the evaluation of microbial diversity using the Shannon-Wiener diversity index. A hypersensitivity test was conducted to assess the safety and compatibility of the plant with bacteria. The study applied the endophytic bacterial formula evenly to the lower and upper surfaces of rice leaves using a sprayer, with treatments administered five times at 56, 62, 67, 72, and 77 days after planting. The results demonstrated that the treatment enhanced the diversity of soil microbial, including genera such as *Paenibacillus*, *Micrococcus*, *Methylobacterium, Streptomyces*, *Actinomyces*, *Acremonium,* and *Penicillium.* The diversity index indicated that treatments from: (i) Petanahan Kebumen; (ii) Karangwangkal Banyumas; (iii) Sumbang Banyumas, and the consortium; (iv) fell into the moderate diversity category. The hypersensitivity test revealed that the isolate coded C104 1 U2 exhibited necrosis symptoms, indicating pathogenicity, while the other isolates showed no necrosis symptoms, suggesting they were beneficial non-pathogenic microbial. Necrosis symptoms indicate the presence of pathogenic microbial, whereas their absence confirms the presence of beneficial microbial. This study provides a scientific foundation for developing innovative agricultural practices to enhance microbial diversity and ecosystem resilience in rice cultivation systems.

Keywords: Characterization, endophytic bacteria, hypersensitivity, soil microbial, soil microbial diversity

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food crop that is essential to the world's population, serving as a primary food source (FAO 2021). In Indonesia, rice is a staple food for nearly 95% of the population, and its demand is expected to increase in line with population growth (Ani et al. 2024). Sustainable rice production activities are critical. Because the current agricultural system faces threats from climate change, soil degradation, and declining biodiversity (Nguyen et al. 2023). This condition is exacerbated by the reliance on chemical fertilizers and pesticides, which leads to environmental degradation and finally impacts on the soil microbial communities (Jin et al. 2022). Innovative and environmentally friendly strategies are urgently needed to enhance soil health and improve rice plant productivity in the face of environmental changes. Rhizomicrobiome engineering using local endophytic bacteria is a sustainable approach that can be applied (Irwandhi et al. 2024).

Endophytic bacteria are a group of microorganisms that inhabit plant tissues without harming their host plants (Afzal et al. 2019). These bacteria play a crucial role in synthesizing phytohormones, enhancing nutrient absorption in plants, and mitigating damage caused by pathogens by producing secondary metabolites, lytic enzymes, and hormone activators (Chaudhary et al. 2022). Indigenous endophytic bacteria from local ecosystems uniquely adapt to specific environmental conditions and host plants. This ability makes them the ideal candidates for biofertilizer development (Kuźniar 2025). Endophyte applications have been shown to stimulate beneficial soil microorganisms, thereby increasing biodiversity and soil resilience. In rice ecosystems, these bacteria can enhance nutrient cycling, suppress pathogenic organisms, and improve plant health, thereby contributing to sustainable crop production (Prihatiningsih et al. 2019, 2020).

Plants play an important role in determining microbial diversity in the rhizosphere. Plant roots cause physical and chemical changes in the rhizosphere that affect microbial diversity in and around the rhizosphere. Microbial populations in the rhizosphere are generally more numerous and diverse than in non-rhizosphere soils. Exudates produced by plant roots influence the activity of rhizosphere microbial. Some rhizosphere microbial play a crucial role in nutrient cycling and soil formation processes, influencing plant growth and microbial activity and acting as biological control agents against root pathogens (Sun et al. 2021).

Soil microbial, such as bacteria and fungi, demonstrate their ability to socialize and interact with other colonies by establishing intercellular communication through a mechanism called Quorum Sensing (QS). Gram-positive and Gram-negative bacteria apply quorum sensing for communication, but they produce different autoinducers. Gram-positive bacteria utilize modified oligopeptides, known as Autoinducer Peptides (AIPs). In comparison, Gram-negative bacteria mainly rely on N-acyl homoserine lactone (AHL) molecules (autoinducer-1, AI-1), while fungi rely on Volatile Aroma Compounds (VOCs) often used in fungal communication between fungi (Verbeke et al. 2017; Heffernan et al. 2023).

Developing and cultivating the Inpari 32 rice variety in Indonesia aims to obtain high rice yields and resistance to biotic stresses (Sitaresmi et al. 2023). Although its application has contributed to increased rice production, maintaining soil health in the Inpari 32 cultivation system remains challenging. The effect of local endophytic bacteria on soil microbial diversity in Inpari 32 land has not been thoroughly studied. Soil microbial diversity plays a crucial role in maintaining the functions of agricultural ecosystems, including nutrient recycling, organic matter decomposition, and pathogen suppression (Gupta et al. 2022). A diverse microbial community will be more resilient to environmental disturbances and provide various ecosystem services important for sustainable agriculture (Pedrinho et al. 2024). The Shannon-Wiener diversity index is a widely used metric for assessing microbial diversity, as it considers both species richness and evenness (Magurran 2013). Additionally, hypersensitivity testing is crucial to ensure that bacterial inoculants do not cause adverse effects on plants or disrupt native microbial communities (Swandi et al. 2019). This study investigated the impact of inoculating native endophytic bacteria on soil microbial diversity in Inpari 32 rice fields. It focused on the application of these bacteria, their effects on soil microbial communities, and their implications for sustainable rice production.

MATERIALS AND METHODS

Location

The research was conducted in Linggasari Village, Kembaran Sub-district, Banyumas District, Central Java, Indonesia, from July to November 2024. Soil analysis was conducted in the Soil Science Laboratory and Plant Protection Laboratory, Faculty of Agriculture, Universitas Jenderal Soedirman, Indonesia. The endophytic bacterial isolates used in this study were obtained from various locations. Isolates A5 and A6 were from Petanahan, Kebumen; KR4 and KR7 were from Karangwangkal, Banyumas; and SB3 were from Sumbang, Banyumas. Below are the original locations of the endophytic bacterial isolates seen in Figure 1.

Isolation and identification of endophytic bacteria

The process of isolation and identification of endophytic bacteria begins with plant root sampling using purposive random sampling method. Isolation was carried out by selecting two healthy rice plants, taking the roots, cleaning them with running water, and weighing 100 g each. The endophytic bacteria isolation process was carried out by sterilizing the root surface using sodium hypochlorite solution, rinsed three times using sterile water, then macerated using a sterile porcelain mortar, and added as much as 100 mL of sterile water. The 1 mL suspension obtained was mixed with 9 mL of sterile water, then incubated at 80°C for 30 minutes to isolate *Bacillus* sp. (Singh et al. 2016). Serial dilutions up to five times were carried out and the results of the fourth and fifth dilutions were grown on Nutrient Agar (NA) media using the spread plate method. Colonies that appeared were observed based on their macroscopic and microscopic morphological characteristics.

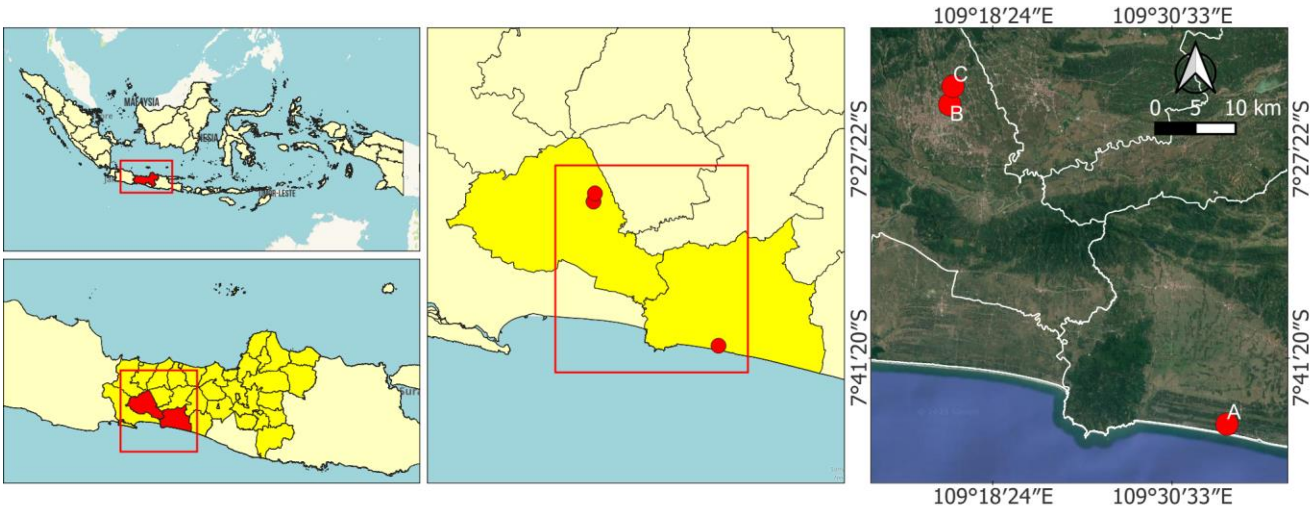

Figure 1. Locations of sources of endophytic bacterial isolates: A. Petanahan, Kebumen; B. Karangwangkal, Banyumas; C. Sumbang, Banyumas in Central Java, Indonesia

Application of endophytic bacteria

This research was conducted using a Randomized Group Design (RGD) consisting of 5 treatments, namely K, A, B, C, and D. Each treatment was repeated 5 times, so there were 25 treatment plots. The treatments were control (K), treatment of endophytic bacteria isolates A5, A6 (A), treatment of endophytic bacteria isolates KR4, KR7 (B), treatment of endophytic bacteria isolate SB3 (C), and consortium of endophytic bacteria isolates A5, A6, KR4, KR7, SB3 (D). Endophytic bacteria were applied by dissolving 250 mL of the endophytic bacteria formula into 5 L of water (0.5%) to create a ready-to-spray solution. The application was made by spraying evenly on the lower and upper surfaces of the leaves five times at the ages of 56, 62, 67, 72, and 77 days after planting using a sprayer. Soil microbial isolation was carried out during the vegetative phase of rice, following one application of endophytic bacteria, and in the generative phase of rice after five applications.

Isolation of soil microbial

A total of 1 g of soil sample was dissolved in 9 mL of distilled water to form a stock solution. The sample solution was subjected to a series of dilutions, ranging from 10^{-1} to $10^{-4.}$ The 10^{-3} and 10^{-4} solutions were spread onto a petri dish containing Potato Dextrose Agar (PDA) medium, each at a volume of 50 µL. Pure bacterial cultures were grown on Nutrient Agar (NA) medium and incubated at room temperature for 24 hours. Pure fungal cultures were grown on a PDA medium and incubated at room temperature for three days.

Characterization of soil microbial

Bacterial characterization was performed on pure isolates exhibiting good colony growth in the culture medium. Characterization of bacterial isolates was carried out macroscopically and microscopically. A macroscopic characterization of bacterial isolates was conducted based on the 7th edition of Bergey's Manual of Determinative Bacteriology. It records the colony shape characteristics (circular, irregular, spindle, filamentous, rhizoid), optical characteristics (transparent, translucent, opaque), colony margin (entire, lobate, undulate, serrate, filamentous, curled), elevation (flat, raised, convex, umbonate, crateriform), colony texture (smooth, dry, mucoid), colony color (white, yellow), and colony size (pinpoint, small, moderate, large) (Breed et al. 1957). Microscopic characterization of the bacterial isolates was performed using Gram staining, catalase testing, endospore testing, and observation of cell morphology under a microscope. The macroscopic characterization of fungal isolates was conducted according to the 2nd edition of Pictorial Atlas of Soil and Seed Fungi, by recording the colony color and shape characteristics. The microscopic characterization of fungal isolates was done by observing hyphae, conidia, and conidiophores (Watanabe 2002).

Diversity index (H')

The diversity of soil microbial will affect the biodiversity of a soil ecosystem. Diverse soil microbial will balance the food chain of soil microorganisms. It is the most widely known and widely used concept of diversity. The Shannon-Wiener index is calculated using the following formula (Shannon 1948):

$$H' = -\sum_{i=1}^{S} (pi)(In\ pi)$$

$$pi = \sum \mathrm{ni}/\mathrm{N}$$

Where: H' = Diversity index Shannon-Wiener; pi = Number of individuals of a species/total number of all species; ni = Number of individuals of the i-th species; N = Total number of individuals.

The criteria for the value of the Shannon-Wiener diversity index (H') are as follows: $H' \leq 1$ = Low diversity; $1 < H' < 3$ = Moderate diversity; $H' \geq 3$ = High diversity.

Hypersensitivity test of soil bacteria

The hypersensitivity test aims to obtain non-pathogenic soil bacteria. It is conducted by growing bacteria in 5 mL of Nutrient Broth (NB) medium and shaking the culture for 24 hours. A 0.3 mL bacterial suspension grown in a Nutrient Broth medium is injected into a jade plant (*Crassula ovata* (Mill.) Druce) leaf. After 5 days, the symptoms are observed; if necrosis occurs, the bacteria are pathogens in plants. If it does not exhibit necrosis symptoms, it may be caused by antagonistic bacteria.

RESULTS AND DISCUSSION

Character of soil microbial in the vegetative phase of rice

The isolation of soil microbial during the vegetative phase of rice revealed the presence of six bacterial isolates and three fungal isolates, highlighting the diversity of the microbial community present in the experimental plot. The bacterial isolates were predominantly Gram-positive, with most exhibiting rod-shaped morphology (bacilli). This finding aligns with previous studies showing that Gram-positive bacteria, especially bacilli, are well-suited to the soil environment due to their robust cell wall structure, which enhances survival under various environmental conditions (Madigan and Martinko 2015).

A total of 9 soil microbial isolates were isolated from all treatment plots (K, A, B, C, and D), indicating the important role of environmental and management factors in shaping soil microbial composition. The differences in soil microbial composition indicate differences in nutrient availability, microbial competition, and the influence of indigenous endophytic bacteria. The treated plots consistently showed a higher number of microbial isolates compared to the control. It is influenced by the ability of endophytic bacteria to produce bioactive compounds, increase nutrient accessibility, and create profitable niches for beneficial microbial (Santoyo et al. 2016; Trivedi et al. 2020). Indigenous endophytic bacteria can also support soil ecosystem resilience through microbial interactions and suppress pathogen populations in the soil (Compant et al. 2016).

Morphological and microscopic analyses of the isolates, illustrated the structural characteristics of the soil microbial community (Figure 2). Macroscopic observation showed that different colony morphologies among isolates, including color, texture, and edge shape variations, indicate functional and genetic diversity within the microbial community. Further microscopic analysis confirmed the dominance of bacilli, consistent with their well-documented role as Plant Growth-Promoting Rhizobacteria (PGPR) and their ability to produce bioactive compounds, including phytohormones and antimicrobial agents (Ni et al. 2024). These findings are consistent with previous studies, which have shown the potential of indigenous microbial applications to enhance soil microbial diversity and ecosystem resilience (Tan et al. 2022). The dominance of Gram-positive bacteria in the treated fields suggests that the applied endophytic bacteria may have created conditions that favor the proliferation of these organisms. This can be attributed to the production of secondary metabolites by endophytic bacteria, which suppress pathogenic microbial while promoting the growth of beneficial species (Narayanan and Glick 2022).

The morphological characteristics of soil bacteria colonies are presented in Table 1 (macroscopically) and Table 2 (microscopically). The six soil bacteria isolates macroscopically showed small colonies. The colonies were bright white and round, with flat elevations and intact edges. Microscopically, it exhibited biochemical characteristics, including Gram-positive staining, catalase positivity, and the formation of endospores. Details of the morphological characteristics of fungi are presented in Table 3. Based on morphological observations during the vegetative phase of rice, six soil bacteria isolates and three soil fungal isolates were identified. The bacterial isolates belonged to genus of *Paenibacillus*, while the fungal isolates belonged to genus of *Acremonium*. The variation in the presence of soil microbial is influenced by plant cultivation, fertilizer application, and the role of the microbes given (Senevirathne et al. 2024).

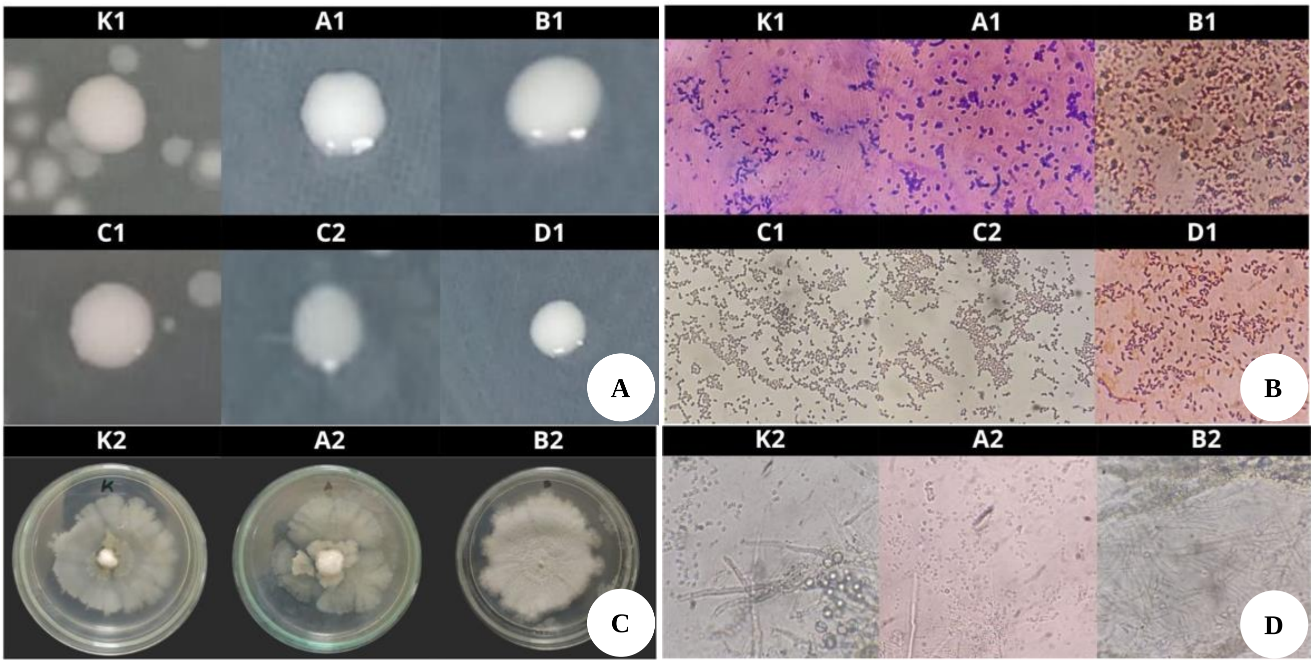

Figure 2. Soil microbial isolation in the vegetative phase of rice: A. Macroscopic soil bacteria; B. Microscopic soil bacteria; C. Macroscopic soil fungi; D. Microscopic soil fungi

Table 1. Macroscopic morphological characteristics of soil bacteria in the vegetative phase of rice

Isolate code	Size	Pigmentation	Optical characteristics	Shape	Elevation	Surface	Margin	Genera
K1	Small	Clear white	Translucent	Circular	Flat	Smooth	Entire	*Paenibacillus*
A1	Small	Clear white	Translucent	Circular	Flat	Smooth	Entire	*Paenibacillus*
B1	Small	Clear white	Translucent	Circular	Flat	Smooth	Entire	*Paenibacillus*
C1	Small	Clear white	Translucent	Circular	Flat	Smooth	Entire	*Paenibacillus*
C2	Small	Clear white	Translucent	Circular	Flat	Smooth	Entire	*Paenibacillus*
D1	Small	Clear white	Translucent	Circular	Flat	Smooth	Entire	*Paenibacillus*

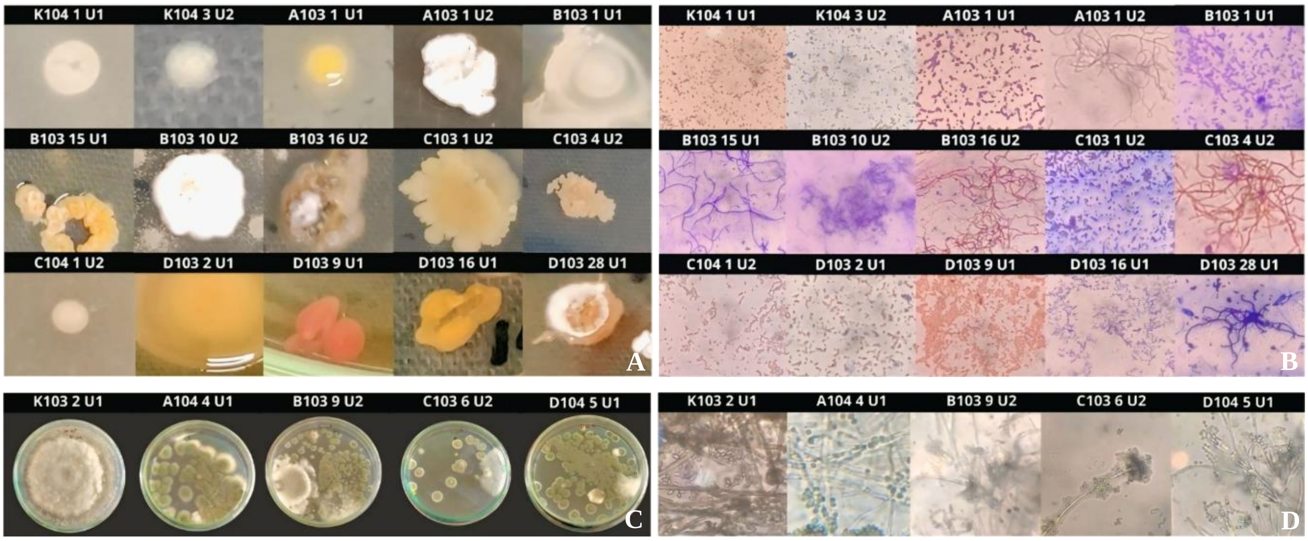

Figure 3. Soil microbial isolation in the generative phase of rice: A. Macroscopic soil bacteria; B. Microscopic soil bacteria; C. Macroscopic soil fungi; D. Microscopic soil fungi

Table 2. Biochemical and microscopic morphological characteristics of soil bacteria in the vegetative phase of rice

Isolate code	Catalase	Gram staining	Endospores	Cell shape	Genera
K1	+	+	+	Bacilli	*Paenibacillus*
A1	+	+	+	Bacilli	*Paenibacillus*
B1	+	+	+	Bacilli	*Paenibacillus*
C1	+	+	+	Bacilli	*Paenibacillus*
C2	+	+	+	Bacilli	*Paenibacillus*
D1	+	+	+	Bacilli	*Paenibacillus*

Table 3. Morphological characteristics of soil fungi in the vegetative phase of rice

Isolate code	Color	Colony shape	Hyphae	Conidium	Conidiophores	Genera
K2	White	Flat, protruding in the middle, like cotton	Septate, upright, hyaline	Single-celled, hyaline, cylindrical	Upright	Acremonium
A2	White	Flat, protruding in the middle, like cotton	Septate, upright, hyaline	Single-celled, hyaline, cylindrical	Upright	Acremonium
B2	White	Flat, protruding in the middle, like cotton	Septate, upright, hyaline	Single-celled, hyaline, cylindrical	Upright	Acremonium

Character of soil microbial in the generative phase of rice

Soil microbial in the generative phase of rice after indigenous endophytic bacterial treatment were successfully isolated from as many as 15 bacterial isolates and 5 fungal isolates. The results of macroscopic and microscopic soil microbial isolation are shown in Figure 3. Based on several tests carried out (Tables 4, 5, 6), five soil bacteria genera namely, *Paenibacillus*, *Micrococcus*, *Methylobacterium*, *Streptomyces*, and *Actinomyces*, and two soil fungal genera namely, *Acremonium* and *Penicillium* were identified.

Characteristics of the soil bacteria genus

Genus Paenibacillus *Ash et al., 1994*

Based on the results of macroscopic observations, isolates in the vegetative phase showed similar morphological characteristics. Meanwhile, isolates after treatment with codes K104 1 U1, K104 3 U2, B103 1 U1, and C104 1 U2 had round, regular (circular) and irregular (irregular) colonies, even edges (entire) and grooved (lobate), smooth surfaces, and milky white to yellowish white. Microscopic observations revealed rod-shaped, Gram-positive, catalase-positive cells with endospores. The results of the observations are based on the identification of bacteria in 7th edition of Bergey's Manual of Determinative Bacteriology (Breed 1957), where this genus is described as Gram-positive, rod-shaped, catalase-positive, and possessing endospores. This genus is widespread and can be found in the soil. Based on research by Grady et al. (2016), *Paenibacillus* sp. can be found in the soil or around the roots of plants that act as Plant Growth-Promoting Rhizobacteria (PGPR) by binding nitrogen and phosphate solvents, producing Indole Acetic Acid (IAA), and siderophores, and as inducers of systemic resistance (induced systemic resistance). According to Grady et al. (2016), more than 20 *Paenibacillus* sp. can bind free nitrogen.

Table 4. Macroscopic morphological characteristics of soil bacteria in the generative phase of rice

Isolate code	**Size**	**Pigmentation**	**Optical characteristics**	**Shape**	**Elevation**	**Surface**	**Margin**	**Genera**
K104 1 U1	Small	Clear white	Opaque	Circular	Flat	Smooth	Entire	*Paenibacillus*
K104 3 U2	Small	Clear White	Opaque	Circular	Flat	Smooth	Entire	*Paenibacillus*
A103 1 U1	Small	Yellow	Translucent	Circular	Raised	Smooth	Entire	*Micrococcus*
A103 1 U2	Small	Clear white	Opaque	Irregular	Umbonate	Wrinkled	Undulate	*Streptomyces*
B103 1 U1	Large	Clear white	Translucent	Irregular	Flat	Smooth	Lobate	*Paenibacillus*
B103 15 U1	Small	Yellow with clear white in the middle	Opaque	Irregular	Umbonate	Wrinkled	Undulate	*Streptomyces*
B103 10 U2	Moderate	Clear white	Opaque	Irregular	Umbonate	Wrinkled	Undulate	*Actinomyces*
B103 16 U2	Small	Green with clear white in the middle	Opaque	Irregular	Umbonate	Wrinkled	Undulate	*Streptomyces*
C103 1 U2	Moderate	White yellowish	Opaque	Irregular	Flat	Smooth	Lobate	*Micrococcus*
C103 4 U2	Small	White	Opaque	Irregular	Umbonate	Wrinkled	Undulate	*Actinomyces*
C104 1 U2	Small	White	Translucent	Circular	Raised	Smooth	Entire	*Paenibacillus*
D103 2 U1	Moderate	White yellowish	Opaque	Circular	Raised	Smooth	Entire	*Micrococcus*
D103 9 U1	Small	Pink	Opaque	Circular	Raised	Smooth	Entire	*Methylobacterium*
D103 16 U1	Small	Yellow	Opaque	Circular	Raised	Smooth	Entire	*Micrococcus*
D103 28 U1	Pinpoint	White with brown in the middle	Opaque	Irregular	Umbonate	Wrinkled	Undulate	*Streptomyces*

Table 5. Biochemical and microscopic morphological characteristics of soil bacteria in the generative phase of rice

Isolate code	**Catalase**	**Gram staining**	**Endospores**	**Cell shape**	**Genera**
K104 1 U1	+	+	+	Bacilli	*Paenibacillus*
K104 3 U2	+	+	+	Coccobacilli	*Paenibacillus*
A103 1 U1	+	+	+	Coccobacilli	*Micrococcus*
A103 1 U2	+	+	-	Filamentous	*Streptomyces*
B103 1 U1	+	+	+	Bacilli	*Paenibacillus*
B103 15 U1	+	+	-	Filamentous	*Streptomyces*
B103 10 U2	-	+	-	Filamentous	*Actinomyces*
B103 16 U2	+	+	-	Filamentous	*Streptomyces*
C103 1 U2	+	+	+	Bacilli	*Micrococcus*
C103 4 U2	-	+	-	Filamentous	*Actinomyces*
C104 1 U2	+	+	+	Bacilli	*Paenibacillus*
D103 2 U1	+	+	-	Bacilli	*Micrococcus*
D103 9 U1	+	-	-	Bacilli	*Methylobacterium*
D103 16 U1	+	+	-	Coccobacilli	*Micrococcus*
D103 28 U1	+	+	-	Filamentous	*Streptomyces*

Table 6. Morphological characteristics of soil fungi in the generative phase of rice

Isolate code	**Color**	**Colony shape**	**Hyphae**	**Conidium**	**Conidiophores**	**Genera**
K103 2 U1	White	Flat, protruding in the middle, like cotton	Septate, upright, hyaline	Single-celled, hyaline, cylindrical	Upright	*Acremonium*
A104 4 U1	Green	Flat, fibrous, and velvety texture	Long filamentous, branched, multinucleated, and septate	Chained, round, hyaline	Upright, branched	*Penicillium*
B103 9 U2	Green	Flat, fibrous, and velvety texture	Long filamentous, branched, multinucleated, and septate	Chained, round, hyaline	Upright, branched	*Penicillium*
C103 6 U2	Green	Flat, fibrous, and velvety texture	Long filamentous, branched, multinucleated, and septate	Chained, round, hyaline	Upright, branched	*Penicillium*
D104 5 U1	Green	Flat, fibrous, and velvety texture	Long filamentous, branched, multinucleated, and septate	Chained, round, hyaline	Upright, branched	*Penicillium*

Genus Micrococcus *Anon.*

Based on the results of macroscopic observations of the treatment isolates in the generative phase of codes A103 1 U1, D103 2 U1, C103 1 U2, and D103 16 U1, the colonies were round, regular (circular), and irregular (irregular) with flat edges (entire) and lobed, smooth surfaces, and yellow. The microscopic observations revealed that the isolates were cocci, Gram-positive, and catalase-positive but did not possess endospores. The results of the observations are based on the identification of bacteria in 7th edition of Bergey's Manual of Determinative Bacteriology, where this genus is described as Gram-positive and round-celled.

Most are found in tetrads or irregular groups, catalase positive, and do not have endospores. This genus is widespread in various terrestrial and aquatic ecosystems. According to research by Patel et al. (2021), *Micrococcus* is a Plant Growth-Promoting Bacteria (PGPB) and biological control agent against soil-borne pathogens. *Micrococcus* can produce Indole Acetic Acid (IAA), ammonia, potassium solubilizers, and phosphate solubilizers, which directly enhance plant growth.

Genus Methylobacterium *Patt et al., 1976*

Based on the results of macroscopic observations of isolate code D103 9 U1, the colony is circular with flat edges (intact), a smooth surface, and pink. The microscopic observations show that the cells are rod-shaped, Gram-negative, catalase-positive, and do not have endospores. The results of the observations are based on the identification of bacteria in the Breed et al. 1957 (7th edition of Bergey's Manual of Determinative Bacteriology). The genus is Gram-negative, rod-shaped, catalase-positive, and does not form endospores. This genus can be found on the surfaces of leaves, soil, dust, and freshwater. According to research by Dourado et al. (2015), *Methylobacterium* strains are reported to produce phytohormones, including cytokinins and auxins, which stimulate cell division and elongation, respectively. *Methylobacterium* can produce IAA, indicating that its inoculation can increase the concentration of IAA in plants and encourage increased plant growth.

Genus Streptomyces *Waksman & Henrici, 1943*

Based on the results of macroscopic observations of isolates in the generative phase of plants with isolate codes A103 1 U2, B103 15 U1, B103 16 U2, and D103 28 U1, the colonies are irregularly shaped with wavy edges, wrinkled surfaces, and are white, yellow, green, and brown. The microscopic observations revealed that the isolates are filamentous, Gram-positive, and catalase-positive and do not form endospores. The results of the observations are based on identifying bacteria in the 7th edition of Bergey's Manual of Determinative Bacteriology (Breed et al. 1957), where this genus is characterized as Gram-positive, filamentous, catalase-positive, and does not form endospores. This genus inhabits the soil surrounding plant roots, known as the rhizosphere, as well as niches within and between root cells, referred to as endophytic compartments. According to research by Vurukonda et al. (2018), *Streptomyces* endophytes contribute to the growth of their host plants as plant growth promoters, thereby increasing the ability of their hosts to survive in stressful environmental conditions. The interaction between IAA-producing bacteria and host plants is important in plant diversity.

Genus Actinomyces *Harz, 1877*

Based on the results of macroscopic observations of isolates treated in the generative phase with isolate codes B103 10 U2 and C103 4 U2, the colonies formed were irregular in shape with wavy edges, wrinkled surfaces, and white. The microscopic observations revealed that the isolates were filamentous, Gram-positive, catalase-negative, and lacked endospores. The results of these observations are based on the identification of bacteria in the 7th edition of Bergey's Manual of Determinative Bacteriology, where this genus is described as Gram-positive, rod-shaped, filamentous, with colonies in the form of branched hyphal tissue, catalase-negative, and lacking endospores. This genus is widespread and can be found in the soil. According to research by AbdElgawad et al. (2020), *Actinomyces* plays a role in the decomposition of organic matter and nitrogen fixation (approximately 15%), as well as serving as a phosphate solvent. *Actinomyces* is known to produce various antibiotics, biological control agents, and plant growth-promoting chemicals.

Characteristics of the soil fungi genus obtained

Genus Acremonium *Link, 1809*

The results of macroscopic observations of isolates in the generative phase in the isolated code K103 2 U1 showed that the colonies were flat, protruding in the middle, and had a cotton-like texture. Meanwhile, microscopic observations revealed that the isolate had septate, upright hyaline hyphae, cylindrical conidia, single-celled hyaline spores, and upright conidiophores. These were identified by consulting the Watanabe 2002 (2nd edition of Pictorial Atlas of Soil and Seed Fungi), which describes this genus as having upright, septate, hyaline conidiophores (phialids). Phialosporous conidia are clavate, cylindrical or elliptical, hyaline, single-celled, and usually biguttulate. The hyphae formed are often crustaceans. According to research by Han et al. (2020), members of the genus *Acremonium* fungi are the dominant endophytes that can be isolated. This fungus increases antifungal activity in host plants. Members of the genus *Acremonium* are generally found in corn and rice.

Genus Penicillium *Link, 1809*

Macroscopic observations of isolates in the generative phase in isolate codes A104 4 U1, B103 9 U2, C103 6 U2, and D104 5 U1 showed that the colonies were flat, fibrous, and velvety textured. Meanwhile, microscopic observations revealed that the isolates possess long, branched, multinucleated, septate fibrous hyphae, conidia arranged in chains, and conidiophores that are upright and branched, with round, hyaline conidia. The results of the observations are by the identification of fungi in the Watanabe 2002 (2nd edition of Pictorial Atlas of Soil and Seed Fungi), where this genus has hyaline, upright, branched conidiophores like penicillate at the top with 2-3 setulae, vertical phialides on each metal, and conidial heads that are somewhat aggregated, compact, and composed of conidia bound to each phialide. Phialids taper gradually or are cylindrical with pointed tips. Phylosporous conidia have a hyaline or yellowish-brown mass, elliptical or oval in shape, single-celled, and rough or slightly serrated on the surface. According to research by Han et al. (2020), *Penicillium* is a rhizosphere fungus that effectively suppresses various damping-off and fusarium wilt diseases. Additionally, these fungi can stimulate plant growth.

Diversity index

The results of the calculation of soil microbial diversity using the Shannon-Wiener index in each treatment showed that the diversity was low and moderate. The low H' index value of the vegetative phase of rice was found in the Control, A, B, C, and D treatments with H' index values of 0.08, 1.10, 1.13, 0.00, and 0.00, respectively. The moderate H' index value of the generative phase of rice was found in the control, A, B, C, and D treatments with H' index values of 1.33, 1.36, 1.24, 1.00, and 1.56, respectively. The results of the soil microbial diversity calculation for the vegetative and generative phases of rice are presented in Table 7.

Application of endophytic bacteria in treatment A increased the H' index value from 0.10 to 1.36, treatment B from 0.13 to 1.36, treatment C from 0 to 1.00, and treatment D from 0 to 1.56. This was caused by one of the environmental factors, namely soil. Soil quality significantly impacts the biological conditions of the soil, particularly the presence of microbial in the plant rhizosphere. The treatment of endophytic bacterial consortium showed the highest increase in bacterial diversity among the others. According to Prayudyaningsih et al. (2015), the rhizosphere is a part of the soil around the plant roots. The microbial population in the rhizosphere is generally more numerous and diverse than in non-rhizosphere soil. The abundance of rhizosphere bacteria can be increased by applying endophytes, especially in the form of consortia. The endophyte application aims to stimulate the stability of microbial abundance in the soil. Endophytes can degrade several organic compounds in the soil, thereby enhancing the availability of food sources for various soil microbial, which maximizes the function of endophytes.

Hypersensitivity test of soil bacteria

A hypersensitivity test using *C. ovata* (Figure 4; Tables 8 and 9) against 22 bacterial isolates revealed that two bacterial isolates, with isolate codes *Xanthomonas oryzae* (Xoo) as a control and C104 1 U2, were pathogenic. This was indicated by necrosis on *C. ovata* leaves after 5 days of incubation. Hypersensitivity reactions are characterized by rapid and localized cell death following bacterial inoculation. This reaction occurs in infected plants during the introduction of pathogens and is also an effort to inhibit pathogen growth (Balint-Kurti 2019).

Hypersensitivity test using *C. ovata*, according to Umesha et al. (2008) plants of the Crassulaceae family are succulent plants, have thick enough leaves that store water and bacterial cells can be easily infiltrated. The microclimate in the mesophyll of the leaves of the *C. ovata* plant offers suitable conditions for bacterial proliferation resulting in the expression of hypersensitivity reactions.

Table 7. Soil microbial diversity index in the vegetative and generative phases of rice

Treatment	Vegetative phase		Generative phase	
	Soil microbial diversity (H')	**Criteria**	**Soil microbial diversity (H')**	**Criteria**
Control	0.08	Low	1.33	Moderate
A	0.10	Low	1.36	Moderate
B	0.13	Low	1.24	Moderate
C	0	Low	1.00	Moderate
D	0	Low	1.56	Moderate

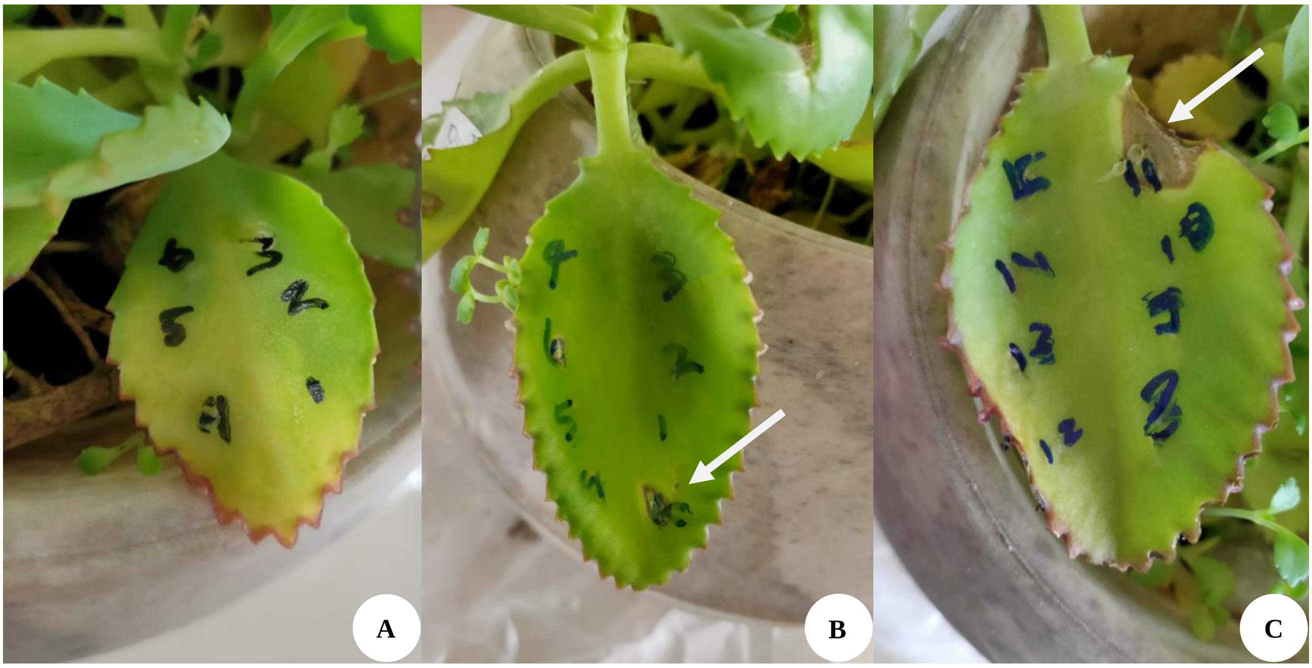

Figure 4. Hypersensitivity test results: A. Soil bacterial isolates in the vegetative phase of rice; B. Soil bacterial isolates in the generative phase of rice (codes 1 to 7); C. Soil bacterial isolates in the generative phase of rice (codes 8 to 15). The arrow on the hypersensitivity test results indicates that the isolate is a pathogen

Table 8. Results of hypersensitivity tests of soil bacterial isolates found in the vegetative phase of rice

Test code	Isolate code	Hypersensitivity tests
1	K	-
2	A	-
3	B	-
4	C	-
5	C2	-
6	D	-

Table 9. Results of hypersensitivity tests of soil bacterial isolates found in the generative phase of rice

Test code	Isolate code	Hypersensitivity tests
K (control)	**Xoo**	+
1	K104 1 U1	-
2	K104 3 U2	-
3	A103 1 U1	-
4	A103 1 U2	-
5	B103 1 U1	-
6	B103 15 U1	-
7	B103 10 U2	-
8	B103 16 U2	-
9	C103 1 U2	-
10	C103 4 U2	-
11	**C104 1 U2**	+
12	D103 2 U1	-
13	D103 9 U1	-
14	D103 16 U1	-
15	D103 28 U1	-

In conclusion, the isolation of soil microbial during the vegetative and generative phases of rice plants revealed an increase in the diversity of soil microbial, comprising the genera *Paenibacillus*, *Micrococcus*, *Methylobacterium*, *Streptomyces*, *Actinomyces*, *Acremonium*, and *Penicillium*. The diversity index indicated that treatments A, B, C, and D fell into the moderate category. The highest increase in soil microbial diversity was observed in the treatment with the endophytic bacterial consortium. The results of the hypersensitivity test showed symptoms of necrosis in isolate C104 1 U2, while the other isolates did not exhibit these symptoms. This indicates that there is only one pathogenic soil microbe, and other microbes have the potential to develop as antagonistic agents.

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REFERENCES

AbdElgawad H, Abuelsoud W, Madany MMY, Selim S, Zinta G, Mousa ASM, Hozzein WN. 2020. *Actinomycetes* enrich soil rhizosphere and improve seed quality as well as productivity of legumes by boosting nitrogen availability and metabolism. Biomolecules 10 (12): 1675. DOI: 10.3390/biom10121675.

Afzal I, Shinwari ZK, Sikandar S, Shahzad S. 2019. Plant beneficial endophytic bacteria: Mechanisms, diversity, host range and genetic determinants. Microbiol Res 221: 36-49. DOI: 10.1016/j.micres.2019.02.001.

Ani SW, Darwanto DH, Waluyati LR, Masyhuri. 2024. Regeneration of rural rice farmers in Central Java Province. Environ Chall 16: 100971. DOI: 10.1016/j.envc.2024.100971.

Balint-Kurti P. 2019. The plant hypersensitive response: Concepts, control and consequences. Mol Plant Pathol 20 (8): 1163-1178. DOI: 10.1111/mpp.12821.

Breed RS, Murray EGD, Smith NR. 1957. Bergey's Manual of Determinative Bacteriology. 7th Edition, The Williams & Wilkins Company, Baltimore.

Chaudhary P, Agri U, Chaudhary A, Kumar A, Kumar G. 2022. Endophytes and their potential in biotic stress management and crop production. Front Microbiol 13: 933017. DOI: 10.3389/fmicb.2022.933017.

Compant S, Saikkonen K, Mitter B, Campisano A, Mercado-Blanco J. 2016. Editorial special issue: Soil, plants and endophytes. Plant Soil 405: 1-11. DOI: 10.1007/s11104-016-2927-9.

Dourado MN, Camargo Neves AA, Santos DS, Araújo WL. 2015. Biotechnological and agronomic potential of endophytic pink-pigmented methylotrophic *Methylobacterium* spp. Biomed Res Intl 2015: 909016. DOI: 10.1155/2015/909016.

FAO. 2021. The State of Food and Agriculture 2021. Food and Agriculture Organization of the United Nations, Rome. DOI: 10.4060/cb4476en.

Grady EN, MacDonald J, Liu L, Richman A, Yuan ZC. 2016. Current knowledge and perspectives of *Paenibacillus*: A review. Microb Cell Fact 15 (1): 203. DOI: 10.1186/s12934-016-0603-7.

Gupta A, Singh UB, Sahu PK, Paul S, Kumar A, Malviya D, Singh S, Kuppusamy P, Singh P, Paul D, Rai JP, Singh HV, Manna MC, Crusberg TC, Kumar A, Saxena AK. 2022. Linking soil microbial diversity to modern agriculture practices: A review. Intl J Environ Res Public Health 19 (5): 3141. DOI: 10.3390/ijerph19053141.

Han L, Zhou X, Zhao Y, Zhu S, Wu L, He Y, Ping X, Lu X, Huang W, Qian J, Zhang L, Jiang X, Zhu D, Luo C, Li S, Dong Q, Fu Q, Deng K, Wang X, Wang L, Peng S, Wu J, Li W, Friml J, Zhu Y, He X, Du Y. 2020. Colonization of endophyte *Acremonium* sp. D212 in *Panax notoginseng* and rice mediated by auxin and jasmonic acid. J Integr Plant Biol 62 (9): 1433-1451. DOI: 10.1111/jipb.12905.

Heffernan D, Pilz M, Klein M, Haack M, Race AM, Brück T, Qoura F, Strittmatter N. 2023. Screening of Volatile Organic Compounds (VOCs) from liquid fungal cultures using ambient mass spectrometry. Anal Bioanal Chem 415 (18): 4615-4627. DOI: 10.1007/s00216-023-04769-6.

Irwandhi I, Khumairah FH, Sofyan ET, Ukit U, Satria RE, Salsabilla A, Sauri MS, Simarmata T. 2024. Innovative regenerative technologies for enhancing resilience in salinity-stressed rice fields along the Indonesian coast: Promoting net-zero farming practices to adapt to climate change. J Sustain Agric Environ 3 (4): e70026. DOI: 10.1002/sae2.70026.

Jin Q, Wang C, Sardans J, Vancov T, Fang Y, Wu L, Huang X, Gargallo-Garriga A, Peñuelas J, Wang W. 2022. Effect of soil degradation on the carbon concentration and retention of nitrogen and phosphorus across Chinese rice paddy fields. Catena 209: 105810. DOI: 10.1016/j.catena.2021.105810.

Kuźniar A, Kruczyńska A, Włodarczyk K, Vangronsveld J, Wolińska A. 2025. Endophytes as permanent or temporal inhabitants of different ecological niches in sustainable agriculture. Appl Sci 15 (3): 1253. DOI: 10.3390/app15031253.

Madigan MT, Martinko JM. 2015. Brock Biology of Microorganisms. 14th Edition. Pearson Education Limited, London.

Magurran AE. 2013. Measuring Biological Diversity. John Wiley & Sons, Hoboken.

Narayanan Z, Glick BR. 2022. Secondary metabolites produced by plant growth-promoting bacterial endophytes. Microorganisms 10 (10): 2008. DOI: 10.3390/microorganisms10102008.

Nguyen TT, Grote U, Neubacher F, Rahut DB, Do MH, Paudel GP. 2023. Security risks from climate change and environmental degradation: Implications for sustainable land use transformation in the Global South. Curr Opin Environ Sustain 63: 101322. DOI: 10.1016/j.cosust.2023.101322.

Ni S, Wu Y, Zhu N, Leng F, Wang Y. 2024. *Bacillus licheniformis* YB06: A rhizosphere–genome-wide analysis and plant growth-promoting analysis of a plant growth-promoting rhizobacterium isolated from *Codonopsis pilosula*. Microorganisms 12 (9): 1861. DOI: 10.3390/microorganisms12091861.

Patel P, Patel K, Dhandhukia P, Thakker JN. 2021. Plant growth promoting traits of marine *Micrococcus* sp. with bio-control ability against *Fusarium* in chickpea plant. Vegetos 34 (1): 94-101. DOI: 10.1007/s42535-021-00191-4.

Pedrinho A, Mendes LW, de Araujo Pereira AP, Araujo ASF, Vaishnav A, Karpouzas DG, Singh BK. 2024. Soil microbial diversity plays an important role in resisting and restoring degraded ecosystems. Plant Soil 500: 325-349. DOI: 10.1007/s11104-024-06489-x.

Prayudyaningsih R, Nursyamsi, Sari R. 2015. Advantaging soil microorganism on the rhizosphere of tuber crop under the shade of community forest stand in South Sulawesi. Pros Sem Nas Masy Biodiv Indon 1 (4): 954-959. DOI: 10.13057/psnmbi/m010453. [Indonesian]

Prihatiningsih N, Djatmiko HA, Erminawati. 2019. Bio-management of anthracnose disease in chilli with microencapsulates containing *Bacillus subtilis* B298. IOP Conf Ser: Earth Environ Sci 250: 012041. DOI: 10.1088/1755-1315/250/1/012041.

Prihatiningsih N, Djatmiko HA, Lestari P. 2020. Screening of competent rice root endophytic bacteria to promote rice growth and bacterial leaf blight disease control. J HPT Tropika 20 (1): 78-84. DOI: 10.23960/j.hptt.12078-84.

Santoyo G, Moreno-Hagelsieb G, Orozco-Mosqueda Mdel C, Glick BR. 2016. Plant growth-promoting bacterial endophytes. Microbiol Res 183: 92-99. DOI: 10.1016/j.micres.2015.11.008.

Senevirathne RAGHL, Premarathna M, Balasooriya BLWK, Seneviratne G. 2024. Impact of endophytic and soil microbes on rice grain yield under modern bio-organo mineral fertilizer. Proc 22nd Agric Res Symp 321: 325.

Shannon CE. 1948. A mathemaical theory of communication. Bell Syst Technol J 27 (3): 379-423. DOI: 10.1002/j.1538-7305.1948.tb01338.x.

Singh D, Yadav DK, Chaudhary G, Rana VS, Sharma RK. 2016. Potential of *Bacillus amyloliquefaciens* for biocontrol of bacterial wilt of tomato incited by *Ralstonia solanacearum*. J Plant Pathol Microbiol 7: 327. DOI: 10.4172/2157-7471.1000327.

Sitaresmi T, Nafisah N, Susanto U, Rahmini R, Widyastuti Y, Kusbiantoro B, Wahab MI, Hairmansis A, Nugraha Y. 2023. Deciphering genetic-by-environment interaction, pests and disease's reaction, grain quality, and response to fertilizer for targeting high-yielding rice varieties. Ecol Genet Genom 28: 100189. DOI: 10.1016/j.egg.2023.100189.

Sun H, Jiang S, Jiang C, Wu C, Gao M, Wang Q. 2021. A review of root exudates and rhizosphere microbiome for crop production. Environ Sci Pollut Res Intl 28 (39): 54497-54510. DOI: 10.1007/s11356-021-15838-7.

Swandi MK, Mubarik NR, Tjahjoleksono A. 2019. Rhizobacterial inoculants: The formulation as biofertilizer and its application on chili plants (*Capsicum annum* L.). Malays J Microbiol 15 (1): 44-51. DOI: 10.21161/mjm.112517.

Tan C, Kalhoro MT, Faqir Y, Ma J, Osei MD, Khaliq G. 2022. Climate-resilient microbial biotechnology: A perspective on sustainable agriculture. Sustainability 14 (9): 5574. DOI: 10.3390/su14095574.

Trivedi P, Leach JE, Tringe SG, Sa T, Singh BK. 2020. Plant–microbiome interactions: From community assembly to plant health. Nat Rev Microbiol 18: 607-621. DOI: 10.1038/s41579-020-0412-1.

Umesha S, Richardson PA, Kong P, Hong CX. 2008. A novel indicator plant to test the hypersensitivity of phytopathogenic bacteria. J Microbiol Methods 72 (1): 95-97. DOI: 10.1016/j.mimet.2007.11.002.

Verbeke F, De Craemer S, Debunne N, Janssens Y, Wynendaele E, Van de Wiele C, De Spiegeleer B. 2017. Peptides as quorum sensing molecules: Measurement techniques and obtained levels in vitro and in vivo. Front Neurosci 11: 183. DOI: 10.3389/fnins.2017.00183.

Vurukonda SSKP, Giovanardi D, Stefani E. 2018. Plant growth promoting and biocontrol activity of *Streptomyces* spp. as endophytes. Intl J Mol Sci 19 (4): 952. DOI: 10.3390/ijms19040952.

Watanabe T. 2002. Pictorial Atlas of Soil and Seed Fungi: Morphologies of Cultured Fungi and Key to Species. CRC Press, Florida.