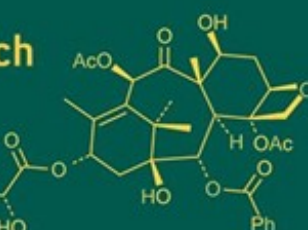
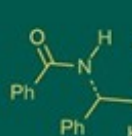


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Multitrait characterization of salt-tolerant plant growth-promoting bacteria under salinity stress

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ABSTRACT

Soil salinity is a major constraint on global crop productivity, making salt-tolerant plant growth-promoting bacteria (PGPB) a promising, eco-friendly solution for mitigating salt stress in crops. This study characterized salt-tolerant PGPB isolated from rhizosphere soil and root/shoot samples of rice grown in salt-affected fields, evaluating their growth-promoting functions under increasing salinity. Fifteen morphologically distinct isolates (IIRRSTB-1 to IIRRSTB-15) were screened for hydrogen cyanide production, phosphate and zinc solubilization, ACC deaminase activity, IAA production, and nitrogenase activity (acetylene reduction assay) under non-saline and saline (5% and 10% NaCl) conditions. All isolates grew luxuriantly at 0% and 5% NaCl, and eight retained this growth at 10% NaCl. At the highest salinity, IIRRSTB-2 and IIRRSTB-12 retained all five qualitative PGP traits, followed by IIRRSTB-1 and IIRRSTB-7 with four traits each. Quantitatively at 10 EC, IIRRSTB-2 recorded the highest values for HCN (35.1), zinc solubilization (19.9), ACC deaminase (84.5), nitrogenase activity, and IAA production (53.4 $\mu\text{g mg}^{-1}$ protein), while IIRRSTB-1 (156.0) and IIRRSTB-7 (148.1) excelled in phosphate solubilization. IIRRSTB-2 as a strong candidate for further molecular characterization and subsequent pot- and field-scale validation as a bioinoculant to support sustainable rice cultivation in salt-affected agricultural systems.

Keywords: Salt-tolerant plant growth-promoting bacteria, Salinity stress, Bioinoculant, Rhizosphere soil, Rice

Introduction

Soil salinity ranks among the most pressing threats to global agriculture and food security. Excess soluble salts compromise soil structure and fertility, suppress plant growth, depress crop yields, and disturb soil microbial communities (Tarolli *et al.*, 2024). This salinization arises from a combination of natural drivers arid climates, scarce rainfall, and high evaporation and anthropogenic pressures, including improper irrigation, inadequate drainage, and overuse of fertilizers (Tarolli *et al.*, 2024). Salt stress is a leading constraint on agricultural output, contributing, together with drought, to an estimated 20-50% of global crop losses (Ha-Tran *et al.*, 2021). Secondary salinization has already compromised nearly one-fifth of irrigated land worldwide (Mishra *et al.*, 2023), and the arid and semi-arid regions most susceptible to this damage span roughly 40% of Earth's land surface, sustaining close to two billion people (Hanin *et al.*, 2016). With climate change and intensifying agricultural practices compounding the problem (Mukhopadhyay *et al.*, 2020), developing sustainable mitigation strategies has become essential to securing food production and long-term agricultural resilience (Mukhopadhyay *et al.*, 2020).

Among the eco-friendly solutions being explored, plant growth-promoting bacteria (PGPB) have gained recognition as a powerful biological tool for easing salinity stress in crops (Kumar *et al.*, 2020) [1]. These salt-tolerant bacteria help plants cope with saline conditions through several mechanisms producing 1-aminocyclopropane-1-carboxylate deaminase, indole-3-acetic acid, antioxidants, extracellular polymeric substances, and volatile organic compounds (Kumar *et al.*, 2020) [1]. Because such microbes enhance plant salt tolerance and help recover yields in saline-prone environments, inoculating crops with them is becoming increasingly valuable in modern agronomy (Ilangumaran *et al.*, 2017) [2]. Halo-tolerant plant growth-promoting rhizobacteria, in particular, serve as effective agents against both biotic and abiotic stresses while boosting productivity in salt-affected farming systems (Kumawat *et al.*, 2023). Deployed as green bioinoculants, they show real promise for reinforcing plant defenses under salt stress and helping meet the world's growing food demand (Ha-Tran *et al.*, 2021).

It is within this context that the present study set out to characterize salt-tolerant plant growth-promoting bacteria isolated from rhizosphere soil and root/shoot samples collected from salt-affected rice fields (Multitrait Characterization of Salt-Tolerant Plant Growth-Promoting Bacteria under Salinity Stress, n.d.). Fifteen isolates (IIRRSTB-1 through IIRRSTB-15) were systematically assessed for a range of plant growth-

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promoting traits hydrogen cyanide production, phosphate and zinc solubilization, ACC deaminase activity, IAA production, and nitrogenase activity under both non-saline and saline conditions (5% and 10% NaCl), using standard protocols (Multitrait Characterization of Salt-Tolerant Plant Growth-Promoting Bacteria under Salinity Stress). This multitrait screening across increasing salinity levels was designed to screen out isolates capable of sustaining their growth-promoting functions under salt stress, laying the groundwork for their potential development as bioinoculants in sustainable crop production across saline environments.

Materials and methods

Sample collection

Rhizosphere soil and root/shoot samples were collected from salt-affected rice fields at Agricultural Research Station (ARS) Machilipatnam, ARS Kunaram, ARS Kampasagar (Nelaguda and adjoining villages), and Krishi Vigyan Kendra (KVK) Gangavathi during the rice-growing season, and transported under refrigeration in sterile, labelled polythene bags to ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad.

Isolation and purification

Serial dilutions of rhizosphere soil and surface-sterilized plant tissues were plated on nutrient agar (NA) with 5 % and 10 % NaCl. Morphologically distinct colonies were purified by repeated streaking and maintained on NA slants at 4 °C; 15 isolates (IIRRSTB-1 to IIRRSTB-15) were selected for further study.

Salt-tolerance screening

Overnight cultures were spot-inoculated (6 µl) on NA plates with 0 %, 5 %, and 10 % NaCl, incubated at 28 ± 2 °C for 48 h, and scored visually on a 1-5 scale (++++ = luxuriant growth).

Morphological characterization

Colony form, margin, elevation, texture, and appearance were recorded for all isolates after 48 h on NA medium.

Plant growth-promoting trait assay

Isolates were assessed qualitatively and quantitatively for HCN production, phosphate and zinc solubilization, ACC deaminase activity, and indole-3-acetic acid (IAA) production under non-saline, 5 % and 10 % NaCl, following standard protocols (Bakker & Schippers, 1987; Pikovskaya, 1948; Fasim *et al.*, 2002; Honma & Shimomura, 1978; Gordon & Paleg, 1957); nitrogenase activity was additionally measured via the acetylene reduction assay (ARA).

Results

Morphological characterization of salt-tolerant isolates

The fifteen cultures exhibited wide variation in colony form, margin, elevation, texture, and appearance (as shown in Table 1, Fig. 1). As these traits are descriptive rather than quantitative, no isolate was ranked as high- or low-performing on morphological grounds. Round colonies were the most prevalent, recorded for seven isolates (IIRRSTB-6, 7, 8, 11, 12, 13, and 14), followed by irregular colonies in five isolates (IIRRSTB-1, 3, 4, 5, and 15) (as shown in Table 1). Oval, rhizoid, and punctiform forms were restricted to IIRRSTB-2, IIRRSTB-9, and IIRRSTB-10, respectively (as shown in Table 1). Convex elevation was the dominant type, observed in eleven isolates (IIRRSTB-2, 3, 5, 6, 7, 8, 10, 11, 13, 14, and 15), whereas raised elevation was confined to IIRRSTB-1, 4, 9, and 12 (as shown in Table 1). In terms of appearance, opaque/white colonies were most frequent (IIRRSTB-2, 3, 4, 6, 8, 10, and 12), followed by milky white (IIRRSTB-1 and 5), creamy opaque (IIRRSTB-7 and 13), creamy, white, milky, and opaque (as shown in Table 1).

Comparative salt-tolerance performance

Under both control and 5 % NaCl conditions, all fifteen cultures displayed luxuriant growth (++++) (as shown in Table 2). Differences among isolates became apparent only at 10 % NaCl: the high-performing group (IIRRSTB-1, 2, 4, 7, 9, 11, 12, and 13) sustained luxuriant growth (++++), the intermediate-performing group (IIRRSTB-3, 6, 8, and 14) showed good growth (+++), and the low-performing group (IIRRSTB-5, 10, and 15) exhibited only moderate growth (++) (as shown in Table 2). Notably, no isolate showed poor growth (+) at 10 % NaCl (as shown in Table 2).

Qualitative PGP activity under salinity stress

At 10 % NaCl, HCN production was retained by the high-performing isolates IIRRSTB-1, 2, 4, 7, 9, 12, and 13 (+) (Fig. 7), while the remaining eight isolates were negative (−) (as shown in Table 3). Phosphate-solubilizing activity persisted only in IIRRSTB-1, 2, 4, 7, and 13 (+) (Fig. 2), and zinc-solubilizing activity only in IIRRSTB-2, 6, and 12 (+) (as shown in Table 3, Fig. 4). ACC-deaminase activity was retained by IIRRSTB-1, 2, 4, 7, 9, and 12 (+) (Fig. 5), and IAA production by IIRRSTB-1, 2, 7, and 12 (+) (Fig. 3) (as shown in Table 3).

Considering all five qualitative traits collectively at 10 % NaCl, IIRRSTB-2 and IIRRSTB-12 emerged as the most consistently high-performing isolates, retaining activity for HCN, phosphate solubilization, zinc solubilization, ACC deaminase, and IAA (as shown in Table 3). IIRRSTB-1 and IIRRSTB-7 retained four traits, IIRRSTB-4 retained three, IIRRSTB-9 and IIRRSTB-13 retained two, and IIRRSTB-6 retained one; the remaining isolates (IIRRSTB-3, 5, 8, 10, 11, 14, and 15) were uniformly negative for all five traits and were therefore categorized as low-performing for qualitative PGP retention (as shown in Table 3).

Quantitative PGP performance at 10 EC

HCN production was highest in IIRRSTB-2 (35.1) and IIRRSTB-13 (33.7), with intermediate activity in IIRRSTB-12 (27.0), IIRRSTB-7 (15.0), IIRRSTB-1 (14.5), and IIRRSTB-9 (9.6), and the lowest detectable value in IIRRSTB-4 (6.1); all other isolates were non-detectable (as shown in Table 4).

Phosphate solubilization was highest and comparable in IIRRSTB-1 (156.0) and IIRRSTB-7 (148.1), followed by IIRRSTB-2 (108.8); lower activity was recorded for IIRRSTB-13 (19.5) and IIRRSTB-4 (7.5), with no detectable activity in the remaining isolates (as shown in Table 4). Zinc solubilization was detected exclusively in IIRRSTB-2 (19.9), IIRRSTB-6 (14.8), and IIRRSTB-12 (10.0) (as shown in Table 4).

ACC-deaminase activity was maximal in IIRRSTB-2 (84.5) and IIRRSTB-7 (68.2), intermediate in IIRRSTB-4 (19.1), and comparable at lower values in IIRRSTB-9 (12.1) and IIRRSTB-12 (12.0), with the lowest detectable activity retained by IIRRSTB-1 (6.0); the remaining isolates were non-detectable (as shown in Table 4). Acetylene-reduction activity was retained by only three isolates — IIRRSTB-2, IIRRSTB-4, and IIRRSTB-12 (Fig. 6) while all other cultures were non-detectable across salinity levels (as shown in Table 4). IAA production ($\mu\text{g mg}^{-1}$ protein) was highest in IIRRSTB-2 (53.4) and IIRRSTB-7 (41.0), with lower detectable activity in IIRRSTB-12 (25.0) and IIRRSTB-1 (8.9); the remaining isolates were non-detectable (as shown in Table 4).

Across the high-stress conditions examined, IIRRSTB-2 proved the most consistently high-performing isolate: it retained luxuriant growth (++++) at 10 % NaCl (as shown in Table 2), remained positive for all five qualitative PGP traits (as shown in Table 3), and recorded the highest quantitative values at 10 EC for HCN production (35.1), zinc solubilization (19.9), ACC deaminase (84.5), acetylene-reduction activity, and IAA production (53.4) (as shown in Table 4).

IIRRSTB-1 and IIRRSTB-7 performed strongly but in a more trait-specific manner, particularly for phosphate solubilization at 10 EC (156.0 and 148.1, respectively) (as shown in Table 4). IIRRSTB-12 was equally resilient, retaining all five qualitative traits (as shown in

Table 3) together with detectable quantitative activity for HCN (27.0), zinc solubilization (10.0), ACC deaminase (12.0), acetylene-reduction activity, and IAA (25.0) at 10 EC (as shown in **Table 4**).

Low-performing status varied with the endpoint considered: at 10 % NaCl, IIRRSTB-5, 10, and 15 were low-performing, showing only moderate growth (as shown in **Table 2**); for quantitative PGP activity at 10 EC, IIRRSTB-3, 5, 8, 10, 11, 14, and 15 formed the low-performing group, being non-detectable for the measured parameters (as shown in **Table 4**). IIRRSTB-4, 6, 9, and 13 exhibited intermediate or trait-specific performance rather than uniformly low activity. These classifications reflect laboratory observations at 10 % NaCl or 10 EC and do not establish statistical superiority or field performance.

Discussion

Salinity stress remains a major limiting factor for crop productivity worldwide, and salt-tolerant plant growth-promoting rhizobacteria (PGPR) are increasingly recognized as a promising biological approach for enhancing crop performance in saline agro-ecosystems (Shultana *et al.*, 2022) [3]. Since native strains isolated from saline habitats tend to outperform those from non-saline environments in boosting plant salt resistance (Shultana *et al.*, 2020) [4], recovering indigenous halotolerant PGPR from salt-affected rice fields is a sensible starting point for developing effective bio-inoculants.

In this study, 15 morphologically distinct isolates (IIRRSTB-1 to IIRRSTB-15) were obtained from rhizosphere soil and root/shoot tissue of rice grown in saline fields, using nutrient agar supplemented with 5% and 10% NaCl. This approach to recovering diverse halotolerant bacteria from saline environments echoes previous work: Ramadoss *et al.* (2013) [5] isolated 84 strains from saline habitats, Albdaoui *et al.* (2019) [6] recovered 74 isolates from durum wheat growing near the Dead Sea, and Pallavi *et al.* (2023) [7] obtained 156 isolates from the Sundarbans mangroves. Similarly, halotolerant rice-associated PGPR including *Pseudomonas stutzeri* and *Klebsiella pneumoniae* have been reported to improve seedling growth under saline conditions (Khumairah *et al.*, 2022) [8]. The colony-level diversity observed here (variation in shape, margin, elevation, texture, and surface appearance) reflects the standard use of morphological characterization as a first-pass screening tool in rhizobacterial studies (Damodaran *et al.*, 2013; Pallavi *et al.*, 2023) [9, 7].

Growth response to increasing salinity. All 15 isolates grew vigorously at 0% and 5% NaCl, with clear differentiation emerging only at 10% NaCl: eight isolates sustained luxuriant growth, four showed good growth, and three showed only moderate growth. This pattern aligns with earlier reports Ramadoss *et al.* (2013) [5] found that while all 84 isolates thrived at 5% NaCl, only a quarter tolerated 20% NaCl, and Pallavi *et al.* (2023) [7] noted that just 20 of 156 isolates survived 15% (2.56 M) NaCl. Similar tolerance thresholds have been documented for multifunctional PGPR tested across 5-9% NaCl (Oo *et al.*, 2020) [10] and for biofilm-forming rhizobacteria capable of surviving 5%, 10%, and 15% NaCl (Haque *et al.*, 2020) [11]; Sultana *et al.* (2019) likewise recovered rice-associated isolates tolerant of up to 2.60 mol L⁻¹ NaCl. The ability of most isolates here to grow at 10% NaCl (~1.7 M) therefore confirms genuine halotolerance consistent with their saline paddy origin.

Strain-dependent retention of PGP traits. A key outcome of this study is that select isolates preserved multiple plant growth-promoting (PGP) traits despite salt stress. IIRRSTB-2 and IIRRSTB-12 remained positive for all five qualitative traits at 10% NaCl; IIRRSTB-1 and IIRRSTB-7 retained four; IIRRSTB-4 retained three; IIRRSTB-9 and IIRRSTB-13 retained two; IIRRSTB-6 retained one; and the remaining seven isolates (IIRRSTB-3, 5, 8, 10, 11, 14, and 15) lost all activity. Harboring multiple PGP traits within a single strain is widely considered advantageous for biofertilizer development (Gupta *et al.*, 2019) [12], and multi-trait screening is the accepted strategy for identifying strong halotolerant candidates (Khan *et al.*, 2021) [13]. Salinity is known to modulate trait expression in a strain-specific manner (Oliva *et al.*, 2023) [14], and nutrient-solubilizing ability generally weakens as NaCl concentration rises (Dragojević *et al.*, 2023) [15] a

trend that mirrors the progressive decline in activity observed among the lower-performing isolates in this collection.

Hydrogen cyanide production. HCN, a volatile metabolite produced by genera such as *Bacillus*, *Pseudomonas*, *Alcaligenes*, *Aeromonas*, and *Rhizobium* (Martínez-Viveros *et al.*, 2010) [16], contributes to biocontrol of soil-borne pathogens (Elshahat *et al.*, 2016; Shah *et al.*, 2021; Fasusi *et al.*, 2023) [18, 17]. Only seven isolates retained HCN activity at 10% NaCl, with IIRRSTB-2 (35.1) and IIRRSTB-13 (33.7) recording the highest values at 10 dS m⁻¹. Notably, none of the isolates in Ramadoss *et al.* (2013) [5] produced HCN at all, making the HCN-positive strains identified here a relatively rare and potentially valuable biocontrol resource under saline conditions.

Phosphate and zinc solubilization. Phosphate-solubilizing bacteria are particularly important components of salt-tolerant microbial communities, since salinity reduces phosphorus availability in soil (Çelik *et al.*, 2024) [19]. Only five isolates retained qualitative phosphate solubilization at 10% NaCl, with IIRRSTB-1 (156.0) and IIRRSTB-7 (148.1) showing the highest quantitative activity, followed by IIRRSTB-2 (108.8) at 10 dS m⁻¹. These values are broadly comparable to previous reports of salt-tolerant phosphate solubilizers, such as *Enterococcus faecium* and *Pantoea dispersa*, which promoted mungbean growth under salinity (Kumawat *et al.*, 2021) [20], and *Bacillus halotolerans* AD9, which solubilized 86 ± 3.06 µg mL⁻¹ phosphate under high-salt conditions (Kapadia *et al.*, 2022) [21]. Zinc solubilization was even more restricted, retained only by IIRRSTB-2 (19.9), IIRRSTB-6 (14.8), and IIRRSTB-12 (10.0). This trait carries agronomic significance because saline, sodic, and calcareous soils frequently induce zinc deficiency (Acharya *et al.*, 2024) [22]; PGPR can improve zinc bioavailability through organic-acid secretion, proton extrusion, and siderophore-mediated chelation (Otlewska *et al.*, 2020) [23]. The dual retention of phosphate- and zinc-solubilizing activity by strains such as IIRRSTB-2 under salt stress strengthens their potential as multifunctional inoculants.

ACC deaminase activity. ACC deaminase degrades the ethylene precursor 1-aminocyclopropane-1-carboxylate into ammonia and α-ketobutyrate, thereby reducing stress-induced ethylene levels and protecting root growth (Nadeem *et al.*, 2010; Kumar *et al.*, 2020; Ilangumaran *et al.*, 2017) [24, 1, 2]. Bacterial inoculants producing this enzyme reliably alleviate salinity stress by lowering ethylene output (Gupta *et al.*, 2019) [12], and this capacity persists in at least some halotolerant PGPR from saline habitats (Etesami *et al.*, 2018) [25]. Here, six isolates retained ACC deaminase activity at 10% NaCl, led by IIRRSTB-2 (84.5) and IIRRSTB-7 (68.2) at 10 dS m⁻¹. The co-occurrence of ACC deaminase and IAA production in strains like IIRRSTB-2 is especially valuable, as these traits work together to stimulate root development (Gupta *et al.*, 2019) [12].

Nitrogenase activity. Nitrogen-fixing capacity, assessed via the acetylene reduction assay (ARA), was retained only by IIRRSTB-2, IIRRSTB-4, and IIRRSTB-12. ARA remains a standard method for estimating nitrogen-fixation potential in PGPR collections (de Souza *et al.*, 2014; Khumairah *et al.*, 2022) [8]. Non-symbiotic nitrogen fixation is well documented in genera such as *Azospirillum*, *Azotobacter*, *Burkholderia*, *Herbaspirillum*, *Bacillus*, and *Paenibacillus* (Otlewska *et al.*, 2020) [23], and diazotrophic activity has previously been detected in rice tissue using this assay (Rangiaroen *et al.*, 2014). Inoculation with diazotrophic consortia has been shown to boost rice yield while cutting nitrogen fertilizer requirements (Ríos-Ruiz *et al.*, 2020) [26], highlighting the practical value of the nitrogenase-positive isolates identified in this study. However, ARA is a short-term enzymatic assay and does not, on its own, provide a definitive measure of biological nitrogen fixation (Boddey *et al.*, 1987) [27].

IAA production. Indole-3-acetic acid is the most commonly reported PGP trait among halotolerant bacteria (Pallavi *et al.*, 2023) [7]. It supports lateral root formation, root-hair elongation, and improved water and nutrient uptake under stress (Etesami *et al.*, 2018; Ramasamy *et al.*, 2023; Otlewska *et al.*, 2020) [25, 28, 23], and IAA-producing halotolerant strains have been shown to enhance wheat root and shoot elongation under salinity (Otlewska *et al.*, 2020) [23]. Only four iso

lates in this study retained IAA production at 10% NaCl, with IIRRSTB-2 (53.4 $\mu\text{g mg}^{-1}$ protein) and IIRRSTB-7 (41.0) recording the highest levels at 10 dS m^{-1} values comparable to those reported for *Bacillus* sp. AD32 (42.67 $\pm$ 1.20 $\mu\text{g mL}^{-1}$) by Kapadia *et al.* (2022) [21] and to the top IAA producers characterized by Pallavi *et al.* (2023) [7].

IIRRSTB-2 as the elite multifunctional isolate. Considering all measured parameters together, IIRRSTB-2 stood out as the most consistently high-performing strain: it sustained luxuriant growth at 10% NaCl, retained all five qualitative PGP traits, and recorded the highest quantitative values for HCN, zinc solubilization, ACC deaminase, nitrogenase activity, and IAA production. Single strains combining multiple PGP functions have proven effective elsewhere—for instance, *Curtobacterium albidum* SRV4, which produces EPS alongside nitrogen fixation, IAA, and ACC deaminase, helped mitigate rice salinity stress (Kumar *et al.*, 2020) [1]. Multi-trait-selected halotolerant isolates have similarly alleviated salt stress in soybean at concentrations up to 240 mM NaCl (Khan *et al.*, 2021) [13], while multifunctional

consortia (*Pseudomonas* sp. P8, *Peribacillus* sp. P10, and *Streptomyces* sp. X52) showed promise in saline maize soils (Peng *et al.*, 2021) [29], and native PGPR consortia have outperformed commercial biofertilizers under saline conditions (Karnwal, 2023). Given this precedent, the promising strains identified here particularly IIRRSTB-2 warrant molecular identification through 16S rRNA sequencing, as done in comparable studies (Albdaiwi *et al.*, 2019; Pallavi *et al.*, 2023; Khan *et al.*, 2021; Patani *et al.*, 2023) [6, 7, 13, 30], followed by pot and field validation.

Study limitations. It should be emphasized that these classifications rest solely on laboratory observations at 10% NaCl or 10 dS m^{-1} and do not establish statistical superiority or predict field performance. Further work assessing additional traits such as EPS production and biofilm formation, both of which support PGPR survival and Na^+/K^+ homeostasis under salinity (Shultana *et al.*, 2020; Mohammed, 2018) [4, 31] alongside in-planta inoculation trials, will be necessary to fully establish the agronomic potential of the IIRRSTB collection, and of the elite strain IIRRSTB-2 in particular.

Table 1. Morphological characterization of salt tolerant bacteria

S. No	Cultures	Colony characteristics				
		Form	Margin	Elevation	Texture	Appearance
1	IIRRSTB-1	Irregular	Filamentous	Raised	Dry Mucoid	Milky White
2	IIRRSTB-2	Oval	Entire Smooth	Convex	Glossy	Opec/White
3	IIRRSTB-3	Irregular	Entire Smooth	Convex	Slimy Moist	Opec/White
4	IIRRSTB-4	Irregular	Undulated wavy	Raised	Slimy Moist	Opec/White
5	IIRRSTB-5	Irregular	Undulated Wavy	Convex	Slimy Moisture	Milky White
6	IIRRSTB-6	Round	Serrated	Convex	Slimy Moisture	Opec/White
7	IIRRSTB-7	Round	Filamentous	Convex	Shiny Viscous	Creamy Opec
8	IIRRSTB-8	Round	Entire (Smooth)	Convex	Shiny Viscous	Opec/White
9	IIRRSTB-9	Rhizoid	Lobate	Raised	Dry Mucoid	Creamy
10	IIRRSTB-10	Punctiform	Undulate	Convex	Slimy Moisture	Opec/White
11	IIRRSTB-11	Round	Undulate	Convex	Slimy Moist	White
12	IIRRSTB-12	Round	Entire Smooth	Raised	Dry Mucoid	Opec/White
13	IIRRSTB-13	Round	Undulate Wavy	Convex	Slimy Moist	Creamy Opec
14	IIRRSTB-14	Round	Entire Smooth	Convex	Slimy Moist	Milky
15	IIRRSTB-15	Irregular	Undulate Wavy	Convex	Slimy Moist	Opaque

Table 2. Screening of salt tolerant bacteria on different salt concentrations

S. No	Cultures	Microorganism	Control (Non saline)	5% NaCl	10% NaCl
1	IIRRSTB-1	AMP351	++++	++++	++++
2	IIRRSTB-2	AMP162	++++	++++	++++
3	IIRRSTB-3	AMP353	++++	++++	+++
4	IIRRSTB-4	GKS5	++++	++++	++++
5	IIRRSTB-5	B21KPS5A	++++	++++	++
6	IIRRSTB-6	B21KPS5A1	++++	++++	+++
7	IIRRSTB-7	B21KPS5A2	++++	++++	++++
8	IIRRSTB-8	B21KPS5	++++	++++	+++
9	IIRRSTB-9	B23KPS7	++++	++++	++++
10	IIRRSTB-10	B23KPS6	++++	++++	++
11	IIRRSTB-11	19AKS21	++++	++++	++++
12	IIRRSTB-12	19AKS12	++++	++++	++++
13	IIRRSTB-13	19AKS2B13	++++	++++	++++
14	IIRRSTB-14	19AKS24	++++	++++	+++
15	IIRRSTB-15	19AKS25	++++	++++	++

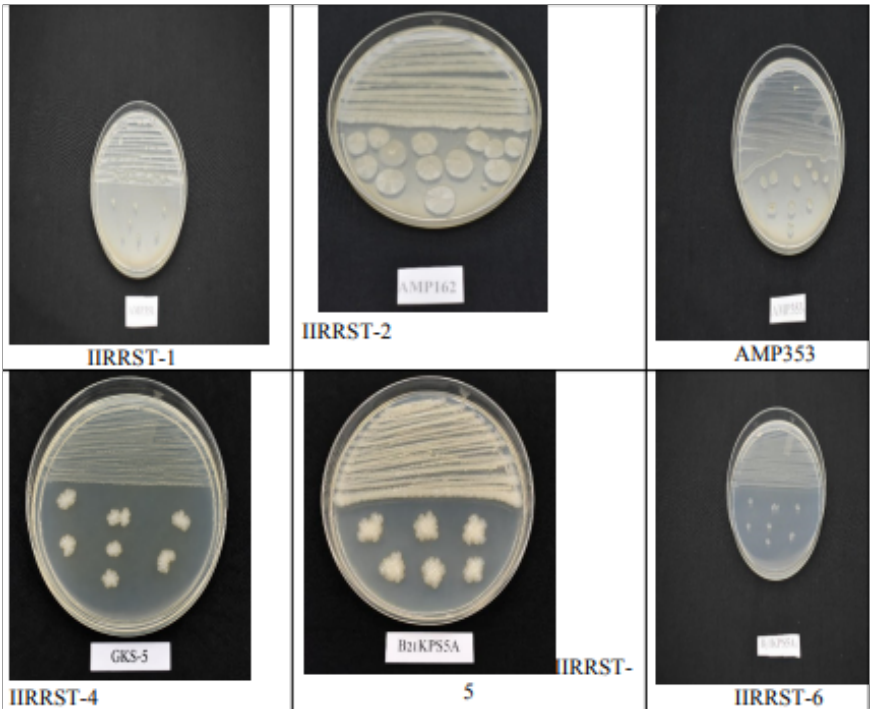
+ = Poor growth, ++ = Moderate growth, +++ = Good growth, ++++ = Luxuriant growth

Table 3. Qualitative assessment of plant growth promoting traits of salt tolerant bacterial isolates under different salt concentrations

S. No	Microorganism	Cultures	HCN Production			PSB			ZnSB			Acc deaminase			IAA		
			Control (Non saline)	5% NaCl	10% NaCl	Control (Non saline)	5% NaCl	10% NaCl	Control (Non saline)	5% NaCl	10% NaCl	Control (Non saline)	5% NaCl	10% NaCl	Control (Non saline)	5% NaCl	10% NaCl
1	AMP351	IIRSTB-1	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+
2	AMP162	IIRSTB-2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	AMP353	IIRSTB-3	+	+	-	+	+	-	-	-	-	+	-	-	+	+	-
4	GKS5	IIRSTB-4	+	+	+	+	+	+	+	-	-	+	+	+	+	-	-
5	B21KPS5A	IIRSTB-5	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-
6	B21KPS5A1	IIRSTB-6	+	-	-	-	-	-	+	+	+	+	+	+	+	-	-
7	B21KPS5A2	IIRSTB-7	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+
8	B21KPS5	IIRSTB-8	+	-	-	+	+	-	-	-	-	-	-	-	+	-	-
9	B23KPS7	IIRSTB-9	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-
10	B23KPS6	IIRSTB-10	+	+	-	+	+	-	+	-	-	+	-	-	-	-	-
11	19AKS21	IIRSTB-11	-	-	-	+	+	-	+	+	-	-	-	-	+	+	-
12	19AKS12	IIRSTB-12	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+
13	19AKS2B13	IIRSTB-13	+	+	+	+	+	+	+	-	-	+	-	-	+	+	-
14	19AKS24	IIRSTB-14	+	-	-	+	+	-	+	-	-	+	-	-	+	+	-
15	19AKS25	IIRSTB-15	+	-	-	+	+	-	+	+	-	+	-	-	+	+	-

Table 4. Quantitative assessment of plant growth promoting traits of salt tolerant bacterial isolates under different salt concentrations

S. No	Cultures	HCN production			P-solubilization			Zn-solubilization			ACC deaminase			Acetylene Reduction Activity			IAA production (µg/mg protein)		
		C	5 EC	10 EC	C	5 EC	10 EC	C	5 EC	10 EC	C	5 EC	10 EC	C	5 EC	10 EC	C	5 EC	10 EC
1	IIRRSTB-1	30.6	21.2	14.5	220.0	175.0	156.0	25.0	ND	ND	13.0	11.2	6.0	ND	ND	ND	20.3	15.8	8.9
2	IIRRSTB-2	62.2	50.2	35.1	150.1	121.3	108.8	30.6	24.1	19.9	109.8	92.3	84.5	180	158	132	87.3	73.4	53.4
3	IIRRSTB-3	25.0	12.0	ND	35.6	11.0	ND	ND	ND	ND	12.7	ND	ND	ND	ND	ND	14.6	9.4	ND
4	IIRRSTB-4	12.6	7.4	6.1	12.4	9.7	7.5	ND	ND	ND	35.1	27.6	19.1	145	126	101	15.1	ND	ND
5	IIRRSTB-5	13.2	ND	ND	ND	ND	ND	18.0	ND	ND	44.3	ND	ND	ND	ND	ND	26.5	ND	ND
6	IIRRSTB-6	34.6	ND	ND	ND	ND	ND	31.0	22.4	14.8	64.3	21.1	ND	ND	ND	ND	31.2	ND	ND
7	IIRRSTB-7	35.0	22.0	15.0	176.1	152.5	148.1	ND	ND	ND	89.7	85.1	68.2	ND	ND	ND	60.0	48.0	41.0
8	IIRRSTB-8	36.8	ND	ND	23.1	17.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	28.1	ND	ND
9	IIRRSTB-9	23.1	16.4	9.6	45.6	20.1	ND	12.2	8.1	ND	31.5	30.0	12.1	ND	ND	ND	37.1	18.9	ND
10	IIRRSTB-10	23.1	16.5	ND	36.1	21.9	ND	ND	ND	ND	33.7	ND	ND	ND	ND	ND	ND	ND	ND
11	IIRRSTB-11	ND	ND	ND	34.7	13.1	ND	16.2	11.2	ND	ND	ND	ND	ND	ND	ND	36.2	20.0	ND
12	IIRRSTB-12	50.0	35.0	27.0	35.0	ND	ND	24.0	16.0	10.0	24.1	18.0	12.0	120	103	82	40.0	32.0	25.0
13	IIRRSTB-13	46.3	40.1	33.7	30.1	24.1	19.5	15.6	ND	ND	55.8	ND	ND	ND	ND	ND	56.7	34.8	ND
14	IIRRSTB-14	37.7	ND	ND	42.1	31.3	ND	25.2	ND	ND	60.4	ND	ND	ND	ND	ND	43.7	40.0	ND
15	IIRRSTB-15	48.3	ND	ND	51.9	36.6	ND	30.6	21.7	ND	46.7	ND	ND	ND	ND	ND	38.7	25.5	ND



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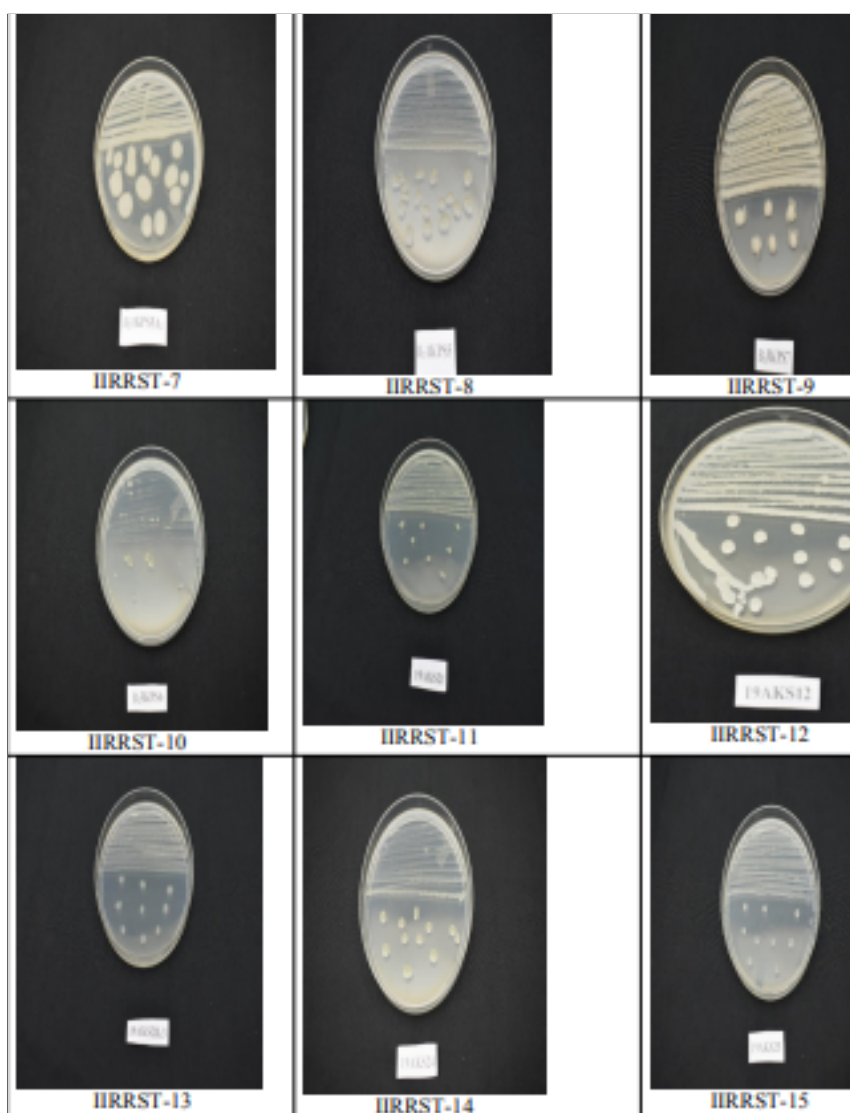


Figure 1. Morphological characterization of salt tolerant bacteria

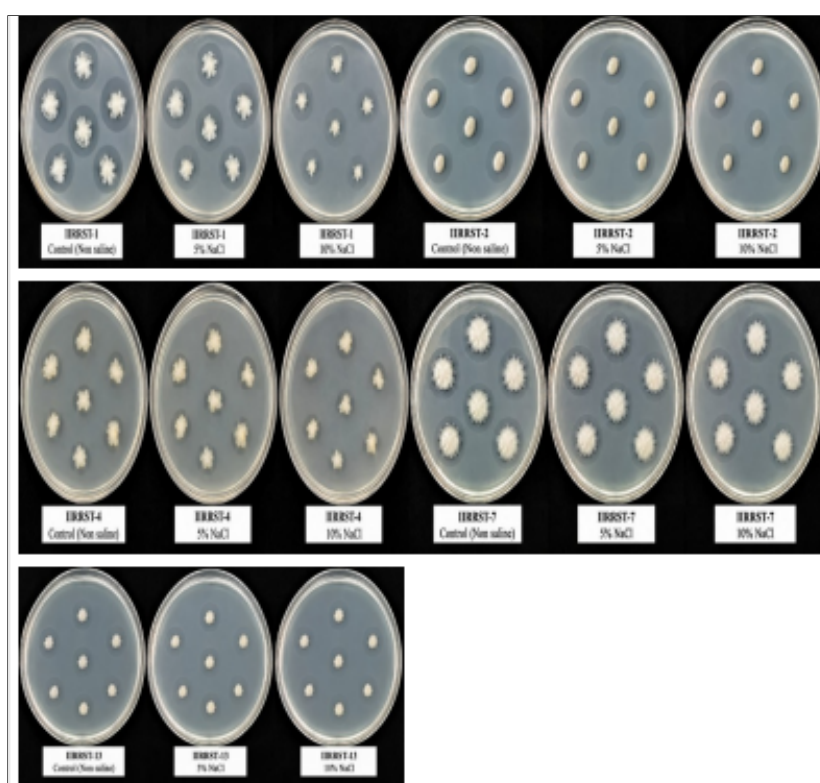


Figure 2. Qualitative estimation for Phosphorus solubilization of salt tolerant bacterial isolates under different salt concentrations

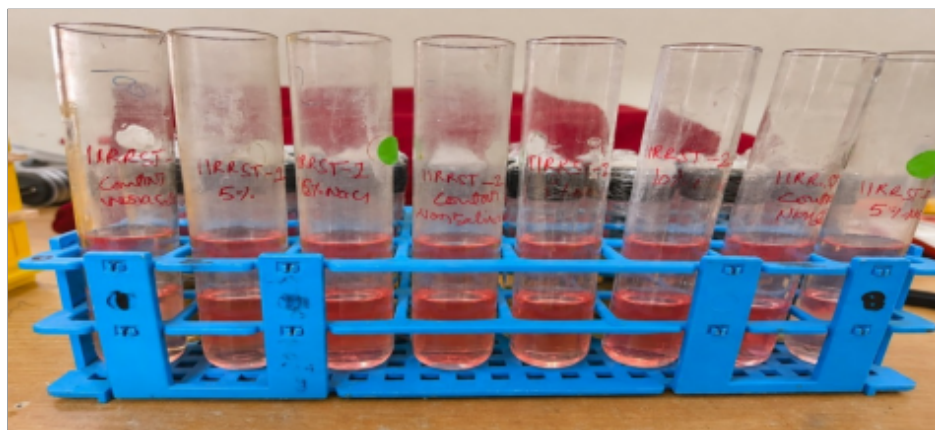


Figure 3. Development of pink coloration indicating IAA production by salt tolerant bacterial isolates under different salt concentrations

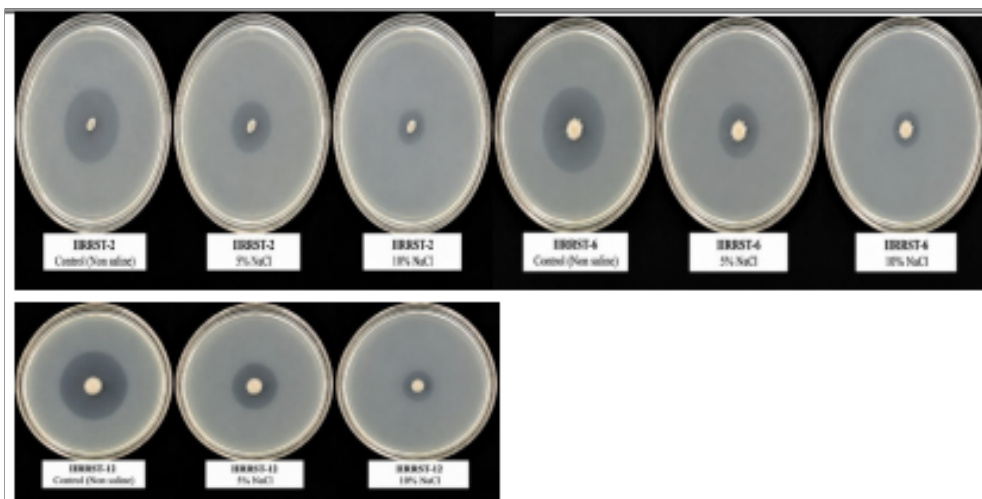


Figure 4. Qualitative estimation for Zinc solubilization of salt tolerant bacterial isolates under different salt concentrations

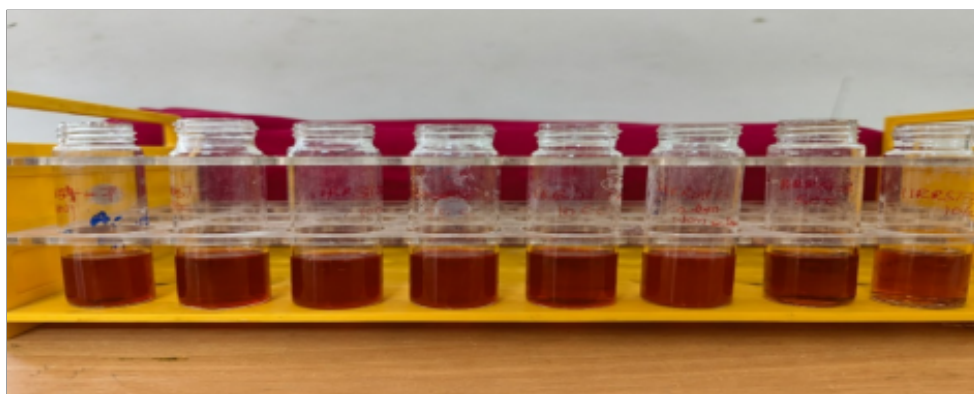


Figure 5. ACC deaminase activity of salt tolerant bacterial isolates under different salt concentrations



Figure 6. Quantification of Acetylene reduction activity estimation of salt tolerant bacterial isolates under different salt concentrations

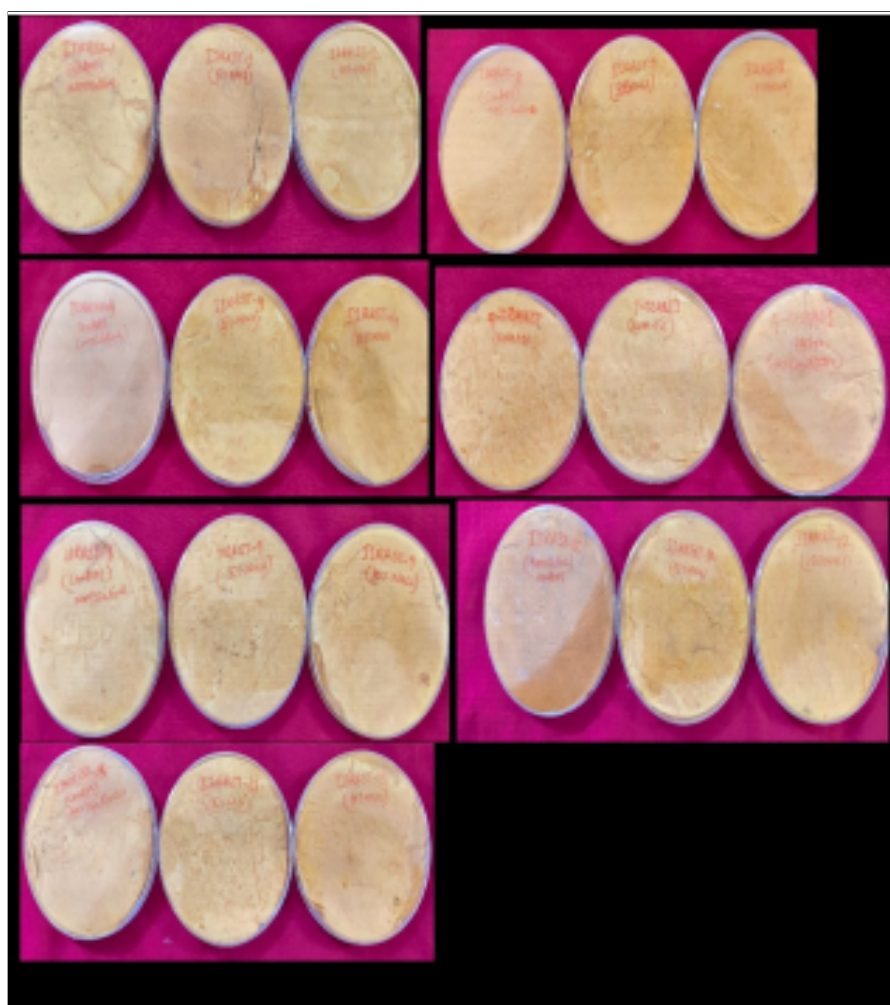


Figure 7. Qualitative estimation of HCN production salt tolerant bacterial isolates under different salt concentrations

Conclusion

This study successfully recovered 15 halotolerant bacterial isolates (IIRRSTB-1 to IIRRSTB-15) from the rhizosphere and root/shoot tissues of rice grown in salt-affected fields. All isolates grew luxuriantly at 0% and 5% NaCl, and eight sustained this growth along with multiple PGP functions at 10% NaCl, confirming genuine halotolerance consistent with their saline paddy origin. Multifunctional trait expression varied by strain: IIRRSTB-2 and IIRRSTB-12 retained all five qualitative traits at 10% NaCl, while seven isolates lost activity entirely, among quantitative measures at 10 EC, IIRRSTB-2 was the most consistently high-performing isolate, recording the highest values for HCN, zinc solubilization, ACC deaminase, ARA, and IAA

production, while IIRRSTB-1 and IIRRSTB-7 excelled at phosphate solubilization. Identifying strains combining multiple PGP traits under salt stress particularly IIRRSTB-2 offers a clear advantage for biofertilizer development, though these findings reflect laboratory conditions only. Molecular identification and pot/field trials are needed to confirm the agronomic potential of the IIRRSTB collection for saline soils.

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