ISOLATION AND SCREENING OF BACTERIA WITH PLANT GROWTH PROMOTION TRAITS FROM IRON SAND SOILS OF CENTRAL JAWA SOUTH COAST

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Abstract. Isolation and screening of bacteria with plant growth-promoting traits from iron sand soils have been conducted. The traits examined included nitrogen fixation, IAA production, phosphate and potassium solubilization, and siderophore production. The physical and chemical properties of the iron sand soil of Central Java's south coast showed that air and soil temperatures were 31–39°C and 29–35°C, pH levels ranging from 6.5 to 7.8, and humidity levels between 15% and 100%. Sixteen bacterial isolates were identified, with population densities ranging from 5.0 x 10^2 to 3.5 x 10^5 CFU. g^{-1} of soil. Out of these, 11 isolates demonstrated nitrogen-fixing abilities. Two isolates, PS2Y2 and KS1C4, produced IAA at concentrations of 20.6 ppm and 11.73 ppm, respectively. All isolates were capable of solubilizing phosphate, with solubilization index (SI) ranging from 1.09 to 2.81. One isolate, CS1P1, exhibited a strong ability to solubilize potassium, with an SI value of 6.39. Additionally, three isolates PS3Y4, KS1K1, and CS1P1 were able to produce siderophores.

Keywords: isolation, iron sand soils, plant growth-promoting bacteria

A. Introduction

Iron sand soil is a mineral-rich material formed by volcanic activity, carried by rivers, and deposited along coastal areas. In Indonesia, iron sand deposits are found on the west coast of Sumatra, southern Java, Sulawesi, Nusa Tenggara, and Maluku [1]. Along the south coast of Central Java, iron sand soils form coastal dunes, which help protect the coastline by absorbing energy from winds, tides, and waves. This type of soil is classified as marginal because it has low organic matter, poor fertility, limited cation exchange and water-holding capacity, and low nitrogen content. It also contains high concentrations of iron (14.6% to 56.75%) and experiences significant daily temperature fluctuations (29–45°C) [2] [3]. These factors limit the soil's capacity to support high populations and activity levels of organisms [4].

In such environmentally stressed soils, the number of culturable bacteria can be as low as 10^4 cells per gram of soil. The abundance and diversity of bacteria in the soil are influenced by factors such as temperature, moisture, salinity, and chemical composition, as well as the types and amounts of plants present [5]. Despite these challenges, microorganisms that thrive in iron sand soils have adapted to tolerate extreme conditions of temperature, humidity, nutrients, and salinity. Bacteria capable of nitrogen fixation, phosphate and potassium solubilization, and the production of phytohormones, ACC-deaminase, and siderophores show promise as plant growth-promoting agents. These microorganisms have significant potential for use in improving dry and saline soils.

Plant growth-promoting bacteria (PGPB) are microorganisms that enhance plant growth. These bacteria include free-living species, those that form specific symbiotic relationships with plants (e.g., *Rhizobium* spp. and *Frankia* spp.), bacterial endophytes that colonize the interior tissues of plants, and cyanobacteria. PGPB are associated with various plant species and are well-known for promoting crop growth by efficiently colonizing roots and synthesizing growth-promoting compounds such as indole-3-acetic acid (IAA), gibberellic acid (GA3), and 1-aminocyclopropane-1-carboxylate deaminase [6]. PGPB are generally classified into three categories. The first group consists of free-living bacteria that interact with plants under favorable conditions. The second group includes bacteria that reside in the rhizosphere (the soil zone near plant roots) or the phyllosphere (the surface of plant leaves). The third group comprises endophytic bacteria, which form stable associations with the internal tissues and organs of plants [7]. Common bacterial genera that exhibit plant growth-promoting properties include *Azospirillum*, *Azotobacter*, *Pseudomonas*, *Bacillus*, *Serratia*, *Streptomyces*, *Acinetobacter*, and *Arthrobacter* [8].

The objectives of this research were to isolate bacteria with plant growth-promoting traits from iron sand soils along the coasts of Purworejo, Kebumen, and Cilacap, and to evaluate their ability to fix nitrogen (N2), solubilize phosphate (P) and potassium (K), produce IAA, and produce siderophores.

B. Methods

1. Soil sampling and isolation of bacteria

Iron sand soils were collected from three coastal locations: Munggangsari Coast in Purworejo District (7°50'28" S, 109°52'8" E), Cemara Sewu Coast in Kebumen District (7°46'22" S, 109°34'15" E), and Srandil Coast in Cilacap District (7°41'35" S, 109°11'33" E). The samples were taken from a depth of approximately 10 cm. Ten grams of each soil sample were suspended in 90 mL of sterile distilled water in an Erlenmeyer flask and thoroughly mixed using a magnetic stirrer. From this suspension, 1 mL was transferred to 9 mL of sterile distilled water in a test tube, and serial dilutions were performed up to 10^{-5}. For the last two dilutions, 1 mL of the diluted sample was placed into sterile Petri dishes, followed by the addition of 18 mL of selective media, which were then gently mixed. Different selective media were used to isolate and screen bacteria with plant growth-promoting traits: yeast extract mannitol agar (YEMA) for *Rhizobium* [8], Ashby's Mannitol Agar for *Azotobacter* [9], Pikovskaya's medium for phosphate-solubilizing bacteria [10], Alexandrov's medium for potassium-solubilizing bacteria [11], Caceres (RC) medium for *Azospirillum* [12], and nutrient agar for total bacterial counts. The Petri dishes were incubated at room temperature for 2–3 days. Colonies of *Rhizobium* on YEMA medium were white, translucent, elevated, and mucilaginous. *Azotobacter* colonies on Ashby's medium appeared smooth, transparent, and round, with entire edges and raised elevations. Phosphate-solubilizing bacteria on Pikovskaya's medium and potassium-solubilizing bacteria were identified by clear zones around their colonies. *Azospirillum* colonies on Caceres medium appeared red or scarlet, flat, and bright.

2. Assay of plant growth-promoting traits of bacterial isolates

The ability of bacterial isolates to fix nitrogen was tested on an NfB semisolid medium. A loopful of each bacterial isolates was stab inoculated into the medium, which was then incubated for three days at room temperature. The positive results were indicated by the growth of bacteria just under the subsurface of the medium, forming a characteristic pellicle associated with *Azospirillum*, or on the surface, indicating the presence of other nitrogen-fixing bacteria [13]. Additionally, a color change in the NfB medium from yellowish green to blue suggested bacterial activity in fixing atmospheric nitrogen [14].

To evaluate the ability of bacterial isolates to solubilize phosphate, Pikovskaya Agar was used. A loopful of each isolate was spot-inoculated onto the agar and incubated for 3 to 5 days at room temperature. The presence of clear zones around the bacterial colonies indicated successful phosphate solubilization. Similar methods were employed to test the ability of the isolates to solubilize potassium and produce siderophores; Alexandrov Agar was used for potassium solubilization, while Chrom Azurol Sulfate Agar was utilized for siderophore production. The solubilization index (SI) for phosphate and potassium, as well as the production of siderophores, was calculated by measuring the total diameter (colony + clear zone) and dividing it by the colony diameter [15] [16].

Additionally, all bacterial isolates were screened for indole-3-acetic acid (IAA) production [16]. Each bacterial culture was inoculated in nutrient broth containing tryptophan (1 mg/mL) and incubated at room temperature for three days. After incubation, the cultures were centrifuged at 3000 rpm for 30 minutes. One millilitre of the supernatant was mixed with 2 mL of Salkowski reagent (50 mL of 35% perchloric acid combined with 1 mL of 0.5% FeCl3). The development of a pink color in the mixture indicated the production of IAA. The optical density (OD) of the sample was measured at 530 nm using a spectrophotometer, and the level of IAA produced was estimated based on a standard IAA graph.

C. Results and Discussion

1. Isolation of bacteria

The physical properties of iron sand soil along the south coast of Central Java revealed that air temperatures ranged from 29 to 34°C, while soil temperatures varied from 29 to 39°C. Soil humidity levels ranged from 10% to 100%, and the pH of the soil was between 6.5 and 8.0. Most coastal areas were characterized by low organic matter content, low fertility, and a limited cation exchange capacity, resulting in a decline in both micro and macronutrient availability [17].

In total, 16 bacterial isolates were obtained from the iron sand soils: 7 isolates from Purworejo Coast, 4 from Kebumen Coast, and 5 from Cilacap Coast. Most of these bacterial isolates were Gram-positive, with the exceptions being the PS1Y1 and PS3Y4 isolates, which exhibited a rod-shaped morphology. Furthermore, the isolates demonstrated one or more traits associated with plant growth-promoting bacteria (Table 1). Notably, most of the isolates were capable of fixing atmospheric nitrogen, as evidenced by tests conducted in NfB semisolid medium.

The isolates also demonstrated the ability to solubilize insoluble phosphate, with solubilization index (SI) ranging from 1.09 to 2.81 (Table 1). Based on these SI values, 12 of the bacterial isolates were classified as having intermediate solubilization ability. According to [10], strains can be categorized as low (SI < 2.00), intermediate (2.00 < SI < 4.00), or high (SI > 4.00) in their phosphate solubilization capacity.

Phosphorus is the second most essential nutrient required by plants for optimal growth, playing a crucial role in various metabolic processes, including energy transfer, signal transduction, respiration, macromolecular biosynthesis, and photosynthesis [18]. In the soil, 95–99% of phosphorus exists in insoluble, immobilized, or precipitated forms, making it difficult for plants to absorb. Plants can only take up phosphate in the forms of monobasic ($H_2PO_4^-$) and dibasic (HPO_4^{2-}) ions. Therefore, the solubilization of phosphorus by phosphate-solubilizing bacteria is a critical characteristic of plant growth-promoting bacteria (PGPB). Many soil bacteria synthesize low molecular weight organic acids, such as gluconic acid, to effectively solubilize inorganic phosphorus [19].

Potassium is an essential nutrient vital for plant resistance and growth. In this study, 6 out of 16 bacterial strains isolated from iron sand soils demonstrated the ability to solubilize potassium. Among these, five isolates exhibited a high capacity for potassium solubilization,

with solubilization index (SI) values ranging from 4.05 to 6.39 (Table 1). In a related study involving 24 bacterial isolates obtained from rice rhizospheres, 7 were identified as potassium-solubilizing bacteria, with the highest SI recorded at 5.3 [11]. Another study reported that 15 out of 41 isolates showed potassium solubilization on solid medium; among these, 53.33% were classified as low (SI < 2.00), 33.33% as intermediate (2.00 < SI < 4.00), and 13.33% as high [20].

Additionally, screening of the bacteria from iron sand soils for IAA production revealed that only two isolates (PS2Y2 and KS1C4) were capable of producing IAA, with concentrations of 20.60 ppm and 11.73 ppm, respectively (Table 1). These findings indicate that although IAA-producing bacteria can be isolated from iron sand soils, they may not interact directly with plant roots. IAA is a crucial regulator of plant growth, involved in various metabolic processes, including cell elongation and division, apical dominance, tropism, and vascular differentiation [21]. The synthesis of IAA by bacteria occurs from tryptophan, which serves as a precursor through three distinct pathways [22], and the concentration of IAA produced is determined by the characteristics of each bacterial strain rather than the concentration of tryptophan.

Iron is the fourth most abundant element in the Earth's crust and is essential for the growth of living organisms, as it acts as a cofactor for enzymes involved in numerous metabolic processes. However, iron is primarily unavailable to plants and microorganisms in the soil because it exists in the ferric form (Fe^{3+}), which is insoluble at physiological pH [5]. To address this challenge, microorganisms, including bacteria, have developed mechanisms to acquire iron by releasing siderophores, which are low molecular weight iron-chelating compounds. These siderophores effectively sequester insoluble ferric iron from various environments. The results of the screening of iron sand soil bacteria for siderophore production showed that four isolates were capable of producing siderophores, with SI values ranging from 2.16 to 2.90 (Table 1). This indicates that these bacterial isolates possess an intermediate ability to produce siderophores. In a related study, 17 siderophore-producing bacterial isolates were obtained from the rhizosphere of groundnut (*Arachis hypogea L.*) grown in calcareous soil, assessed using the CAS method [23]. Among these, four isolates demonstrated exceptional performance, yielding approximately 60–80% siderophores under pH 8, utilizing sucrose as a carbon source and $(NH_4)_2SO_4$ as a nitrogen source at 37°C. The efficient siderophore-producing bacteria were identified as *Bacillus licheniformis*, *Bacillus subtilis*, and *Ochrobactrum grignonense* based on 16S rDNA sequencing.

Table 1. Results of testing the plant growth-promoting traits of bacterial isolates

Sampling Location	Isolate Code	Cell Morphology		N_2-Fixation	Solubilization Index (SI)		IAA Production (ppm)	Siderophore Production (IS)
		Gram	Form		Phosphate	Potassium		
Purworejo Coast	PS1Y1	-	rod	+	2.22	-	-	-
	PS1Y3	+	rod	+	1.73	-	-	-
	PS2Y2	+	rod	+	2.16	-	20.60	2.90
	PS2Y6	+	rod	+	2.11	-	-	-
	PS2P1	+	rod	-	2.27	4.05	-	-
	PS2P2	+	rod	-	2.58	5.12	-	-
	PS3Y4	-	rod	+	2.19	1.96	-	2.45
Kebumen Coast	KS1K1	+	cocci	-	2.10	4.97	-	-
	KS1C4	+	rod	+	1.53	4.43	11.73	-
	KS2C1	+	rod	+	1.09	-	-	-
	KS3K3	+	cocci	-	2.15	-	-	-
Cilacap Coast	CS1P1	+	cocci	-	2.81	6,39	-	2.16
	CS2Y9	+	cocci	+	2.15	-	-	2.23
	CS2Y10	+	rod	+	2.11	-	-	-
	CS2C8	+	cocci	+	2.13	-	-	-
	CS3C7	+	short rod	+	2.12	-	-	-

Table 2. Number of total bacterial population in iron sand soils

Sampling Location	Total Bacterial Population (CFU.g^{-1} soil)
Purworejo Coast	$2.0 \times 10^4 - 3.5 \times 10^5$
Kebumen Coast	$5.0 \times 10^2 - 1.5 \times 10^5$
Cilacap Coast	$1.5 \times 10^4 - 2.0 \times 10^5$

The total bacterial population in iron sand soils was measured to range from 5.0×10^2 to 3.5×10^5 CFU g^{-1} soil (Table 2), indicating a relatively low abundance of bacteria. Iron sand soils are characterized by low organic matter content, reduced water-holding capacity, low nitrogen content, low cation exchange capacity, and high salinity. Consequently, the bacteria that thrive in these soils have developed tolerance to these challenging environmental conditions. [24] reported that a total of 1,231 bacterial operational taxonomic units (OTUs) were identified from the roots of *Calotropis gigantea*, while 1,419 OTUs were found in the roots of *Spinifex littoreus*. The most abundant bacterial phyla associated with the roots of *C. gigantea* were Proteobacteria (38.25%), Actinobacteria (30.17%), Firmicutes (10.87%), and Bacteroidota (5.68%). Among these, certain bacteria, such as *Bacillus idriensis*, were observed to be sensitive to soil salinity levels; however, this bacterium has been reported to promote growth in canola plants [25].

The findings of this investigation indicate that bacterial isolates with plant growth-promoting traits can be successfully isolated from iron sand soils. These isolates show potential for development into biofertilizers suitable for use in marginal soils, particularly following strain improvement. Further studies on the ability of the bacterial isolates to promote plants and their identities need to be done.

D. Conclusion

It can be concluded that 16 bacterial strains with plant growth-promoting traits were isolated from the iron sand soils of Central Java's south coast. Among these, several isolates demonstrated significant potential as candidates for promoting plant growth. Specifically, 11 isolates were capable of fixing nitrogen (N_2) qualitatively, while all isolates exhibited the ability to solubilize phosphorus (P), with SI values ranging from 1.09 to 2.81. Additionally, six isolates were able to solubilize potassium (K), with SI values ranging from 1.96 to 6.39. Furthermore, two isolates produced IAA at concentrations of 11.73 ppm and 20.60 ppm, and five isolates demonstrated the ability to produce siderophores, with SI values between 2.16 and 2.90.

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