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Isolation and Biochemical Characterization of Salt Tolerant, IAA-Producing PGPR from Kaipad Paddy Fields of Kerala

Sharmila S[1], Laija S. Nair[2] and Sandhia G. S.*[3]

[1-3] P. G. Department of Botany and Research Centre, University College, Thiruvananthapuram - 695 034, Kerala, India

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Abstract

Soil salinity is a major constraint to agriculture in coastal regions adversely affecting plant growth and crop yield. Halotolerant microorganisms adapted to saline conditions offer potential for the development of biological strategies in salt-affected soils. In particular, halotolerant plant growth–promoting rhizobacteria (PGPR) with indole-3-acetic acid (IAA) production potential can mitigate salinity stress and enhance crop performance. The present study aimed to isolate halotolerant bacteria from Kaipad saline paddy fields in Kerala, screen them for IAA production, and characterize selected isolates morphologically and biochemically. Four potent bacterial isolates were obtained and evaluated for colony morphology, Gram reaction, motility, and key biochemical traits including catalase, oxidase, amylase, esculin hydrolysis, H_2S gas production, and acid production from carbohydrates. All the four isolates exhibited halotolerance and produced IAA under saline conditions indicating their potential for further evaluation as plant growth-promoting candidates. These findings suggest that the isolated halotolerant, IAA-producing rhizobacteria are promising candidates for development as bioinoculants to improve crop productivity in salinity-affected agricultural systems.

Key words: Halotolerant plant growth promoting Rhizobacteria, Kaipad fields, Salinity stress, IAA production

Soil harbours highly diverse and functionally important microbial communities, comprising bacteria, fungi, viruses, and other microorganisms that occupy a wide range of ecological niches. The distribution and activity of these microorganisms are strongly influenced by physicochemical properties of soil, including pH, moisture, organic matter, nutrient availability, and plant-derived residues [1]. Among these microorganisms, rhizosphere-associated bacteria are particularly important because of their close association with plant roots and their ability to influence plant growth and nutrient acquisition. Saline soils represent challenging environments for crop production, particularly in coastal regions, where excessive salt accumulation can adversely affect plant growth and productivity [2]. At the same time, such environments can serve as reservoirs of indigenous microorganisms possessing adaptations that enable them to survive under salinity stress.

The present study focused on the isolation and characterization of plant growth-promoting rhizobacteria (PGPR) from saline-prone rice fields of the Kaipad ecosystem in Kerala, India. Rhizospheric soil samples were collected from rice-growing areas and processed by serial dilution and plating on appropriate selective media for the recovery of bacterial isolates. The isolates were evaluated for important plant growth-promoting attributes and particular emphasis was given to IAA production because bacterial auxin can stimulate root development and thereby potentially improve plant establishment under stressful environmental conditions. IAA-producing halotolerant PGPR may help plants cope with salinity stress by promoting root development, improving water and nutrient acquisition, and modulating plant defence responses.

The isolation of indigenous, salt-tolerant PGPR possessing multiple plant growth-promoting characteristics highlights the potential of the Kaipad soil microbiome as a source of agriculturally valuable microbial resources. Such bacteria may have potential applications in developing bioinoculants for improving crop performance in salt-affected soils [3-5]. Their ability to promote plant growth while functioning under environmental stress could contribute to reduced dependence on chemical inputs and support sustainable agricultural production. Furthermore, the characterization of native soil microorganisms provides valuable insight into microbial contributions to nutrient cycling, organic matter transformation, plant–microbe interactions, and the development of climate-resilient agricultural systems [6].

MATERIALS AND METHODS

Isolation of soil microorganisms

Soil microorganisms were isolated by the serial dilution and spread-plate method using Luria- Bertani (LB) agar medium. Briefly, 1 g of each soil sample was transferred into 10 mL of sterile distilled water and vigorously shaken for 15 min to facilitate the release and homogenization of microorganisms from the soil matrix. The suspension was subsequently vortexed to obtain a uniform microbial dispersion and serially diluted up to 10^{-6}. An aliquot of 0.1 mL from each dilution was aseptically transferred onto LB agar plates and

***Correspondence to:** Sandhia G. S, E-mail: gssandhia@gmail.com; Tel: +91 8547520570

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evenly distributed over the surface using a sterile L-shaped glass spreader. The inoculated plates were incubated at 37 °C for 48 h. Following incubation, morphologically distinct and well-isolated colonies were selected and purified by repeated subculturing. Pure cultures were maintained at 4 °C for subsequent characterization and experimental analysis [7].

Identification and characterization of bacterial isolates

The purified bacterial isolates were initially characterized according to their colony morphology and cellular characteristics. Colony features, including shape, size, pigmentation, and other observable morphological properties, were recorded. Cellular morphology and Gram reaction were determined by Gram staining to differentiate the isolates into Gram-positive and Gram-negative groups. Further phenotypic characterization was carried out using standard biochemical assays, including indole production, methyl red (MR), Voges–Proskauer (VP), citrate utilization, and carbohydrate fermentation tests. All biochemical tests were performed according to established microbiological procedures and previously reported protocols [8, 9]. The combined morphological, microscopic, and biochemical characteristics were used for the preliminary identification and differentiation of the bacterial isolates prior to further molecular characterization.

RESULTS AND DISCUSSION

Physicochemical characteristics of the soil

Rhizospheric soil collected from the Kaipad field in Kerala was analyzed for selected physicochemical parameters. The soil recorded a pH of approximately 6.5, indicating a slightly acidic to near-neutral condition that can support the availability of several essential nutrients and the establishment of diverse microbial populations. Soil pH is considered one of the major determinants of microbial community composition and activity because it influences nutrient availability, microbial physiology, and interactions between microorganisms and soil components [10]. The soil moisture content was 45.8%, and the temperature at the time of sampling was 32 °C. Adequate soil moisture is essential for microbial growth and metabolic activity because it facilitates substrate diffusion and nutrient transformation. However, the influence of moisture on microbial activity is also dependent on soil aeration and the availability of oxygen within the soil matrix [11-13]. The observed physicochemical conditions therefore provided a potentially suitable environment for the survival and proliferation of cultivable rhizospheric bacteria.

Isolation and characterization of bacterial isolates

Culture-dependent isolation and subsequent screening of the rhizospheric soil samples yielded four distinct bacterial isolates, designated ST2, ST16, ST21, and ST22. The isolates were purified through repeated subculturing and exhibited consistent morphological characteristics. Gram staining revealed that ST2 and ST16 were Gram-negative, motile, rod-shaped, and non-spore-forming, whereas ST21 and ST22 were Gram-positive, motile, rod-shaped, and spore-forming. The observed differences in Gram reaction and spore-forming ability indicate phenotypic diversity among the recovered isolates and provided useful preliminary characteristics for their differentiation and subsequent identification [14]. The occurrence of physiologically diverse bacterial populations in the rhizosphere is consistent with the complex interactions between plants, soil properties, and microorganisms that influence the structure of rhizospheric microbial communities [15-16].

Table 1 Colony morphology and gram staining of the four selected bacterial strains

Strain	ST2	ST16	ST21	ST22
Colony shape	Circular	Circular	Circular	circular
Cell shape	Rod	Rod	Rod	Rod
Colony margin	Entire	Entire	Entire	Entire
Elevation	Raised	Flat	Flat	raised
Motility	+ve	+ve	+ve	+ve
Spore former	-ve	-ve	+ve	+ve
Gram's reaction	-ve	-ve	+ve	+ve

Table 2 Biochemical characterization of bacterial isolate

S. No.		ST2	ST16	ST21	ST22
1.	Growth on Macconkey Agar medium	+	-	+	+
2.	Indole test	+	+	+	+
3.	Methyl red test	-	-	-	-
4.	Voges Proskauer test	+	-	+	-
5.	Citrate test	+	-	-	+
6.	Casein test	-	-	-	-
7.	Starch hydrolysis	-	+	-	+
8.	Gelatin hydrolysis	-	-	-	-
9.	Nitrate reduction	-	-	-	-
10.	Catalase	+	+	+	+
11.	Oxidase	+	+	+	+
12.	Esculin hydrolysis	-	-	-	+
13.	H_2S gas production	+	+	+	+
14.	Urease	-	-	-	-

Biochemical characterization further demonstrated distinct metabolic characteristics among the four isolates. All isolates exhibited catalase and oxidase activity and were capable of utilizing xylose and mannitol with acid production. Growth on MacConkey agar was observed for ST2, ST21, and ST22, whereas ST16 did not exhibit growth under the tested conditions. The remaining carbohydrate fermentation profiles and biochemical reactions of the isolates are presented in (Table

2). Collectively, these characteristics provided a preliminary phenotypic profile for differentiating the isolates. Nevertheless, biochemical characteristics alone cannot establish definitive taxonomic identity because phenotypic traits may overlap among closely related bacterial taxa. Therefore, molecular identification, particularly through 16S rRNA gene sequencing, is necessary to confirm the taxonomic affiliation of the isolates.

The recovery of four morphologically and physiologically distinct bacterial strains highlights the usefulness of culture-based techniques for obtaining cultivable members of the rhizospheric microbiota. Rhizospheric microorganisms contribute substantially to nutrient cycling, organic matter transformation, and plant–microbe interactions, while environmental conditions influence their distribution and functional properties [17]. The isolates obtained in the present study may therefore represent potentially important members of the cultivable bacterial community associated with the Kaipad rice ecosystem. Their subsequent screening for plant-growth-promoting characteristics, including phosphate solubilization, siderophore production, ACC deaminase activity, Indole-3-acetic acid (IAA) production, and salinity tolerance, will provide further insight into their potential application as Plant growth-promoting rhizobacteria (PGPR) for improving plant performance under saline conditions [18-19].

Acid production from carbohydrate

S. No.		ST2	ST16	ST21	ST22
1.	Fructose	-	-	-	-
2.	Salicin	-	-	-	-
3.	Rhamnose	-	-	-	-
4.	Mannitol	+	+	-	+
5.	Xylose	+	+	+	+
6.	Lactose	-	-	-	-
7.	Galactose	-	+	-	-
8.	Inositol	-	-	-	-
9.	Sorbitol	-	-	-	-
10.	Sucrose	-	-	-	-

CONCLUSION

Soil-associated bacteria represent an important microbial resource with potential roles in maintaining soil health and promoting plant growth. The bacterial isolates obtained from agricultural soils exhibited diverse plant growth-promoting characteristics along with the ability to tolerate environmental stresses. These functional attributes suggest that such microorganisms may contribute to nutrient availability, plant development, and improved plant adaptation to challenging environmental conditions through beneficial plant–microbe interactions. The findings emphasize the value of naturally occurring soil bacteria as potential microbial resources for developing sustainable approaches to crop production. Their use as bioinoculants may provide an environmentally compatible approach to complement conventional agricultural inputs. Further investigations involving molecular characterization, elucidation of functional mechanisms, greenhouse evaluation, and field-level validation are necessary to establish their effectiveness and consistency under different agricultural conditions. The findings provide a basis for further research into indigenous halotolerant bacteria as potential bioinoculants for sustainable rice cultivation in saline environments.

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