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Isolation and Screening of Stress-Tolerant PGPR with ACC Deaminase Activity from the Rhizosphere of Plants Grown in Arid and Saline Soils of Kerala

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Abstract

Soil microorganisms, particularly those inhabiting the rhizosphere, play a crucial role in promoting plant growth and enhancing tolerance to environmental stress. In the present study, bacterial isolates were obtained from rhizospheric soils of crops cultivated in saline and drought-prone agricultural fields of Kerala, India. Using the serial dilution technique, distinct bacterial colonies were isolated and purified. Two isolates, designated AS11 and AC21, were selected for further analysis based on colony characteristics. Morphological and biochemical characterization revealed that both isolates were Gram-negative, motile, rod-shaped, and non-spore-forming. Carbohydrate utilization profiles and additional biochemical tests indicated that isolate AS11 belongs to the genus *Caballeronia*, while isolate AC21 was identified as a member of the genus *Enterobacter*. These genera are well-documented for their plant growth-promoting traits, including ACC deaminase activity, making them promising candidates for development as bioinoculants in stress-affected agricultural systems.

***Key words*:** ACC deaminase, Plant growth-promoting bacteria (PGPB), Rhizosphere, Soil, Drought stress

Microorganisms are ubiquitous, inhabiting nearly every corner of the biosphere—from soil and water to extreme environments such as hot springs and deep subterranean rocks, even up to nineteen kilometres beneath the Earth's surface. Among these habitats, soil represents one of the most biologically active ecosystems, harbouring diverse microbial communities including bacteria, fungi, and viruses [1]. The abundance and composition of these microorganisms vary depending on soil properties such as pH, moisture content, organic matter, and the type of plant residues present [2].

The present investigation focuses on the exploration and identification of beneficial soil microorganisms, particularly plant growth-promoting rhizobacteria (PGPR), from challenging environmental conditions such as arid and saline soils. These stress-prone environments are common in certain regions of Kerala, especially in coastal and semi-arid inland zones, where soil salinity and drought significantly hinder agricultural productivity [3]. The rhizosphere—the soil region closely associated with plant roots—is a hotspot of microbial activity and a key target for isolating stress-resilient microorganisms that assist plant survival under such adverse conditions. The primary objective of the study is to isolate PGPR strains that not only exhibit tolerance to abiotic stresses like high salinity and drought but also possess 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity. ACC deaminase is an important enzyme produced by certain soil bacteria which helps plants mitigate the negative effects of abiotic stresses. Under stress, plants typically produce ethylene, a hormone that can inhibit root elongation and plant growth. PGPRs with ACC deaminase can break down ACC, the precursor to ethylene, thereby lowering ethylene levels and allowing the plant to grow better even under stress conditions [4-5].

The process begins with the collection of rhizospheric soil samples from plants naturally growing in arid and saline regions of Kerala. These sites are ideal for sourcing hardy bacterial strains that have evolved mechanisms to survive extreme conditions. Once collected, soil samples are serially diluted and cultured on selective media to isolate diverse bacterial colonies. These isolates are then subjected to preliminary screening for plant growth-promoting traits, such as phosphate solubilization, indole-3-acetic acid (IAA) production, siderophore production, and nitrogen fixation ability. A crucial part of the screening involves identifying ACC deaminase activity using minimal medium supplemented with ACC as the sole nitrogen source. Only bacteria capable of utilizing ACC can grow on this medium, thus indicating the presence of ACC deaminase. Further biochemical and molecular characterization (such as 16S rRNA sequencing) may be employed to identify and classify the most promising strains at the species level [6-7].

The selected PGPR strains are then tested for their tolerance to abiotic stresses like high salt concentration (NaCl), drought simulation using polyethylene glycol (PEG), and high temperature. Their ability to colonize roots and promote plant growth under controlled greenhouse conditions is evaluated

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using model plants or crop species native to Kerala. Parameters such as shoot and root length, fresh and dry biomass, and physiological markers of stress tolerance are recorded. This study holds immense agricultural significance, particularly in the context of climate-resilient farming. By identifying indigenous, stress-tolerant PGPR with ACC deaminase activity, it becomes possible to develop bio-inoculants or microbial consortia that can be applied to crops growing in marginal lands. These biofertilizers can reduce dependency on chemical inputs, improve soil health, and enhance the sustainability of crop production in saline and drought-prone regions of Kerala and beyond [8]. Soil microorganisms play a vital role in maintaining ecological balance and supporting life on Earth. They contribute to soil fertility by decomposing organic matter, recycling nutrients, and participating in biogeochemical cycles. The metabolic activities of soil microbes produce a range of beneficial substances that enhance nutrient availability, promote plant growth, and contribute to the stabilization of greenhouse gases [9-12]. Given their importance in sustainable agriculture, studying soil microbial communities is essential. In the present study, soil samples were collected from saline and arid regions of Kerala. Bacterial isolates were obtained and characterized to explore their potential roles in soil health and plant growth promotion.

MATERIALS AND METHODS

Isolation of soil microorganisms

Soil microorganisms were isolated using the serial dilution technique on Dworkin and Foster (DF) minimal salt agar medium. One gram of soil from each sample was suspended in 10 mL of sterile distilled water and shaken vigorously for 15 minutes, followed by vortexing to ensure uniform dispersion. The resulting suspension was serially diluted up to 10^{-6}. From each dilution, 0.1 mL was aseptically spread onto DF agar plates using a sterile L-shaped glass spreader. The plates were incubated at 37 °C for 24 hours. Distinct and morphologically prominent colonies were selected, sub-cultured, and maintained at 4 °C for further analysis.

Identification and characterization of bacterial isolates

The bacterial isolates were initially characterized based on their morphological features, including shape, size, and cellular arrangement, along with Gram staining to differentiate between Gram-positive and Gram-negative bacteria. Further biochemical characterization was performed using standard tests such as Indole production, Methyl Red (MR), Voges-Proskauer (VP), Citrate utilization, and carbohydrate fermentation profiles, following established protocols [13-15].

RESULTS AND DISCUSSION

Soil samples were collected from the rhizosphere of plants in an agricultural field in Kerala. The physicochemical properties of the soil were analysed. The pH of the soil was found to be approximately 6.5, which is within the optimal range (5.5–7.5) for nutrient availability, as reported by the Queensland Department of Environment and Heritage Protection. The soil moisture content was 45.8%, and the temperature recorded at the time of sampling was 32 °C. These conditions are favorable for microbial activity and nutrient cycling, thereby promoting plant growth. Higher moisture content facilitates microbial proliferation, which in turn enhances nutrient availability to plants.

Table 1 Morphological and biochemical characterization of bacterial isolates from soil

Isolate	Gram reaction	Spore former	Motility	Morphology	Indole	Methyl red	Citrate	Urea
AS11	-	-	+	Rods	-	+	-	-
AC21	-	-	+	Rods	-	+	+	-

Biochemical analysis of isolates

Soil samples collected from agricultural fields in Kerala were subjected to serial dilution, and two morphologically distinct colonies, designated as AS11 and AC21, were isolated and purified through repeated sub-culturing. Both isolates were subjected to Gram staining and a series of biochemical tests. Microscopic examination revealed that both isolates were Gram-negative, motile, rod-shaped, and non-spore-forming (Table 1).

Biochemical characterization, including sugar fermentation profiling, indicated that isolate AC21 exhibited robust fermentation of various sugars, whereas isolate AS11 showed limited fermentative activity. Based on the biochemical characteristics and sugar utilization patterns, isolate AC21 was tentatively identified as *Enterobacter* sp., and AS11 as *Caballeronia* sp. (Table 2). These findings are consistent with previous reports, such as Bhattacharya *et al*. [16], who isolated microorganisms like *Escherichia coli*, *Micrococcus* sp., *Escherichia* sp., and *Staphylococcus* sp. from agricultural soils. Similarly, Jasuja [17] reported the isolation of a diverse range of bacterial and fungal species from polyhouse soils through the application of the serial dilution technique. This method enabled the enumeration and identification of cultivable microbial populations, thereby providing insights into the microbiological profile and potential plant-growth-promoting organisms present in controlled agricultural environments.

Table 2 Carbohydrate fermentation of bacterial isolates from soil

Isolate	Fructose	Salicin	Rhamnose	Mannitol	Xylose	Galactose	Sucrose	Sorbitol	Identification of genus
AS11	+	+	-	-	-	-	-	+	Caballeronia
AC21	+	+	+	+	+	-	+	-	Enterobacter

CONCLUSION

Soil harbours a vast and diverse community of microorganisms, including bacteria that play essential roles in maintaining soil health and supporting plant growth. While some bacterial species are highly sensitive to environmental fluctuations, others are remarkably resilient, capable of surviving under extreme conditions such as heat, cold, and desiccation. Certain bacteria also exhibit plant-specific associations, contributing to plant-microbe interactions within the soil food web. A diverse bacterial population enhances soil functionality and can naturally suppress root-borne diseases.

The present study demonstrates that agricultural soils from Kerala harbour beneficial bacterial strains with potential plant growth-promoting (PGP) traits. These isolates may be valuable candidates for development as bioinoculants to enhance sustainable agricultural productivity.

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