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Characterization of Rhizosphere Bacteria in the Rice Fields of the Sawah Tambak Rice-Fish Farming System

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ABSTRAK

The Sawah Tambak Rice-Fish Farming System is a land-use practice that alternates between rice cultivation and fish farming depending on the season. During the dry season, the land is used for growing rice, while in the rainy season, it functions as a fishpond. Successful rice cultivation in this system relies on beneficial rhizosphere bacteria that interact with plant roots through biological, physical, and chemical processes. This study aims to characterize bacteria isolated from the rhizosphere of rice plants (*Oryza sativa* L.) in this system and evaluate their ability to produce indole-3-acetic acid (IAA) and solubilize phosphate. Bacterial colony identification was performed using the streak plate method, IAA production was measured via spectrophotometry, and phosphate solubilization was assessed using the phosphate solubilization index (PSI). The results showed diverse bacterial isolates with distinct morphological, biochemical, and physiological characteristics. Several isolates exhibited strong IAA production, with B3 reaching the highest concentration (18.29 ppm) on the sixth day. Phosphate-solubilizing bacteria (PSB) were also identified, with B4 and S5 showing the highest PSI values of 6.06 and 5.3, respectively. These findings suggest that rhizosphere bacteria in the Sawah Tambak system have the potential to enhance rice growth by improving phosphorus availability and producing plant growth hormones, contributing to sustainable and environmentally friendly rice cultivation.

Keywords: Indole-3-acetic acid, *Oryza sativa* L., Phosphate solubilization, Rice-field farming, Rhizosphere bacteria.

ABSTRAK

Sistem Pertanian Sawah Tambak adalah praktik penggunaan lahan yang bergantian antara budidaya padi dan perikanan tergantung pada musim. Pada musim kemarau, lahan digunakan untuk menanam padi, sedangkan pada musim hujan, lahan berfungsi sebagai tambak. Keberhasilan budidaya padi dalam sistem ini bergantung pada keberadaan bakteri rizosfer yang bermanfaat, yang berinteraksi dengan akar tanaman melalui proses biologis, fisik, dan kimia. Penelitian ini bertujuan untuk mengkarakterisasi bakteri yang diisolasi dari rizosfer tanaman padi (*Oryza sativa* L.) dalam sistem ini serta mengevaluasi kemampuannya dalam menghasilkan asam indole-3-asetat (IAA) dan melarutkan fosfat. Identifikasi koloni bakteri dilakukan dengan metode gores, produksi IAA diukur menggunakan spektrofotometri, dan pelarutan fosfat dinilai berdasarkan indeks pelarutan fosfat (PSI). Hasil penelitian menunjukkan bahwa isolat bakteri yang ditemukan memiliki karakteristik morfologi, biokimia, dan fisiologi yang beragam. Beberapa isolat menunjukkan produksi IAA yang tinggi, dengan isolat B3 mencapai konsentrasi tertinggi (18,29 ppm) pada hari keenam. Bakteri pelarut fosfat (PSB) juga teridentifikasi, dengan isolat B4 dan S5 memiliki nilai PSI tertinggi, masing-masing 6,06 dan 5,3. Temuan ini menunjukkan bahwa bakteri rizosfer dalam sistem Sawah Tambak berpotensi meningkatkan pertumbuhan padi dengan meningkatkan ketersediaan fosfor dan menghasilkan hormon pertumbuhan tanaman, sehingga berkontribusi pada budidaya padi yang berkelanjutan dan ramah lingkungan.

Kata kunci: Asam indole-3-asetat, Bakteri rizosfer, *Oryza sativa* L., Pertanian sawah tambak, Pelarutan fosfat.

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I. INTRODUCTION

1. Research Background

The Sawah Tambak Rice-Fish Farming System is a land-use strategy that adapts to seasonal variations, where rice is cultivated during the dry season, and the land is converted into fishponds during the rainy season (FAO, 2001). This system is widely implemented in several regions of Indonesia, particularly in lowland areas with ample water resources, maximizing land productivity by producing both rice and fish throughout the year (Nurhidayati et al., 2020). In areas such as Lamongan, Sidoarjo, and Pasuruan, which practice the Sawah Tambak system, rainwater is the primary water source for rice cultivation. However, prolonged dry seasons can lead to limited water availability and reduced water quality due to sedimentation and waste accumulation in rivers. Conversely, during the rainy season, river water becomes more turbid as it carries silt and debris from surrounding areas, impacting field conditions (Prawiro, 2019).Rice cultivation in the Sawah Tambak system depends on fertile soil enriched with beneficial rhizosphere bacteria. The rhizosphere harbors various bacterial species that support rice growth, including *Pseudomonas fluorescens* and *Bacillus subtilis* (Amruta et al., 2016; Guyasa et al., 2018). These microorganisms enhance root development by producing indole-3-acetic acid (IAA) and improving plant health. The composition of rhizosphere bacteria varies based on soil conditions, such as salinity levels (Susilowati et al., 2015). Additionally, these microbes play crucial roles in phosphate solubilization and nitrogen fixation, which are essential for rice growth in the Sawah Tambak system(Hussein & Joo, 2015; Widawati, 2015). The diversity of rhizosphere bacteria in this system is influenced by soil properties, including pH and organic matter content. These microbes not only stimulate root growth but also facilitate phosphate solubilization, making vital nutrients more available for rice plants.Despite the significance of rhizosphere bacteria, research on their characterization in Sawah Tambak rice fields remains scarce. Therefore, further studies are necessary to isolate and analyze IAA-producing and phosphate-solubilizing microorganisms in this environment. Such research is expected to provide valuable insights into rhizosphere bacterial communities in Sawah Tambak rice fields and their potential to enhance rice productivity, ultimately contributing to the sustainability of integrated agricultural systems across different regions.

II. RESEARCH METHOD

Place and Time

This research was carried out from September 2022 to January 2023 in Lamongan, East Java, Indonesia. Sample analysis was conducted in the Microbiology Laboratory at Universitas Muhammadiyah Lamongan.

Material and Equipment

This study utilized various equipment, including autoclave, test tubes, Erlenmeyer flasks, analytical balance, inoculating loops, hot plate, aluminum foil, plastic wrap, Petri dishes, microscope, writing instruments, glass slides, test tube racks, L-rods, micropipettes, Laminar Air Flow cabinet, and microtubes. The materials used comprised Nutrient Agar (NA) medium, a 0.9% NaCl physiological saline solution, soil samples, alcohol, Gram stain, 3% hydrogen peroxide (H_2O_2) solution, L-tryptophan, Salkowski's reagent, and solid Pikovskaya medium.

Research Stages

Sample Collection

Soil samples were collected from rice fields practicing the Rice-Fish Farming System at three locations in Lamongan Regency, Indonesia: Sukodadi (S), Babat (B), and

Kedungpring (K) subdistricts. Samples were taken from five points at each location, specifically from the rhizosphere area at a depth of 5-10 cm (Fallo et al., 2015).

Rhizosphere Bacteria Isolation

Under aseptic conditions, 10 grams of soil sample from the five sampling points were combined and diluted with 90 ml of physiological saline solution (0.9% NaCl). This composite solution was then subjected to serial dilution up to 10-7. From the last three dilutions, 0.1 ml was plated on sterile solid Nutrient Agar (NA) and spread using an L-rod spreader. The cultures were incubated for 24 hours at 37 °C (Astriani, 2015). Distinct colonies were selected from each plate and labeled according to their location: Sukodadi (S), Babat (B), and Kedungpring (K). The isolates were then characterized through Gram staining and observed under a microscope at 1000x magnification. Catalase activity was tested using 3% H_2O_2 (Pollack et al., 2018).

III. RESULTS AND DISCUSSION

Bacterial isolates from the rhizosphere of rice plants in the Sawah Tambak Rice-Fish system showed diverse morphological, microscopic, and biochemical characteristics (**Table 1**). Colony margins varied as umbonate, lobate, circular, irregular, punctiform, and undulate, with colors white, yellow, and green. Microscopic analysis revealed cocci and bacilli in different arrangements. Gram staining showed both Gram-positive and Gram-negative bacteria.Biochemical testing showed variations in catalase activity, an enzyme that breaks down hydrogen peroxide into water and oxygen. Most isolates tested positive for catalase, indicating their ability to tolerate oxidative stress, which could be beneficial for plant-microbe interactions in fluctuating environmental conditions (Rajkumar et al., 2017). The diversity of bacterial isolates found in the Sawah Tambak system suggests that the rhizosphere hosts a wide range of microorganisms, potentially contributing to plant growth promotion through mechanisms such as phosphate solubilization, nitrogen fixation, and phytohormone production. Further studies are necessary to assess their functional roles in enhancing rice productivity and soil fertility in this integrated farming system.

Table 1 macroscopic and microscopic observation of bacterial isolate

Bacteria Isolate	Macroscopic		Microscopic		Catalase activity
	Colony Shape	Colour	Cell Shape	Gram	
S1	Umbonate	White	Cocci (Tetrads)	-	+
S2	Umbonate	Green	Cocci (Staphylococci)	+	+
S3	Lobate	White	Cocci (Staphylococci)	-	+
S4	Circular	White	Cocci (Streptococci)	-	+
S5	Circular	Yellow	Bacilli (Streptobacilli)	+	-
S6	Irregular	Yellow	Cocci (Staphylococci)	+	-
S7	Punctiform	White	Bacilli (Streptobacilli)	-	-
S8	Punctiform	Yellow	Cocci (Streptococci)	+	+
B1	Umbonate	White	Cocci (Streptococci)	+	-
B2	Umbonate	Green	Cocci (Tetrads)	+	-
B3	Lobate	White	Cocci (Diploccci)	-	+
B4	Circular	White	Cocci (Streptococci)	+	-
B5	Circular	Yellow	Bacilli (Coccobacilli)	-	+
K1	Umbonate	White	Cocci (Streptococci)	+	+
K3	Lobate	White	Cocci (Streptococci)	+	-
K4	Circular	White	Bacilli	-	-
K5	Circular	Yellow	Cocci (Streptococci)	-	+
K11	Undulate	White	Cocci (Streptococci)	+	+
K12	Umbonate	Yellow	Cocci (Streptococci)	-	+

IAA Activity

The selection of IAA-producing bacteria was conducted on 19 isolates. The absorbance values of these isolates were measured at a wavelength of approximately 535 nm, and the

IAA concentration was calculated using the equation obtained from the IAA standard curve. IAA-producing bacteria can be observed through the highest absorbance values, and the color of the sample isolates changed to pink. From the selection of IAA-producing bacteria, six isolates with the highest substrate concentration values were identified: S7 (23.683 ppm), S8 (14.746 ppm), B3 (34.936 ppm), B2 (28.657 ppm), K1 (26.569 ppm), and K5 (24.366 ppm) (**Figure 1a**).

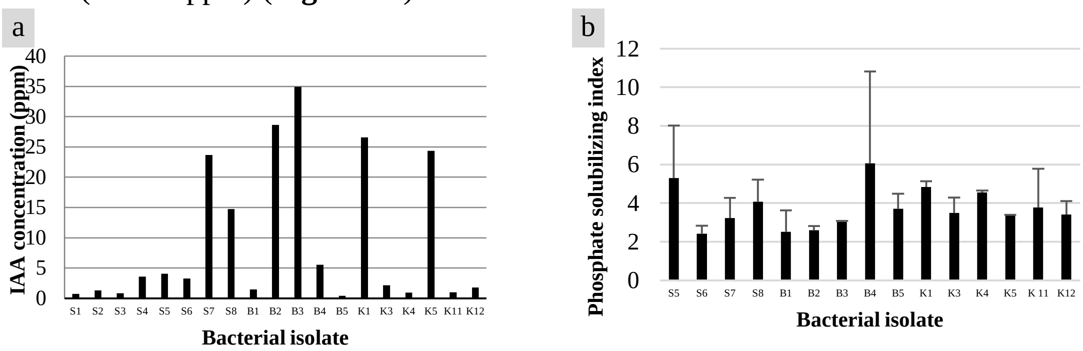

Figure 1. a. auxin-producing bacterial selection, b. phosphate-solubilizing bacteria (no significant different between sample – P<0,05).

The IAA-producing activity of bacterial isolates is shown in **Table 2**. During the first five days of incubation, IAA production remained stagnant. However, on the sixth day, production increased, with B3 reaching the highest concentration (18.29 ppm). On the sixth day, S7 (17.75 ppm), K1 (17.68 ppm), and K5 (17.5 ppm) showed peak concentrations. The results are consistent with Lwin et al. (2012) that the peak of IAA production is at its peak at 6th day. Indole-3-acetic acid (IAA) production by rhizobacteria is a key mechanism for promoting plant growth (Lebrazi et al., 2020). Various species, including *Rhizobium*, *Pseudomonas*, and *Bacillus*, synthesize IAA through tryptophan-dependent and independent pathways (Kumari et al., 2018). IAA biosynthesis is regulated by genes like *TyrR* and quorum sensing-related genes (Rico-Jiménez et al., 2023) and enhances root development, nutrient uptake, and yield (Etesami et al., 2015). In rice fields, IAA-producing bacteria, such as *Bacillus*, *Pseudomonas*, and *Enterobacter*, improve plant growth (Khoi Nghia, 2017; Susilowati & others, 2015). Rainfed rice fields harbor more effective IAA producers (Haerani et al., 2021) (Haerani et al., 2021). Some strains also control bacterial leaf blight (Prihatiningsih et al., 2020). Screening for IAA production is a useful method for selecting plant growth-promoting bacteria (Etesami et al., 2015).

Table 2. bacterial activity to IAA production through 7 days incubation

Bacterial isolate	IAA Production (ppm)				
	Day 1	Day 3	Day 5	Day 6	Day 7
S7	4,96	5,25	5,98	17,44	17,75
S8	5,68	5,26	6,06	16,60	17,05
B2	4,79	5,54	6,17	15,73	15,79
B3	4,50	5,58	6,24	18,29	16,82
K1	4,83	6,59	6,12	16,81	17,68
K5	4,77	5,17	6,87	17,20	17,51

Phosphate-solubilizing activity

The phosphate-solubilizing test revealed varying solubilization capacities among the isolates, with B4 and S5 exhibiting the highest phosphate solubilization index (PSI) values of 6.06 and 5.3, respectively, though no significant differences were observed (**Figure 1b**). Phosphate-solubilizing bacteria (PSB) play a crucial role in enhancing phosphorus availability for plants, particularly in rice cultivation, by solubilizing inorganic and organic phosphorus through mechanisms such as reducing soil pH via organic acid production and mineralizing organic phosphorus using acid phosphatase (Khan et al., 2009). PSB inoculation has been reported to improve phosphorus uptake, stimulate rice growth, and increase yields (Rosnan et al., 2021). In acid sulfate soils, strains like *Burkholderia thailandensis* and *Sphingomonas pituitosa* can decrease aluminum availability and raise soil pH, benefiting rice growth. Additionally, PSB contribute to plant development by producing indoleacetic acid (Panhwar et al., 2014). The application of PSB in combination with chemical fertilizers offers a sustainable approach to rice cultivation by reducing reliance on inorganic phosphorus fertilizers and mitigating environmental pollution (Ahash et al., 2024).

IV. CONCLUSION

The bacterial isolates from the rhizosphere of rice plants in the Sawah Tambak Rice-Fish system exhibited diverse morphological, microscopic, and biochemical characteristics. Colony variations, Gram-positive and Gram-negative bacteria, and different cellular arrangements were observed. Most isolates tested positive for catalase, indicating oxidative stress tolerance. Several isolates demonstrated strong indoleacetic acid (IAA) production, with B3 showing the highest concentration (18.29 ppm) on the sixth day. Phosphate-solubilizing bacteria (PSB) were also identified, with B4 and S5 exhibiting the highest solubilization index (PSI) values of 6.06 and 5.3, respectively. These findings highlight the potential of rhizosphere bacteria in promoting rice growth by enhancing phosphorus availability and producing plant growth hormones, contributing to sustainable rice cultivation.

REFERENCES

Ahash, S., Manikandan, K., Elamathi, S., Sivasankari, D. T., & Maragatham, S. (2024). Sustainable phosphorus management: Leveraging phosphate solubilizing bacteria for enhanced rice growth.

Amruta, N., Kumar, M. K. P., Narayanaswamy, S., Gowda, M., Channakeshava, B. C., Vishwanath, K., Puneeth, M. E., & Ranjitha, H. P. (2016). Isolation and Identification of Rice Blast Disease - Suppressing Antagonistic Bacterial Strains from the Rhizosphere of Rice.

Astriani, M. (2015). Seleksi Bakteri Penghasil Indole-3-Acetic Acid (Iaa) Dan Pengujian Pada Bibit Kelapa Sawit (Elaeis Guineensis Jacq.). Bogor Agricultural University (IPB).

Etesami, H., Alikhani, H. A., & Hosseini, H. M. (2015). Indole-3-acetic acid (IAA) production trait, a useful screening to select endophytic and rhizosphere competent bacteria for rice growth promoting agents. MethodsX, 2, 72–78.

Fallo, G., Mubarik, N. R., & others. (2015). Potency of auxin producing and phosphate solubilizing bacteria from dryland in rice paddy field. Research Journal of Microbiology, 10(6), 246.

FAO. (2001). Integrated agriculture-aquaculture: a primer (FAO Fisheries and Aquaculture Technical Papers) (Issue 407). Food & Agriculture Org.

Guyasa, I. M., Sadimantara, G. R., Khaeruni, A., Kade, G. A., & Sutariati. (2018). Isolation of bacillus spp and pseudomonas fluorescens from upland rice rhizosphere and its potential as plant growth promoting rhizobacteria for local upland rice (Oryza sativa L.).

Haerani, N., SYAMUN, E., RASYID, B., & HARING, F. (2021). Isolation and characterization of N-fixing and IAA producing rhizobacteria from two rice field agro-ecosystems in South Sulawesi, Indonesia. Biodiversitas Journal of Biological Diversity, 22(5).

Hussein, K. A., & Joo, J. H. (2015). Isolation and characterization of rhizomicrobial isolates for phosphate solubilization and indole acetic acid production. Journal of the Korean Society for Applied Biological Chemistry, 58, 847–855.

Khan, A. A., Jilani, G., Akhtar, M. S., Naqvi, S. M. S., Rasheed, M., & others. (2009). Phosphorus solubilizing bacteria: occurrence, mechanisms and their role in crop production. J. Agric. Biol. Sci, 1(1), 48–58.

Khoi Nghia, N. (2017). Isolation and Characterization of Indole Acetic Acid Producing Halophilic Bacteria from Salt Affected Soil of Rice–Shrimp Farming System in the Mekong Delta, Vietnam. Agriculture, Forestry and Fisheries, 6(3), 69.

Kumari, S., Prabha, C., Singh, A., Kumari, S., & Kiran, S. (2018). Optimization of indole-3-acetic acid production by diazotrophic B. subtilis DR2 (KP455653), isolated from rhizosphere of Eragrostis cynosuroides. International Journal of Pharma Medicine and Biological Sciences, 7(2), 20–27.

Lebrazi, S., Niehaus, K., Bednarz, H., Fadil, M., Chraibi, M., & Fikri-Benbrahim, K. (2020). Screening and optimization of indole-3-acetic acid production and phosphate solubilization by rhizobacterial strains isolated from Acacia cyanophylla root nodules and their effects on its plant growth. Journal of Genetic Engineering and Biotechnology, 18(1), 71.

Lwin, K. M., Myint, M. M., Tar, T., & Aung, W. Z. M. (2012). Isolation of plant hormone (indole-3-acetic acid-IAA) producing rhizobacteria and study on their effects on maize seedling. Engineering Journal, 16(5), 137–144.

Mubarik, N. R., Mursyida, E., & Tjahjoleks, A. (2015). Selection and Identification of Phosphate-Potassium Solubilizing Bacteria from the Area Around the Limestone Mining in Cirebon Quarry. Research Journal of Microbiology, 10(6), 270–279.

Nurhidayati, D. R., Huang, W.-C., Hanani, N., & Sujarwo, S. (2020). Rice-Fish Farming System in Lamongan, East Java, Indonesia: Swot and Profit Efficiency Analysis. Agricultural Socio-Economics Journal, 20(4), 311–318.

Panhwar, Q. A., Naher, U. A., Jusop, S., Othman, R., Latif, M. A., & Ismail, M. R. (2014). Biochemical and molecular characterization of potential phosphate-solubilizing bacteria in acid sulfate soils and their beneficial effects on rice growth. PloS One, 9(10), e97241.

Patten, C. L., & Glick, B. R. (2002). Role of Pseudomonas putida indoleacetic acid in development of the host plant root system. Applied and Environmental Microbiology, 68(8), 3795–3801.

Pollack, R. A., Findlay, L., Mondschein, W., & Modesto, R. R. (2018). Laboratory exercises in microbiology. John Wiley & Sons.

Prawiro, R. H. (2019). Ekologi Lingkungan Pencemaran. Satya Wacana.

Prihatiningsih, N., Adi Djatmiko, H., & Lestari, P. (2020). SCREENING OF COMPETENT RICE ROOT ENDOPHYTIC BACTERIA TO PROMOTE RICE GROWTH AND BACTERIAL LEAF BLIGHT DISEASE CONTROL. Jurnal Hama Dan Penyakit Tumbuhan Tropika, 20(1), 78–84.

Rajkumar, M., Bruno, L. B., & Banu, J. R. (2017). Alleviation of environmental stress in plants: The role of beneficial Pseudomonas spp. Critical Reviews in Environmental Science and Technology, 47(6), 372–407.

Rico-Jiménez, M., Muñoz-Mira, S., Lomas-Mart\'\inez, C., Krell, T., & Matilla, M. A. (2023). Regulation of indole-3-acetic acid biosynthesis and consequences of auxin production deficiency in Serratia plymuthica. Microbial Biotechnology, 16(8), 1671–1689.

Rosnan, N. A., Aziz, K. M., Hashim, M. M., & Nor, M. N. M. (2021). Characterization of effective microorganism (Phosphate Solubilizing Bacteria) isolated from Rice-Field soils. IOP Conference Series: Earth and Environmental Science, 757(1), 12074.

Susilowati, D. N., & others. (2015). Analisis Komunitas dan Fungsi Bakteri Rhizosfer Tanaman Padi Pada Gradien Salinitas Tanah Pesisir. IPB (Bogor Agricultural University).

Vipin, K., & Anshumali. (2012). Phosphate Solubilizing Activity of Some Bacterial Strains Isolated from Chemical Pesticide Exposed Agriculture Soil.

Widawati, S. (2015). The effect of salinity to activity and effectivity phosphate solubilizing bacteria on growth and production of paddy. KnE Life Sciences, 609–612.

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