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Study of different beneficial rhizospheric culturable microbes in rice (*Oryza sativa*) crop in long term fertilizer experiment

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Abstract
Rice (*Oryza sativa* L.) is the staple food for more than two-thirds of the Indian population and plays a pivotal role in food security and the national economy. Long-term fertilizer management significantly alters soil physicochemical properties and microbial diversity, which in turn influences nutrient cycling and crop productivity. The present study was conducted at RARS, Jagtial, using soil samples from a 20-year long-term fertilizer experiment to investigate the microbial diversity and plant growth promoting (PGP) traits of rhizospheric isolates under organic (100% FYM) and inorganic (100% NPK) fertilization practices. Soil samples were collected from the rhizosphere at a depth of 15-30 cm and serial dilutions were plated on selective media for isolation of bacteria, fungi, and actinomycetes. A total of 48 microbial isolates were obtained, of which 43 were purified and characterized based on morphological, cultural, biochemical, and physiological traits following Bergey's Manual. Among these, 42 were Gram-positive and one Gram-negative, with diverse pigmentation and colony morphology. Screening for enzymatic activities revealed that 27 isolates exhibited protease activity, 13 urease production, and 26 hydrocyanic acid (HCN) production, whereas none showed detectable chitinolytic activity under plate assay conditions. Further, PGP screening showed that 17 isolates solubilized phosphate (halo zones 3.7-14.8 mm), 24 isolates solubilized potassium (3.2-15.6 mm), and 19 isolates solubilized zinc (3.2-15.6 mm). Thirteen isolates produced siderophores, 27 produced ammonia, and the majority (22 isolates) synthesized indole acetic acid (IAA), with varying intensities. Notably, isolates RB 40, RB 39, and RB 1 exhibited superior phosphate, potassium, and zinc solubilization respectively, while RB 2, RB 19, RB 37, RA1, and RA2 were strong siderophore producers. The results highlight that long-term nutrient management exerts a significant influence on the rhizospheric microbial community and its functional potential. Several isolates demonstrated multiple beneficial traits, suggesting their potential as biofertilizer candidates to enhance nutrient availability and reduce dependence on chemical inputs. This study emphasizes the importance of integrating microbial inoculants in sustainable rice cultivation systems to improve soil health and crop productivity while minimizing environmental impacts of continuous chemical fertilizer use.

Keywords: Plant growth-promoting rhizobacteria (PGPR), phosphate solubilization, Rhizospheric isolates, Physiological traits

Introduction
Rice (*Oryza sativa*) is the third-largest cereal crop in the world. It is one of the principal cereal crops constituting the main nutrient resources and is a principal food crop of south and south-eastern countries. The crop occupies one third of the world's cultivated area and supplies 25-60% of the calories needed by 2.7 billion people worldwide. It is grown in countries like India, China, Indonesia, Bangladesh, Thailand, Vietnam, Burma, Philippines, Cambodia and Pakistan which are highly populated. It is the staple food for more than two third of the Indian population, thus holds the key for food security and plays a crucial role in the national economy. India stands first in rice area (43.77 M ha), with a production of 118.9 MT and with the productivity of 2,722 kg per ha. In Telangana, rice is cultivated around 1.6 million hectares with production of 13.43 million tones.

Previous studies have reported that microbial populations associated with the nitrogen cycle are positively or negatively affected by long-term chemical or mineral fertilizer application (Singh *et al.*, 2018) [26]. Thus (Wu *et al.*, 2020) [30] reported that soil quality, soil fertility, and the soil

microbial community can be negatively affected by repeatedly over using of chemical fertilizers. (Sun *et al.,* 2015) [28] reported that soil pH was significantly reduced, soil bacterial diversity was decreased, and soil bacterial community composition was significantly changed after long term chemical fertilizer application. However, (Geisseler *et al.,* 2014) [12] reported that in a comparison with a nonfertilized treatment, fertilizer application increased soil microbial biomass by 15.1%.

In rhizosphere, the organic compounds secreted by plants enrich diversified microbial communities to provide beneficial nutrients for plants (Fan *et al.,* 2018) [11]. (Edward *et al.,* 2015) exhaustively characterized the root associated microbiome of rice to support a multistep model for the assembly of a root microbiome from soil. Previous studies reported that the compositions of soil microbial communities in rhizospheric soil were significantly different from those in bulk soil (Zhang *et al.,* 2017) [32]. In the rhizosphere of plants, their roots coordinate development and interact with rhizospheric microorganisms (Kuzyakov *et al.,* 2019) [18]. the structure, composition and functioning of plant-associated rhizospheric microbiota can be shaped by free-living soil microorganisms.

Soil microbiota is also a vital component for various soil functions *viz.* breakdown of soil organic matter, degradation of xenobiotics and configuration of soil aggregates (Stenberg *et al.,* 1999) [27], and they can act as a source and sink for plant nutrients.

Moreover, several environmental factors and management practices are responsible for mineralization of organic matter and accumulation of carbon in soil (Kandeler *et al.,* 1999) [16]. Nutrient management is one of the most important agricultural practices which significantly affect soil microbial abundance, diversity and activity under long-term fertility management (Chinnadurai *et al.,* 2014). However, long-term use of balanced chemical fertilizers of nitrogen (N), phosphorous (P) and potassium (K) may cause less negative impact on soil biological properties than unbalanced fertilization (NP, NK or PK).

Rice rhizosphere and bulk soil methanogens make paddy soils a significant biogenic source of methane (CH_4) release, contributing roughly 5-19% of the world's CH_4 budget. (Bao *et al*., 2016) [2]. However, to date, there are a lack of studies which provide a summary of literature focused on the microbiomes residing in the rice root-related compartments, i.e. endosphere, rhizoplane and rhizosphere. Developments in the field of plant-microbe interactions have demonstrated that the associative soil microbial populations, which govern many of the phenetic features of plants, are influenced by the rice genotypes. For example, by altering root shape, postponing senescence, and lowering heavy metal bioavailability to the host plant, rhizobiomes can assist their host in surviving abiotic challenges (Deepthi *et al.,* 2014) [8]. By engaging in competitive interactions with invasive pathogenic species and obstructing their colonization, the rhizobiome also offers protection against biotic stressors.

Fertilizers that are applied to soil have an aftereffect and leave lasting residuals on crops. Compared with short term tests, long-term positioning tests can be used to objectively characterize the effects of different management measures on physical, chemical, biological properties of soil. Many studies have reported the effects of soil quality, soil fertility, the soil microbial community, and crop growth indicators on yield with long-term chemical fertilizer application. However, few studies (Ran *et al.,* 2021) [22] have investigated the effects of bacterial community structure in rhizospheric and bulk soils after long-term chemical fertilizer application.

Keeping in view of the above facts, it is proposed to study the nutrient fractions and microbial activities in rice-rice cropping system under different nutrient management practices in a 20-year-old long- term fertilizer experiment, located at RARS, Jagtial.

Materials and Methods

1. Soil sample collection

Soil samples were collected from long term fertilizer plots they are 100% organic that is 10^{th} treatment of FYM application and 100% inorganic that is 2^{nd} treatment of NPK application, RARS, Polasa, Jagital. The samples were collected near the rhizospheric region at 15-30 cm of depth and kept in sterilized polythene coveres and brought to the labouratory.

2. Isolation of culturable microorganisms from rhizospheric soil of paddy crop

Sample of 2gm of soil from the root zone was taken and soil and mixed the sample into 9 ml of saline solution and serial dilutions was made up to the concentrations of 10^{-3}, 10^{-4}, 10^{-5}, $10^{-6,}$ 10^{-7}, 10^{-8}, 10^{-9} and spread, the sample on nutrient agar plates for bacterial isolation and on Potato Dextrose agar for Fungal Isolation and Kenknight agar for Actinomycetes isolation and incubated the plates in inverted position at 28^0 c for 24 hours.

3. Purification and maintainance of different bacterial isolates

3.1 Streak Plate Method

A total of 48 varying isolates are obtained of which 42 were purified after morphological studies. Purification of different bacterial isolates was done by quadrant streak plate method by using suitable medium and growth conditions. A portion of the well-isolated colonies was taken and streaked on a suitable medium under aseptic conditions and incubated at 30^0C for 5 to 7 days and then store at 4^0C for further use.

3.2 Maintenance of the isolates

The purified isolates were preserved for further analysis in nutrient agar and starch casein agar slants. The 20% glycerol stocks were also prepared for the preservation of cultures.

4. Characterization of the isolates

The bacterial isolates were identified based on morphological, cultural, physiological and biochemical characteristics according to the standard methods described in Bergey's manual of systematic bacteriology (Holt *et al.,* 1984).

4.1 Cultural and Morphological Characterization

All the isolates were checked for their purity and then studied for colony morphology and pigmentation. The cell shape and gram reaction were also recorded as per the standard procedures given by Barthalomew and Mittewar (1950).

4.2 Colony Morphology

Morphological characteristics of the colony of each isolate was examined by growth of the isolates on their respective medium and incubated them for 5 to 7 days. Cultural characterization of isolates were observed by different characteristics of colonies such as shape, form of colony, surface, margin, pigmentation etc., were recorded.

4.3 Grams staining

A drop of sterile distilled water was placed in the centre of a glass slide. A loopful of freshly grown culture was taken and

mixed with water on the slide, spread into a thin smear by using a sterile inoculation needle. The smear was air-dried and heat-fixed near the flame. To the smear droplets of crystal violet solution were added and kept aside for 30 sec, after that washed gently with a flow of tap water. Then the slide was flooded with iodine solution for 30 sec and drained out with water followed by flooding with a 95 per cent decolourizer for 10 to 15 sec. After that, it was washed with water. The smear was flodded with safranin solution for 30 sec. The slide was washed gently with inflow of tap water and air-dried. Then the slide was examined under a microscope at 100 X power with oil immersion and the data was recorded.

5. Biochemical Characterization and Screening of isolates for Enzyme Activity

Different biochemical tests and enzymatic activity were performed using standard procedures. The protocols which were followed are briefly outlined below.

5.1 Chitinolytic Activity

5.1.1 Preparation of Colloidal Chitin

Colloidal chitin was prepared from the crab shell chitin flakes (Hi-media Chemicals Company, USA) by the modified method of Hsu and Lockwood (1995). Powder the flakes of crab shell chitin by using a mixer. Then 40 gm of Crab shell chitin powder was slowly added to 600 ml of concentrated HCl and kept for vigorous shaking for 60 min at a temperature of 30□C on a rotary shaker. After one hour, the solution was added to 5 litres of distilled water with continuous stirring by using a glass rod such that the chitin gets precipitated. Now centrifuge the solution at 10,000 rpm for 15 min, discard the supernatant and collect the pellet. Continue washing the pellet with 1 N NaOH and three times with sterile water to bring down the pH of the pellet to 7.0. Then pellet is stored at 4 °C for further use.

5.1.2 Qualitative Assay by Colloidal Chitin Agar Media

All the isolates were analysed for the detection of chitinolytic activities. A qualitative assay for detection of chitinase produced by isolated strains were carried out on 0.4% colloidal chitin agar (Appendix -I). Petri plates by visualizing the zone of chitin hydrolysis. Isolates were inoculated in a nutrient broth medium and incubated at 30 °C for 5 to 7 days. After incubation, cultures were streaked on 0.4% colloidal chitin agar petri plates by using a sterile inoculation loop. The inoculated colloidal chitin petri plates were incubated at 30 °C for 4 to 6 days. For visualization of the zone, the plates were flooded with 1% congo red solution for 15 min and then with 1 M NaCl solution for 15 min. The isolates forming a clear zone around the colony was considered positive for chitin hydrolysis (Saima *et al.*, 2013) [23].

5.2 Protease Activity

Skim milk agar plates were prepared and spot inoculated with actively grown culture(s) aseptically and incubated at 30°C for 4 days. Clear zones of hydrolysis around the bacterial cultures indicated positive for casein hydrolysis (Kusuma and Vindhya., 2012).

5.3 Urease Production

Urea agar plates (Appendix -I) were used to conduct the urease production test. Urea agar was autoclaved then media was poured into sterilized Petri plates and allowed to solidify aseptically. All the isolates were streaked under aseptic conditions and kept for incubation at 30 □C for 5 to 6 days. observed the colour difference from yellow to pink or magenta pink indicated as a positive reaction for urease production (Vashist *et al.*, 2013) [29].

5.4 Hydrocyanic Acid Production

The HCN production was tested by the method of Bakker, A.W and Schippers (1987) [3] with few modifications. Medium plates *i.e.*, modified nutrient agar media were prepared by adding 4.4 g of glycine per litre separately. One ml culture of each isolate was spread on respective media plates.

Then a disc of Whatman's no.1 filter paper of the diameter equal to the Petri plate, impregnated with an alkaline picric acid solution (0.2 per cent picric acid (w/v) in 1 per cent sodium carbonate) was placed in the upper surface of the inoculated Petri plates under aseptic condition and wrapped it with a thin film to prevent HCN gas escape (for accurate results). However, the control plate did not receive the inoculum.

The plates were incubated upside down at 30 °C for 6 to 7 days. The plates were observed for the change in colour from yellow to light brown, moderate or strong reddish- brown was taken as positive for HCN production.

5.5 Nitrogen Fixation Efficiency

Nitrogen fixation efficiency was analysed by Acetylene Reduction Assay (ARA) method given by (Bergersen, 1980). The isolates were grown on Rennie semi-solid media (Appendix-1) and incubated for 4 to 5 days at 30 °C. Fully grown slants were used for ARA where the cotton plugs were replaced by sterile Suba-seals, and three replications per tube were maintained. Ten percent of the air was removed from the test tubes and an equal amount of pure acetylene gas was injected, these tubes were incubated for 24 hrs at room temperature. ARA was done by using the gas chromatography (Trace 1100 Thermo Scientific) with a porapak Q column and flame ionization detector and the temperature of the column was maintained at 80 °C. One ml of gas from the culture tubes was injected into the GC where the nitrogen gas was used as carrier gas at the flow rate of 20 ml/min.

6. *Invitro* screening of the isolates for plant growth promoting traits

The pure isolates were screened for plant growth-promoting activities.

6.1 Phosphate Solubilization

Isolates were tested for phosphate solubilization by spot inoculation of pure isolates on Pikovskaya's agar media plates aseptically. The plates were incubated for 6 to 8 days at 30 °C and the zone of phosphate solubilization (mm) formed around the grown colonies was recorded (Pikovskaya, 1948) [20].

6.2 Pottasium Solubilization

Potassium solubilisation was assayed by using Aleksandrov's agar medium (Appendix.1). The isolates were spot inoculated on media plates under aseptic conditions and incubated at 30□C for 2-3 days. The clear zone diameter around the colonies were measured (Prajapati and Modi, 2012) [21].

6.3 Zinc Solubilization

Tris minimal agar medium containing 0.1% of insoluble zinc compounds like Zinc Oxide (ZnO) were used for Zinc solubilisation studies. The isolates were spot inoculated on media plates and incubated at 30 °C for 2 to 3 days. The diameter around the colonies were measured (Saravanan *et al.*, 2003) [24].

6.4 Siderophore Production

Production of siderophores were analysed by the method given by Schwyn and Neilands, (1987) [25] with few modifications. Aqueous Ferric chloride solution was used for qualitative estimation of siderophore production by the isolates. 0.06 g of Chrome Azurol Sulfonate (CAS) was dissolved in 5 ml of double-distilled water (solution I) and mixed with 9 ml of solution II containing 0.0027 g of FeCl3.6H2O in 10 ml of 10 mM HCl. This was added to 0.073 g of hexadecyl trimethyl ammonium bromide (HDTMA) dissolved in 40 ml of double-distilled water. The dark blue colour CAS reagent was autoclaved for 15 min. King's B media (Appendix.1) was prepared separately and to this CAS reagent was added, which gives blue colour to the media. The isolates were spot inoculated on the plates under aseptic conditions and incubated for 3 to 4 days. Observed the orange colour zones around the colonies on the plates which were considered positive for siderophore production.

6.5 Ammonia Production

Ammonia production was done by using peptone water. Peptone water prepared in test tubes with 10 ml volume and sterilized by using an autoclave. Inoculate the culture in each tube and kept for incubation for 2-3 days. After the incubation period centrifuge the culture at 10,000 rpm for 15 min at 4 °C. Supernatant was collected and added the Nessler's reagent and observed for colour change. Moreover, the development of brown to yellow colour in the supernatant was taken as positive for ammonia production.

6.6 Indole Acetic Acid Production

IAA production was estimated by the spectrophotometric method given by Glickmann and Dessaux. The nutrient broth was prepared and autoclaved, which was supplemented with 5 mM of tryptophan. Cultures were inoculated in nutrient broth and incubated in the tubes for 4 to 6 days.

After the incubation period, centrifuge the tubes aT_{10},000 rpm for 20 min, at 4 °C. Take 1 ml of supernatant from each tube and two drops of orthophosphoric acid, followed by 4 ml of Salkowski reagent. Moreover, incubate these tubes for 25 mins, at room temperature. After which readings were taken spectrophotometrically at 530 nm.

6.6.1 Prepared Standard Stock for IAA Production

A standard stock solution of IAA was prepared by taking 100 µg/ml of IAA in 50% ethanol. A standard curve was prepared by taking 0.1, 0.2, 0.3, 0.4 and 0.5 mg/l of standard IAA solution in test tubes. To this 2 ml of distilled water was added followed by 4 ml of Salkowski reagent. These tubes were incubated for 25 min at room temperature, after that optical density was measured at 530nm and the standard graph was prepared.

Results and Discussion

Morphological and cultural characterization of rhizosphere culturable microbial isolates

The purified cultures were streaked on a nutrient agar plate for further studies. After 4 to 6 days of incubation, the isolates were studied for their morphological and cultural characteristics. The results are presented in the table 4.2 according to Bergey's manual of systemic Bacteriology.

From the 48 isolates of culturable microorganisms 43 were gram positive and 5 fungal isolates were gram negative. Of which 24 bacterial isolates were circular in shape and the other are in irregular shape where as the actinomycetes are filamentous shape. These characters are shown in the table 4.2.

Similar morphological characteristics of the isolates varied with the pigmentation which includes grey, cream, yellow, orange, white and ash colour colonies were reported by Nonthakaew *et al.* which supports this research findings.

Table 4.2: Morphological and Cultural Characterization of the Rhizosphere Isolates

S. No	Isolate Name	Gram Reaction	Surface	Form of whole colony	Elevation	Pigmentation
1	RB 1	Positive	Smooth	Circular	Convex	White
2	RB 2	Positive	Smooth	Irregular	Convex	White
3	RB 3	Positive	Smooth	Circular	Convex	Orange
4	RB 4	Positive	Smooth	Circular	Convex	Creamish Orange
5	RB 5	Positive	Smooth	Irregular	Convex	Cream
6	RB 6	Positive	Smooth	Irregular	Convex	Creamish Orange
7	RB 7	Positive	Smooth	Circular	Convex	Yellow
8	RB 8	Positive	Smooth	Irregular	Convex	Orange
9	RB 9	Positive	Smooth	Circular	Convex	Orangish White
10	RB 10	Positive	Smooth	Irregular	Convex	Orangish White
11	RB 11	Positive	Smooth	Irregular	Convex	Cream
12	RB 12	Positive	Smooth	Circular	Convex	White
	Isolate Name	Gram Reaction	Surface	Form of whole colony	Elevation	Pigmentation
13	RB 13	Positive	Smooth	Circular	Convex	Cream
14	RB 14	Positive	Smooth	Circular	Convex	Cream
15	RB 15	Positive	Smooth	Circular	Convex	White
16	RB 16	Positive	Smooth	Circular	Convex	White
17	RB 17	Positive	Smooth	Irregular	Convex	orange
18	RB 18	Positive	Smooth	Circular	Convex	Creamish white
19	RB 19	Positive	Smooth	Irregular	Convex	Light yellow
20	RB 20	Positive	Smooth	Circular	Convex	Cream
21	RB 21	Positive	Smooth	Circular	Convex	Cream
22	RB 22	Positive	Smooth	Circular	Convex	Cream
23	RB 23	Positive	Smooth	Circular	Convex	Orange
24	RB 24	Positive	Smooth	Irregular	Convex	Cream
25	RB 25	Positive	Smooth	Irregular	Convex	Yellow

Biochemical characterization of the rhizosphere isolates

4.3.1 Protease Activity

The isolates were streaked on casein agar plates and the clear zone around the culture was considered for positive for protease enzyme activity are presented in the table 4.3. Among 4 3 isolates 27 isolates were positive where RB 13, RB 14, RB20, RB31, RB36 RB 37 showed significantly high (+++) zone od hydrolysis followed by 13 isolates RB 3,RB 4,RB 6,RB 8,RB 19,RB 24, RB 25,RB 26, RB 32,RB 33,RB 34,RB 38,RB 16 showed moderate(++) zone of hydrolysis and followed by 6 isolates RB 10, RB 11, RB 14, RB 28, RB 30, RB 15 showed low(+) zone of hydrolysis around the streaked isolates.

Urease Activity

The rhizosphere isolates were streaked on urea agar plate, after the incubation period the change in the colour from pink to magenta pink was considered positive for urease production whereas no change in colour was taken as negative are presented in the table 4.3. Out of 43isolates 13 were positive where RB 5, RB 10, RB 19, RB 27, RB 37, RB39, RB 40 showed significantly high (+++) zone of colour change around the colonies followed by 3 isolates RB 29, RB 22 and RA 2 showed moderate (++) zone of magenta pink colour and 3 isolates RB 2, RB 13, and RA 3 depicts low (+) zone of colour change around the colonies.

HCN Production

All the 43 isolates were spread on the nutrient agar plates which was supplemented with glycine separately, after the incubation period the change in colour from yellow to brown was considered for positive for HCN production are given in the table4.3. Out of which only 26 isolates RB 10, RB33, RB 29, RB 14, RB 34, RB 32, RB 15, RB 1, RB 2 RB 16, RB 26, RB 39, RB 5, RB 13, RB 21, RB 26, RB 30, RB 3, RB 36, RB 18, RB 7, RB 31, RB 17, RB 4, RB 9 RA 2 showed light brown to reddish brown colour.

Chitinolytic Activity

Screening of all 43 bacterial isolates which were spot inoculated on colloidal chitin agar had showed negative. The presence of halo zone around spot inoculated bacterial culture (Table 4.3) and it showed as negative. Although the isolates possess various plant growth-promoting and antagonistic traits, chitinase production was not one of them. Keeping the plate assay which relies on visible zone formation, which depends on enzyme diffusion and sufficient degradation of chitin, low-level or non-diffusible enzyme production may not result in a detectable zone, despite the presence of enzymatic activity.

Table 4.3: Screening of isolates for Protease, Urease, HCN and Chitinolytic Activity

Isolate Name	Protease Activity	Score	Urease Activity	Score	HCN Production	Score	Chitinolytic Activity	Score
RB 1	-	0	-	0	1	1	-	0
RB 2	-	0	+	1	1	1	-	0
RB 3	++	2	-	0	1	1	-	0
RB 4	++	2	-	0	1	1	-	0
RB 5	-	0	+++	3	-	0	-	0
RB 6	++	2	-	0	-	0	-	0
RB 7	-	0	-	0	1	1	-	0
RB 8	++	2	-	0	-	0	-	0
RB 9	-	0	-	0	1	1	-	0
RB 10	+	1	+++	3	1	1	-	0
RB 11	+	1	-	0	-	0	-	0
RB 12	-	0	-	0	-	0	-	0
RB 13	+++	3	+	1	-	0	-	0
RB 14	+++	3	-	0	1	1	-	0
RB15	+	1	-	0	1	1	-	0
RB 16	++	2	-	0	1	1	-	0
RB 17	-	0	-	0	1	1	-	0
RB 18	-	0	-	0	1	1	-	0
RB 19	++	2	+++	3	-	0	-	0
RB 20	+++	3	-	0	-	0	-	0
RB 21	-	0	-	0	-	0	-	0
RB 22	-	0	++	2	-	0	-	0
RB 23	-	0	-	0	-	0	-	0
RB 24	++	2	-	0	-	0	-	0
RB 25	++	2	-	0	-	-	-	0
RB 26	++	2	-	0	1	1	-	0
RB 27	-	0	+++	3	-	0	-	0
RB 28	+	1	-	0	1	1	-	0
RB 29	-	0	++	2	-	0	-	0
RB 30	+	1	-	0	1	1	-	0
RB 31	+++	3	-	0	1	1	-	0
RB 32	++	2	-	0	1	1	-	0
RB 33	++	2	-	0	1	1	-	0
RB 34	++	2	-	0	1	1	-	0
RB 35	-	0	+++	3	-	0	-	0
RB 36	+++	3	-	0	1	1	-	0
RB 37	+++	3	+++	3	-	0	-	0
RB 38	++	2	-	0	-	0	-	0
RB 39	-	0	+++	3	-	0	-	0

RB 40	-	0	+++	3	-	0	-	0
RA 1	-	0	-	0	-	0	-	0
RA 2	+++	3	++	2	1	1	-	0
RA 3	+	1	+	1	-	0	-	0

*Values Mentioned in the Table are the Mean of three Replications
"+++": High production, "++": Moderate production, "+": Low production

Characterizing isolates for plant growth poromoting properties

Phosphate solubilization

Phosphate solubilization ability of isolates was performed by spot inoculating the cultures on Pikovskayas agar media containing tricalcium phosphate as insoluble phosphate. Among 43 isolates 17 isolates were shown clear zone of solubilization around the colonies which ranges from 3.7 mm to 14.8mm are presented in the table. The highest zone of solubilization was shown by RB 40 (14.8mm), followed by RB 39(13.6mm), RB 12(12.5mm), RB 16 and 24 (12.3mm), RB 35 and 37(11.8mm), RB 21(9.1mm), RB 3 (7.8mm), RB 4 (7.6mm), RB 8 and 10(7.2mm) RB 1 and RA 2 (6.8mm), RB 35(6.1mm), RB 31 (4.2 mm) and the least has shown by RB 21 (3.7mm). The values for isolates with zone of solubilization is mentioned in the table and the same has depicted in the bar diagram in the fig. The scores were given based on the solubilization index if the S.I is between 0-1.5 the score is 1 and if the S.I is between 1.5 to 2.5 the score is 2 and if it's more them 2.5 then the score is 3.

8.2 Potassium Solibilization

Among 43 isolates screened for potassium solibilization, a total of 24 isolates has shown the clear zone of solubilization around the grown colonies which has a range from 3.2mm to 15.6mm are presented in the table. The isolates which showed highest zone is RB 1 (15.6mm), followed by RB 31 (14.9mm), RB 12(14.7mm), RB 38(14.6mm),RB 27 and 36 shown similar zone of solibilization (14.1 mm), RB 16 (13.8mm), RB 5(12.9mm), RB 13(12.8mm), RB 14(12.6mm), RB 19 and RB 33 (12.4 mm) RB 23 (9.8mm), RB 35(8.7mm), RB6 (8.4mm), RB 18(8.1mm), RB 29 (7.8mm), RB 37(7.5mm), RA 29 6.8mm), RB 4 and RB 30 (5.4mm), RB 40 (4.8mm) whereas the least was shown by RB 2 (3.2 mm). The values for all the isolates with zone of solubilization was mentioned in the table and the same is depicted in pictorial diagram. The scores were given based on the solubilization index if the S.I is between 0-1.5 the score is 1 and if the S.I is between 1.5 to 2.5 the score is 2 and if it's more them 2.5 then the score is 3.

8.3 Zinc Solibilization

Among 43 isolates screened for zinc solubilization, a total of 19 isolates has shown clear zone of solibilization around the colonies which ranges from 3.2 mm to 15.6mm.The highest zone of solibilization was shown by RB 1 (15.6mm), followed by RB 14(14.6mm), RB 16(14.5mm), RB 27 and 28 (13.8mm), RB 30 (13.7mm), RB 22 and 23(12.8mm), RB 32(9.1mm), RB 3 (7.8 mm), RB37(7.6 mm), RB 4 (7.5 mm), RB 8 (7.4 mm), 10(7.4 mm), RB 1(6.8 mm), RA 2(6.8 mm), RB 35(6.5 mm), RB 31 (4.5 mm) and the least has shown by RB 2 (3.2 mm). The values for isolates with zone of solibilization is mentioned in the table and the same has depicted in the bar diagram in the fig. The scores were given based on the solubilization indes if the S.I is between 0-1.5 the score is 1 and if the S.I is between 1.5 to 2.5 the score is 2 and if its more them 2.5 then the score is 3.

Siderophore production

A total of 43 isolates were screened for siderophore production among which 13 has shown positive which are mentioned in the table which were developed orange yellow colour hallow zones around the colonies among which 5 isolates with high (+++)isolates RB 2, RB 19, RB 37, RA1, RA2 with moderate(++) halo zones which includes RB 3, RB 10, RB 11, RB 33 and four with low (+) orange yellow colour zone around the colonies include RB 32, RB 40, RB 23, RB 24 has shown in the plates with orange yellow colour zone around the colonies.

8.5 Ammonia production

All the 43 isolates were screened for ammonia production where only27 isolates were shown brown colour after the addition of Nessler's reagent were the 4 isolates RB 4, RB 6, RB 8 and RB 26 shown high(+++) brown to yellow colour and followed by 12 isolates RB 5, RB 10, RB 11, RB 14, RB 19, RB 22, RB 28, RB 30, RB 35, RA 2, RA 3 has shown moderate (++) and the 12 isolates RB 1, RB 3, RB 7, RB 13, RB 20, RB 21, RB24, RB 27, RB33, RB34, RB37, RA1 has shown less (+) colour change.

8.6 Indole Acetic Acid Production

The bacteria isolates were screened for IAA production by inoculating the isolates in nutrient broth amended with tryptophan. After incubation, orthophosphoric acid and Salkowski reagent were added for pink colour development, Majority of the isolates (22) had produced IAA in low quantity and only 6 isolates have not developed pink colour indicating no IAA production with score 0. Based on the intensity of the pink colour the scores were given for RB as 3 (High production) and for 22 isolates as 2 (moderate production). And the isolates RB 1,13,26,31 has showed less production were given score 1. Three isolates have shown negative results for IAA production. The isolates were divided into three score as 1, 2 and 3 based on the concentrations where as the concentrations from (0-0.035) given as score 1 and 0.035 to 0.50 given as score 2 and more than 0.05 are given as score

Table 4.5: Siderophore Production by isolates

Isolate Name	Yellow Zone	Siderophore Production (S.P.I)	Score
RB 1	0	-	-
RB 2	23	3.2	3
RB 3	16	2.3	2
RB 4	0	-	-
RB 5	0	-	-
RB 6	0	-	-
RB 7	0	-	-
RB 8	0	-	-

RB 9	0	-	-
RB 10	16	2.4	2
RB 11	18	2.4	2
RB 12	0	-	-
RB 14	0	-	-
RB 15	0	-	-
RB 16	0	-	-
RB 17	0	-	-
RB 18	0	-	-
RB 19	28	3.7	3
RB 20	0	-	-
RB 21	0	-	-
RB 22	0	-	-
RB 23	14	1.3	1
RB 24	11	1.1	1
RB 25	0	-	-
RB 26	0	-	-
RB 27	0	-	-
RB 28	0	-	-
RB 29	0	-	-
RB 30	0	-	-
RB 31	0	-	-
RB 32	12	1.4	1
RB 33	15	2.3	2
RB 34	0	-	-
RB 35	0	-	-
RB 36	-	-	-
RB 37	30	3.8	3
RB 38	0	-	-
RB 30	0	-	-
RB 40	13	1.3	1
RA1	17	2.6	3
RA 2	18	2.8	3
RA 3	0	-	-

Values are the means of three replicates
*S.P.I - Siderophore production index

Table 4.4: Solubilization of insoluble phosphate, potassium and zinc solubilization by bacterial isolates

Isolate Name	Phosphate Halo Zone (mm)	Phosphate solubilization index (S.I)*	Score	Potassium Halo Zone (mm)	Potassium Solubilization index (S.I)*	Score	Zinc Halo Zone (mm)	Zinc Solubilization index (S.I)*	Score
RB 1	6.8	1.96	2	15.6	3.6	3	15.6	4.6	3
RB 2	0	-	-	3.2	1.09	1	3.2	1.07	1
RB 3	7.8	2.21	2	0	-	-	7.8	2.60	2
RB 4	7.6	1.92	2	5.4	1.53	1	7.5	2.50	2
RB 5	0	-	-	12.9	3.4	3	0	-	-
RB 6	0	-	-	8.4	2.04	2	0	-	-
RB 7	0	-	-	0	-	-	0	-	-
RB 8	7.2	1.87	2	0	-	-	7.4	1.47	1
RB 9	0	-	-	0	-	-	0	-	-
RB 10	7.2	1.87	2	0	-	-	7.4	1.47	1
RB 11	0	-	-	0	-	-	0	-	-
RB 12	12.5	3.82	3	14.7	3.9	3	0	-	-
RB 13	0	-	-	12.8	2.8	3	0	-	-
RB 14	0	-	-	12.6	2.6	3	14.6	3.87	3
RB15	0	-	-	0	-	-	0	-	-
RB 16	12.3	3.26	3	13.8	3.85	3	14.5	3.83	3
RB 17	0	-	-	0	-	-	0	-	-
RB 18	0	-	-	8.1	2.0	2	0	-	-
RB 19	0	-	-	12.4	2.6	3	0	-	-
RB 20	0	-	-	0	-	-	0	-	-
RB 21	3.7	1.09	1	0	-	-	0	-	-
RB 22	0	-	-	0	-	-	12.8	2.7	3
RB 23	0	-	-	9.8	2.2	2	12.8	2.7	3
RB 24	12.3	3.0	3	0	-	-	0	-	-
RB 25	0	-	-	0	-	-	0	-	-
RB 26	0	-	-	0	-	-	0	-	-

RB 27	0	-	-	14.1	3.7	3	13.8	3.60	3
RB 28	0	-	-	0	-	-	13.8	3.60	3
RB 29	0	-	-	7.8	1.2	1	0	-	-
RB 30	0	-	-	5.4	1.0	1	13.7	3.57	3
RB 31	4.2	1.08	1	14.9	4	3	4.5	1.50	1
RB 32	0	-	-	0	-	-	9.1	2.03	2
RB 33	0	-	-	12.4	2.5	3	0	-	-
RB 34	0	-	-	0	-	-	0	-	-
RB 35	11.8	2.81	3	8.7	1.3	1	6.5	1.17	1
RB 36	0	-	-	14.1	3.4	3	0	-	-
RB 37	11.8	2.81	3	7.5	1.9	2	7.6	1.9	2
RB 38	0	-	-	14.6	3.8	3	0	-	-
RB 39	13.6	3.85	3	0	-	-	0	-	-
RB 40	14.8	3.90	3	4.8	1.08	1	0	-	-
RA 1	0	-	-	0	-	-	0	-	-
RA 2	7.5	1.91	2	6.8	1.1	1	6.8	1.1	1
RA 3	0	-	-	0	-	-	0	-	-

Values are the mean of three replicate
*SI- Solubilization Index

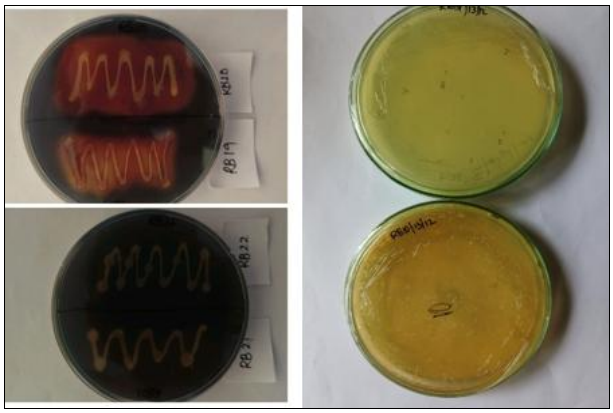
Table 4.6: Solubilization of IAA Production and Ammonia production and N fixation efficiency by bacterial isolates

Isolate Name	IAA Production (mg/ml)	Score	Ammonia Production	Score	Nitrogen Fixation efficiency ($nmolC_2H_4$/mg protein/h)	Score
RB 1	0.034	1	+	1	-	-
RB 2	0.036	2	-	-	-	-
RB 3	0.052	3	+	1	-	-
RB 4	-	-	+++	3	-	-
RB 5	0.036	2	++	2	1.18	3
RB 6	-	-	+++	3	-	-
RB 7	-	-	+	1	-	-
RB 8	0.048	2	+++	3	-	-
RB 9	0.063	3	-	-	-	-
RB 10	0.058	3	++	2	-	-
RB 11	-	-	++	2	-	-
RB 12	0.046	3	-	-	-	-
RB 13	0.034	1	+	1	-	-
RB 14	0.039	2	++	2	-	-
RB15	-	-	-	-	-	-
RB 16	0.037	2	-	-	-	-
RB 17	0.039	2	-	-	-	-
RB 18	0.038	2	-	-	0.91	1
RB 19	0.051	3	++	2	-	-
RB 20	0.042	2	+	1	-	-
RB 21	0.039	2	+	1	-	-
RB 22	-	-	++	2	-	-
RB 23	0.061	3	-	-	-	-
RB 24	0.037	2	+	1	-	-
RB 25	0.041	2	-	-	-	-
RB 26	0.034	1	+++	3	-	-
RB 27	0.035	2	+	1	-	-
RB 28	0.046	2	++	2	-	-
RB 29	0.050	3	-	-	-	-
RB 30	0.034	1	++	2	-	-
RB 31	0.042	2	-	-	-	-
RB 32	0.047	2	-	-	0.92	1
RB 33	0.044	2	+	1	-	-
RB 34	0.035	2	+	1	-	-
RB 35	0.034	1	++	2	-	-
RB 36	0.047	2	-	-	-	-
RB 37	0.053	3	+	1	-	-
RB 38	0.045	2	-	-	-	-
RB 39	0.039	2	-	-	-	-
RB 40	0.033	1	-	-	0.96	2
RA 1	0.035	1	+	1	-	-
RA 2	0.040	2	++	2	-	-
RA 3	0.038	2	++	2	-	-

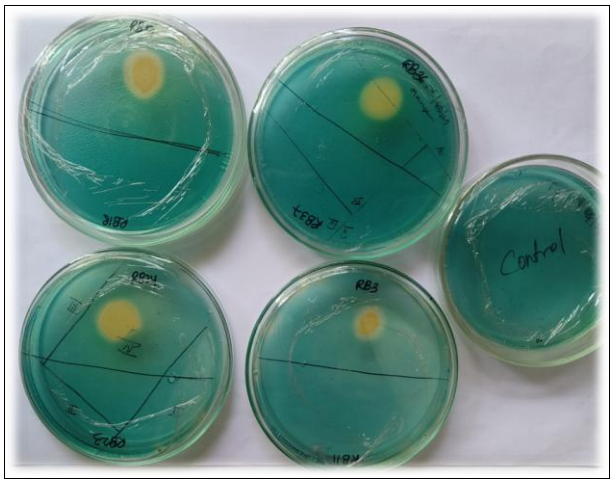
*Values Mentioned in the Table are the Mean of three Replications
"+++": High production, "++": Moderate production, "+": Low production, "-": Negative

Pure culture images

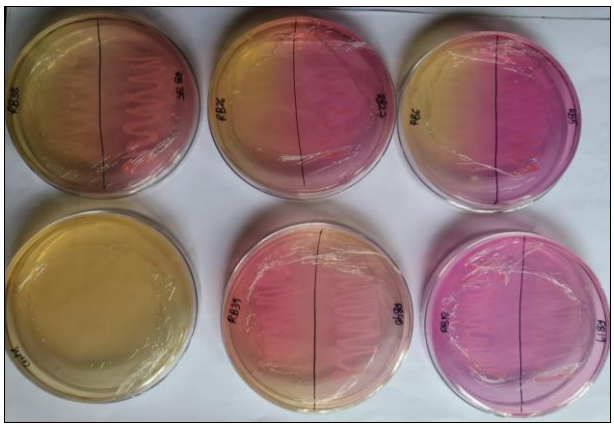
Protease (right) and HCN test images (left)



Siderophore production test



Urease production test



Conclusion

The study characterized by different rhizospheric isolates, highlighting their potential as bio inoculants for sustainable agriculture, promoting plant growth, stress tolerance, and contributing to novel bioinoculant development, while emphasizing the importance of rhizospheric soil as a source of beneficial microorganisms.

References

1. Ashwini G, Kumar S, Singh A, Sharma R. Long-term effects of fertilizer regimes on bacterial communities in paddy soils using NGS sequencing of 16S rRNA genes. J Soil Sci Plant Nutr. 2019;19(3):456-470.
2. Bao Q, Huang Y, Wang F, Nie S, Nicol GW, Yao H, *et al*. Effect of nitrogen fertilizer and/or rice straw amendment on methanogenic archaeal communities and methane production from a rice paddy soil. Appl Microbiol Biotechnol. 2016;100:5989-5998.
3. Bakker AW, Schippers B. Production of hydrogen cyanide by fluorescent *Pseudomonas*. Soil Biol Biochem. 1987;19(5):573-578.
4. Chao C, Liang G, Sun J, Wang X, He P, Zhou W, *et al*. Reduced dependence of rhizosphere microbiome on plant-derived carbon in 32-year long-term inorganic and organic fertilized soils. Soil Biol Biochem. 2015;80:70-78.
5. Chen Y, Tu P, Yang Y, Xue X, Feng Z, Dan C, *et al*. Diversity of rice rhizosphere microorganisms under different fertilization modes of slow-release fertilizer. Sci Rep. 2022;12(1):2694.
6. Chinnaduri A, Girija D, Sidharthan PP, Peedikakkal MP, Nair SS. Variation in soil bacterial communities by long-term application of organic and inorganic fertilizers in rice. J Cereal Res. 2019;11(3):286-292.
7. Cui X, Zhang Y, Gao J, Peng F, Gao P. Long-term combined application of manure and chemical fertilizer sustained higher nutrient status and rhizospheric bacterial diversity in reddish paddy soil of Central South China. Sci Rep. 2018;8(1):16554.
8. Deepthi N, Kumar P, Sharma A. Rhizobiomes assisting host plants in surviving abiotic challenges by altering root shape, postponing senescence, and lowering heavy metal bioavailability. J Plant Stress Adapt. 2014;22(4):345-359.
9. Dhondge HV, Barvkar VT, Paul D, Dastager SG, Pable AA, Nadaf AB. Exploring the core microbiota in scented rice (*Oryza sativa* L.) rhizosphere through metagenomics approach. Microbiol Res. 2022;263:127157.

10. Edwards J, Johnson C, Santos-Medellín C, Lurie E, Podishetty NK, Bhatnagar S, *et al*. Structure, variation, and assembly of the root-associated microbiomes of rice. Proc Natl Acad Sci USA. 2015;112(8):E911-E920.
11. Fan K, Weisenhorn P, Gilbert JA, Shi Y, Bai Y, Chu H. Soil pH correlates with the co-occurrence and assemblage process of diazotrophic communities in rhizosphere and bulk soils of wheat fields. Soil Biol Biochem. 2018;121:185-192.
12. Geisseler D, Scow KM. Long-term effects of mineral fertilizers on soil microorganisms - a review. Soil Biol Biochem. 2014;75:54-63.
13. Guo Y, Song B, Li A, Wu Q, Huang H, Li N, *et al*. Higher pH is associated with enhanced co-occurrence network complexity, stability and nutrient cycling functions in the rice rhizosphere microbiome. Environ Microbiol. 2022;24(12):6200-6219.
14. Herigstad BL, Hamilton M, Heebink B. The spread plate method for the enumeration of bacteria. Infect Control Hosp Epidemiol. 2001;22(8):579-585.
15. Jian N, Jin L, Wang S, Li J, Liu F, Liu Z, *et al*. Reduced chemical fertilizer combined with bio-organic fertilizer affects the soil microbial community and yield and quality of lettuce. Front Microbiol. 2022;13:863325.
16. Kandeler E, Marschner B, Tscherko D. Mineralization of organic matter and accumulation of carbon in soil: the role of environmental factors and management practices. Soil Biol Biochem. 1999;31(8):1127-1138.
17. Kumar U, Nayak AK, Shahid M, Gupta VVSR, Panneerselvam P, Mohanty S, *et al*. Continuous application of inorganic and organic fertilizers over 47 years in paddy soil alters the bacterial community structure and its influence on rice production. Agric Ecosyst Environ. 2018;262:65-75.
18. Kuzyakov Y, Razavi BS. Rhizosphere size and shape: temporal dynamics and spatial stationarity. Soil Biol Biochem. 2019;135:343-360.
19. Li G, Wang K, Qin Q, Li Q, Mo F, Nangia V, *et al*. Integrated microbiome and metabolomic analysis reveal responses of bacterial communities and root exudate composition to drought and genotype in rice (*Oryza sativa* L.). Rice. 2023;16(1):19.
20. Pikovskaya RI. Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. Microbiology. 1948;17:362-370.
21. Prajapati KB, Modi HA. Isolation and characterization of potassium solubilizing bacteria from ceramic industry soil. CIBTech J Microbiol. 2012.
22. Ran X, Zhang Y, Li Q, Wang J. Effects of long-term chemical fertilizer application on bacterial community structure in rhizospheric and bulk soils. 2021.
23. Saima N, Ahmad M, Khan MA. Chitinolytic activity of bacterial isolates: isolation, screening, and characterization. J Appl Microbiol. 2013;114(3):790-798.
24. Saravanan VS, Subramoniam SR, Raj SA. Assessing *in vitro* solubilization potential of different zinc solubilizing bacterial isolates. Braz J Microbiol. 2004;35:121-125.
25. Schwyn B, Neilands JB. Universal chemical receptor for ferric enterobactin. Biochemistry. 1987;26:5364-5370.
26. Singh A, Kumar M, Chakdar H, Pandiyan K, Kumar SC, Zeyad MT, *et al*. Influence of host genotype in establishing root-associated microbiome of indica rice cultivars for plant growth promotion. Front Microbiol. 2022;13:1033158.
27. Stenberg M, Alakukku L, Rosén C. Soil microbiota as a crucial component for soil functions: breakdown of organic matter, degradation of xenobiotics, and configuration of soil aggregates. Soil Biol Biochem. 1999;31(8):1234-1245.
28. Sun R, Wang F, Hu C, Liu B. Metagenomics reveals taxon-specific responses of the nitrogen-cycling microbial community to long-term nitrogen fertilization. Soil Biol Biochem. 2021;156:108214.
29. Vashist A, Kumar V, Singh P. Urease production in bacteria: detection and assay methods. J Microbiol Methods. 2013;94(3):243-250.
30. Wu L, Jiang Y, Zhao F, He X, Liu H, Yu K. Increased organic fertilizer application and reduced chemical fertilizer application affect the soil properties and bacterial communities of grape rhizosphere soil. Sci Rep. 2020;10(1):9568.
31. Yang M, Xu X, Li Z, Meng Y, Yang X, Song X, *et al*. Remote sensing prescription for rice nitrogen fertilizer recommendation based on improved NFOA model. Agronomy. 2022;12(8):1804.
32. Zhang K, Adams JM, Shi Y, Yang T, Sun R, He D, *et al*. Environment and geographic distance differ in relative importance for determining fungal community of rhizosphere and bulk soil. Environ Microbiol. 2017;19(9):3649-3659.
33. Zhang J, Peng H, Ji X, Li C, Li S. Effects of reduced inorganic fertilization and rice straw recovery on soil enzyme activities and bacterial community in double-rice paddy soils. Eur J Soil Biol. 2007;94:103116.