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RESEARCH ARTICLE OPEN ACCESS

Isolation, Characterization, and Investigation on Potential Multi-trait Plant Growth Promoting Rhizobacteria from Wild Banana (*Musa itinerans*) Rhizospheric Soil

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Abstract

The undertaken study was conducted to isolate and characterize the plant growth promoting rhizobacteria from the rhizospheric soil of *Musa itinerans* collected from Zaphumi village, Nagaland, Northeast India. The purified bacterial isolates were screened for plant growth-promoting traits namely phosphate solubilization, IAA production, siderophore production, and ammonia production. Out of the 25 isolates, the three best isolates with maximum growth promoting traits were selected and considered for further study for heavy metal and salinity tolerance. All three isolates were able to produce siderophore, whereas, only isolate EZ30 was able to produce IAA. Phosphate solubilization ability was the highest in EZ27 (272.89±2.46), followed by EZ30 (109.70±5.47) and EZ11(89.12±1.87). The isolates also exhibited variable levels of cadmium (30- 280µg/ml) and salinity resistance (2-14%). Based on 16S-rRNA gene sequence analysis, these bacterial isolates were identified as *Kosakonia arachidis, Pseudomonas putida* and *Pseudomonas monteilii*. The highest salinity tolerance was shown by *P. putida* (14%), whereas *K. arachidis* (4%) and *P. monteilii* (4%) exhibited similar level of tolerance. The cadmium tolerance was the highest for *P. monteilii* (280 µg/ml), followed by *K. arachidis* (80 µg/ml) and *P. putida* (30 µg/ml). Inoculation of *Cicer arietinum* L. with these three isolates significantly enhanced the growth parameter such as shoot and root length ($p \leq 0.05$), root and shoot fresh weight and dry weight ($p \leq 0.05$), except for EZ27 and EZ11 where there was no significant difference in shoot dry weight ($p \geq 0.05$). Overall, the three selected PGPR strains showed potential biofertilizer traits (phosphate solubilizing, IAA producing, siderophore production, salinity, and cadmium tolerant) to be used in the agricultural fields promoting sustainable practices.

Keywords: Cadmium, IAA, PGPR, Phosphate, Rhizospheric Bacteria, *Musa itinerans*, Siderophore

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INTRODUCTION

The present population of the world is 7.9 billion with a rise of 1% from 2021 and it will increase up to 9.7 billion by 2050 according to the United Nation (https://www.UN.org). This significant jump in world population has increased demand for food with scope of further escalation. It is a matter of shared concern for many countries as well as research organizations which have realigned their research objectives on sustainable ways to overcome this issue. Heavy metals such as arsenic (As), cadmium (Cd), chromium (Cr), lead (Pb), mercury (Hg) cause a significant decline in plant growth and crop yield. Cadmium (Cd) is one of the heavy metals that is having higher toxicity (2 to 20 folds higher) compared to other heavy metals, but roots of many plant species' can absorb the easily.[1] A sustainable way to help the agriculture system to mitigate the issues related to increase in population and pollution is the need of the hour and hence there is an urgent requirement for an alternative to synthetic fertilizers and pesticides to increase the food demand without harming the environment. Plant growth-promoting rhizobacteria (PGPR), a group of bacteria residing plant rhizosphere has been eyed upon for the past few decades for their ability to enhance plant growth organically either by direct or indirect methods. Many plants growth-promoting traits like solubilization of phosphates,[2] siderophore production,[3,4] and IAA production[5] are found in different PGPRs. The PGPRs are well known to produce different induced systemic resistance (ISR) elicitor like 1-aminocyclopropane-1-carboxylate (ACC) deaminase, a vital component of plant ethylene levels. With the PGPRs being easily accessible and eco-friendly, it provides with a great alternative to synthetic fertilizers and pesticides.[6] Many manufacturing companies have started manufacturing several environment friendly products such as BlightBan A506, Blue Circle, Conquer, Deny, Diegall, Epic, Galltrol-A, HiStick N/T, Intercept, Kodiak, Nogall, Norbac 84 C, Rhizo-Plus, Serenade, Subtilex, Victus etc.[7] However, it is also understood that replacing synthetic fertilizers and pesticides is not at all conceivable at present and the viable alternative is use of biofertilizers.

Cicer arietinum L (Chickpea) a vital pulse is consumed worldwide as it is an important source of different carbohydrates, better quality of proteins over other pulses.[8] Nevertheless, abiotic factors like drought, salinity, water logging, extreme heat, and freezing frequently restrict chickpea development and yield.[9] Since, it is one of the most demanded cereals; farmers tend to apply large quantities of artificial fertilizers for higher production, disregarding the potentially hazardous effects of it on the environment. Hence, developing a cross-inoculation strategy for chickpea with possible rhizobacteria associated with *Musa itinerans* roots as environmentally benign biofertilizers is of interest. To our knowledge, there have not been any reports of PGPR isolated from *Musa itinerans*, even though various researches have shown the potential advantages of rhizobacteria. Consequently, the objective of the current study was to identify, characterize, and assess the characteristics of bacterial isolates residing in the rhizosphere of the *Musa itinerans* in the Zaphumi region of Nagaland, India, that promote plant growth. This study is an attempt to gain more understanding of the PGPR and its roles in promoting plants growth.

MATERIALS AND METHODS

Rhizospheric Soil Sample Collection

The rhizospheric soil sample was collected from Zaphumi village, Zunheboto district (26°13.487ˈN, 094°28.033ˈE), Nagaland, India. The soil was dugout out ~5-30 cm depth with intact roots. The soils adhered with roots was removed by slightly shaking the roots and roots were then gathered in a clean polythene bag. The sample was used either immediately or kept at 4°C till used. The collected soil was analyzed for quantification of organic carbon following Walkley and Black,[10] available nitrogen using modified Kjeldahl method,[11] available phosphorus through Bray's method for acidic soil,[12] available potassium following Potdar et al.[13] and pH of the soil (Table 1). The moisture content of the soil was done by oven dry method.

Isolation of the Rhizobacteria from the Soil Sample Collected

Ten gm of collected soil was dissolved in 90 ml sterile deionized water and suspended

soil particles by stirring for 10 min. One ml of the suspension was added to 9 ml of the deionized water to make a one-fold dilution; likewise, serial dilution was done for 10^{-2}, 10^{-3}, and 10^{-4}. An aliquot of 20µl of each dilution was spread on Nutrient Agar medium plates Containing agar ($20gL^{-1}$), peptone ($10gL^{-1}$), sodium chloride ($5gL^{-1}$) and yeast extract ($10gL^{-1}$). Based on the morphological characteristics, bacterial isolates were selected and sub-cultured until the pure culture was obtained. The pure isolates were preserved for future use in 80% glycerol stock at -60°C.

Biochemical Characterization

Colony morphology such as colony color, transparency, elevation, margin, and form of the bacterial isolates were recorded. Biochemical activities viz., gram staining, motility test, catalase test, citrate utilization test, methyl red, starch hydrolysis test and sugar fermentation test (Table 2 and Table 3) were done.

Qualitative and Quantitative Estimation of Inorganic Phosphate Solubilization

For the purpose of qualitatively estimating the phosphate solubilization characteristic, bacterial isolates were spot-inoculated on National Botanical Research Institute's Phosphate (NBRIP) growth medium plates. The test isolates were grown for 7 days and incubated at 28±2°C. Positive results were indicated by the halo zone that formed around the colony. (Figure 1 a-c). The Phosphate solubilizing index (PSI) was measured for all the positive isolates. All the observations were made in triplicates. The PSI was measured using the following formula:

Phosphate solubilizing index = Colony diameter + Halo zone / Colony diameter

Quantitative estimation for phosphate solubilization was done in NBRIP liquid medium. As described by Pande et al.,[14] 'P' solubilization was estimated in 10 ml of NBRIP broth (for per liter 10gmglucose;5gm $Ca_3(PO_4)_2$;5gm $MgCl_2$ $6H_2O$;0.25gm $MgSO_4$, $7H_2O$; 0.2 gm KCl; 0.1gm $(NH_4)_2SO_4$; pH 7.0) in test tubes. In each case, 0.025 g of 'P' in insoluble form was used as $Ca_3(PO_4)_2$. The broth without culture inoculated has served as a control. The culture broth was incubated for 8 days at 28±2°C. After incubation, 1 ml of culture broth was taken out and centrifuged at 10,000 rpm for 10 min. 0.1 ml of filtered supernatant was taken and mixed with 0.25 ml of Barton's reagents. Using double distilled water, the final volume was made to 5ml. The developed color (yellow) was measured using Thermo Scientific MultiskanGo spectrophotometer after 10 min of incubation and the amount of P that had been solubilized was inferred from the standard curve. All the experiments were repeated thrice and values were expressed as their Mean Standard error.

Qualitative Estimation of Indole Acetic Acid Production by the Bacterial Isolates

For qualitative estimation of IAA

Table 1. Physicochemical properties of rhizosphericsoil sample

Analysis Parameters	pH	Temp. (°C)	Available nitrogen (ppm)SE*	Available phosphorus (ppm)SE*	Available potassium (ppm)SE*	Organic carbon (%)SE*	Moisture content (%)SE	Soil EC (dS/m)SE*
Soil sample	4.94	26.20	184.74	4.29	161.59	0.71	40.26	0.682

* SE: Standard error from mean

Table 2. Morphological studies of the bacterial isolates

Bacterial isolates	Color	Transparency	Colony elevation	Margin	Form	GenBank Accession No.
Kosakonia arachidis (EZ27)	Off-white	Transparent	Raised	Entire	Round	OL662986
Pseudomonas putida (EZ11)	Off-white	Opaque	Flat	filamentous	round	ON495940
Pseudomonas monteilii (EZ30)	Yellowish	Opaque	Raised	filamentous	Round	OL662939

Table 3. Biochemical test of the bacterial isolates

Bacterial isolates	Gram	Motility	Catalase	Citrate utilization	Methyl red test	Starch hydrolysis test	Sugar Fermentation Test				
							Sucrose	Maltose	Dextrose	Glucose	Mannitol
Kosakonia arachidis (EZ27)	-	+	+	+	-	-	+	+	+	+	+
Pseudomonas putida (EZ11)	-	+	+	+	-	-	-	-	+	+	+
Pseudomonas monteilii (EZ30)	-	+	+	+	-	-	-	-	+	+	-

production, 10 ml of nutrient broth with tryptophan (0.1%) was inoculated with Bacteria isolates and incubated in a shaking incubator for Not two four days at 28±2°C.[15] Ten ml nutrient broth with 0.1% Tryptophan but without bacterial inoculation was maintained as control. On the 4th day, 1 ml of the culture was taken and centrifuged for 10 min @10,000 rpm followed by the supernatant was mixed with 2 ml of the Salkowski reagent to the test tubes labelled with each isolates and after 25 min of incubation; cultures showing pink color formation were identified as positive for IAA production (Figure 1 d-f).

Qualitative Estimation of Siderophore Production

For qualitative analysis of siderophore production, bacterial isolates were grown in Chrome Azurol S (CAS) agar plates and incubated at 28±2°C for 7 days. The formation of an orange halo zone around the bacterial colony indicated positive results (Figure 1 g-i).

Qualitative Estimation of Ammonia Production

Ten ml of peptone broth was inoculated with bacterial isolate using a sterile toothpick and incubated for 4 days in a shaking incubator at 28±2°C. One ml of Nessler's reagent was added to each test tube. Isolates giving light yellow color after adding the reagent was considered as isolates producing a small amount of ammonia whereas isolate producing dark yellow and orange color indicated a medium and high amount of ammonia production, respectively.[16]

Heavy Metal Tolerance (Cadmium)

Bacterial isolates were screened for heavy metal tolerance using minimum inhibitory concentration as described by Yadav et al.[17] Nutrient agar plates supplemented with different concentrations (30, 50, 80, 110, 140, 170, 200, 230, 280 and 320 µg/ml) of cadmium were streaked by the bacterial isolates (Table 4). Inoculated plates were then incubated for 72 h at 28±2°C. A negative control plate i.e., without Cd was also inoculated and incubated.

Salt Tolerance Test

By inoculating the bacterial isolates on nutrient agar plates with various concentrations of NaCl (2-14%, w/v with an increment of 2%), the

Table 4. Qualitative and quantitative analysis of growth-promoting traits of the bacterial isolates

Bacterial isolates	*Psi in NBRIP agar platesSE #	Phosphate solubilization in*** NBRIP broth(µg/ml)SE #	IAA	Siderophore production	Ammonia production	Cadmium resistant (µg/ml)	Salinity resistant test (%)
Kosakonia arachidis (EZ27)	2.8 ± 0.3	272.89±2.46	-	+	++	80	4
Pseudomonas putida (EZ11)	2.4 ± 0.1	89.12± 1.87	-	+	++	30	12
Pseudomonas monteilii (EZ30)	2.7 ± 0.1	109.70±5.47	18.25 ± 0.07	+	+	280	4

'+' Indicates positive, '-'indicates negative, *PSI= phosphate solubilizing index, PSI = Phosphate solubilizing index, ***NBRIP= National Botanical Research Institute's phosphate growth medium, *** IAA= Indole 3 acetic acid, # SE: Standard error from mean

bacteria were tested for salt tolerance as described by Sharma et al.[16] Pure isolates were streaked on media plates and incubated for 5 days at 28±2°C and observed for growth (Table 4).

Molecular Characterization of the Bacterial Isolates

DNA extraction was done by colony PCR method. Freshly grown bacterial colonies were picked by the sterilized toothpick and were suspended in the 60µl lysis buffer (Triton-X 100). Bacterial colonies in the lysing buffer were boiled for 10-15 min in boiling water bath. After 15 min the tubes with bacterial colonies were taken out and kept in the refrigerator for 3- 5 min followed by centrifugation at 10,000 rpm for 2-3 min. The PCR amplification of the target sequence was carried out with 0.6µl of dNTPs, 3µl of buffer, 21.7µl of sterile deionized water, 3µl of the template, 0.6µl of both primers [1492R (5'GGTTACCTTGTTACGACTT3') reverse primer and 18F (5'AGAGTTTGATCCTCAG3') forward primer][18] and 1µl of the T*aq* DNA polymerase. The reaction was performed in the Bio-Rad thermal cycler with 95°C for the early denaturation stage followed by 30 cycles of 94°C for 50 sec, 55°C for 90 sec, 72°C for 1 min, and last extension step at 72°C for 3 min. The amplified product of 16S ribosomal gene sequence was confirmed using agarose gel (1%) electrophoresis and the sequences were analyzed using the NCBI BLAST tool; the acquired gene sequences were compared to others in the GenBank databases. Sequences were then submitted to the NCBI GenBank database and accession numbers were obtained. A phylogenetic tree was made using MEGA11 software (Figure 2).

Seed Sterilization and Bacterization

Cicer arietinum L. seeds were surface sterilized with sodium hypochlorite (2%, v/v) for 2 min followed by ethanol (70%, v/v) for 1 min and washed thoroughly 5-6 times with sterilized deionized water. When 100µl of the aliquot from the most last washing were tested for the bacterial growth, there was no growth, proving that the seeds' surfaces had been completely sterilized. Sterilized seeds were then immersed in each bacterial suspension for 3½ hrs. in shaking conditions at room temperature. After bacterizations, seeds were sown in pots

containing mixture of soil and sand at 1:1 ratio. Soil and sand used for the experiment was sterilized by autoclaving at 121 psi for 15 min three times consecutively and then put in plastic pots with three replicates of each treatment.

Effect of Rhizobacterial Inoculation on the Plant Growth Parameters

The effects of the investigated rhizobacterial isolates on plants growth of model plant *Cicer arietinum* L. in pot experiment are presented in Table 5 and Figure 3. For each treatment 30 seeds were sown in each pot and experiments were repeated thrice. Seedlings were harvested after 30 days of sowing and morphological characteristics were determined. For the purpose, vegetative characteristics including shoot length, root length, root and shoot fresh biomass, and dry biomass were measured after uprooting all the plantlets. Under the control condition, these metrics were compared with PGPR treated and untreated plantlets.

Table 5. Effect of the bacterial inoculation on the plant growth parameters

Isolates	Shoot length (cm)*	Shoot fresh weight (g)*	Shoot dry weight (g)*	Root length (cm)*	Root fresh weight (g)*	Root dry weight (g)*
Control	7.17 ± 0.34d	0.50 ± 0.02d	0.10 ± 0.01cd	10.63 ± 0.27d	0.51 ± 0.03d	0.08 ± 0.01d
EZ30	17.27 ± 3.53ab	1.15 ± 0.22ab	0.24 ± 0.05a	12.00 ± 0.71c	0.85 ± 0.13b	0.18 ± 0.04ab
EZ27	17.90 ± 3.59a	1.25 ± 0.23a	0.20 ± 0.05abc	18.87 ± 0.69a	0.73 ± 0.12c	0.19 ± 0.03a
EZ11	13.10 ± 0.15c	0.84 ± 0.10c	0.17 ± 0.01abc	16.43 ± 0.36b	1.11 ± 0.54a	0.15 ± 0.01c

Control = Uninoculated, * Data was expressed mean values of the 3 replicates with standard error

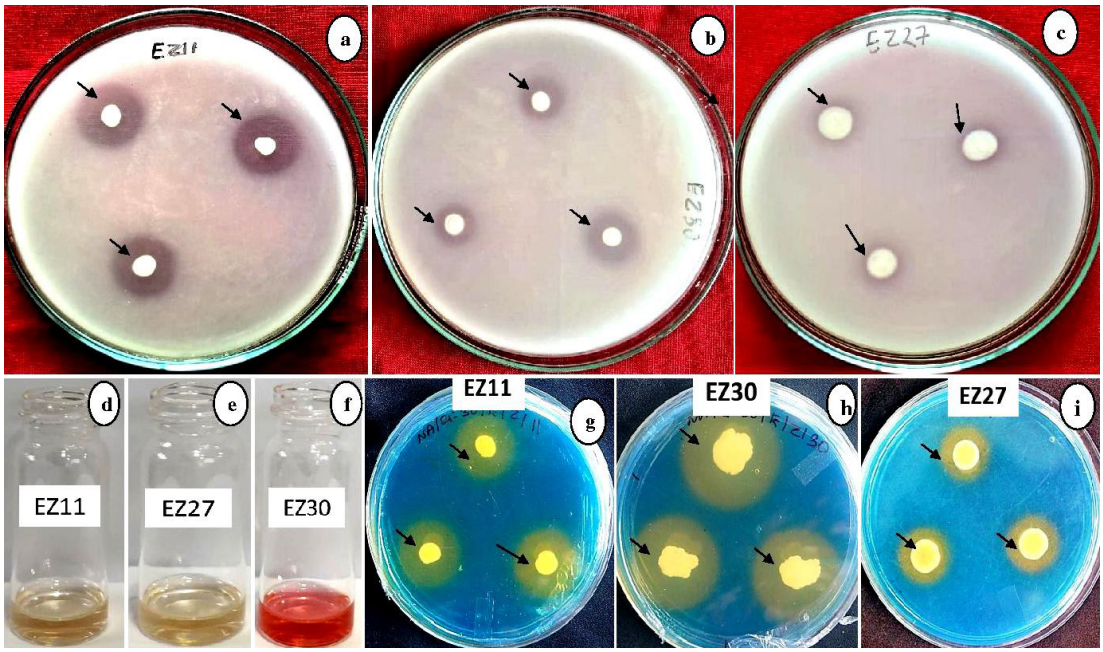

Figure 1. a-c: Phosphate solubilization and formation of halo zone around the bacterial colonies on NBRIP agar medium. a. *Pseudomonas putida*; b. *Pseudomonas monteilii*; c. *Kosakonia arachidis* after 7 days of incubation. d-f: Indole 3 acetic acid production test by d. *Pseudomonas putida*; e. *Kosakonia arachidis*; f. *Pseudomonas monteilii*. g-i: Siderophore production by isolates. g. *Pseudomonas putida*; h. *Pseudomonas monteilii*; i. *Kosakonia arachidis*

Statistical Analysis

The SPSS software was used for statistical analysis of the experimental data. All the reported results are the mean of the three replicates and deviations were calculated as the standard error of the mean (SEM). For assessing the importance treatment effect was done following one-way ANOVA and Least Significance Test (LSD) at the 0.05 level of confidence was used to compare means in cases where the F values were significant. When the P value was ≤ 0.05, differences were deemed significant.

RESULTS AND DISCUSSION

The objective of the present study was to study and provide some insight into the PGPR residing in the rhizosphere of the *Musa itinerans*. The effect of PGPR inoculation on *Cicer arietinum* L. on the growth and development was also studied.

Physicochemical analysis of the soil sample showed that the pH of the soil was 4.94 which indicate an acidic nature of the Zaphumi soil. Electrical conductivity of tested rhizospheric soil was 0.682dS/m which shows highly saline environment of the soil with high soluble salt content. Available nitrogen was found to be 413.82

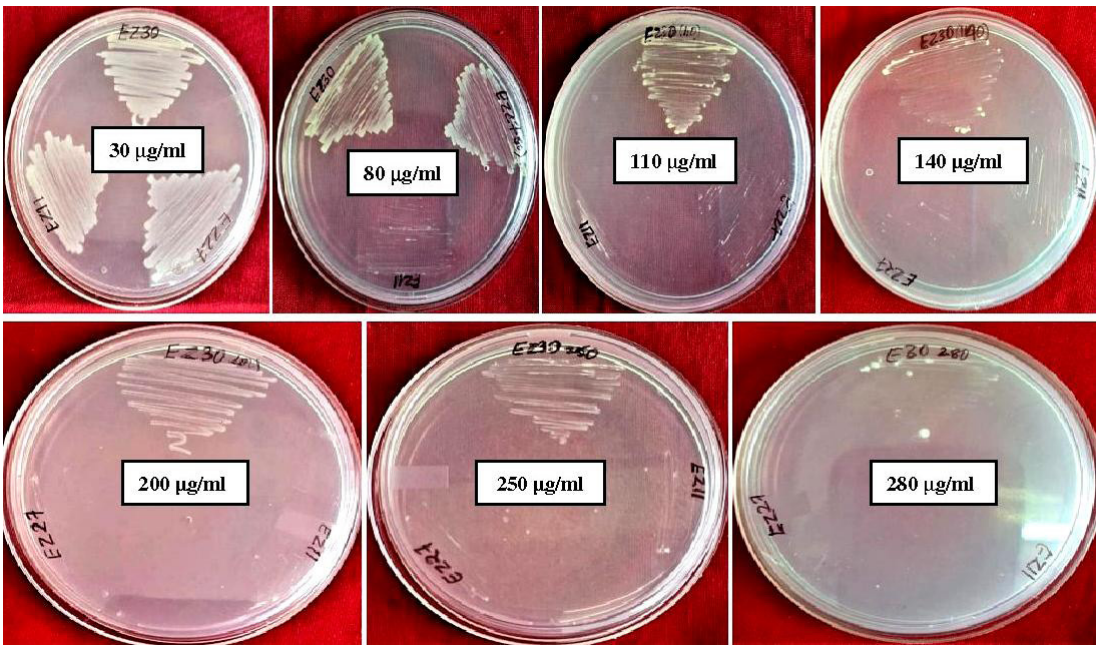

Figure 2. Cadmium tolerance test (Conc. of Cd g/ml) by *Pseudomonas putida* (EZ11), *Pseudomonas monteilii* (EZ30), and *Kosakonia arachidis* (EZ27)

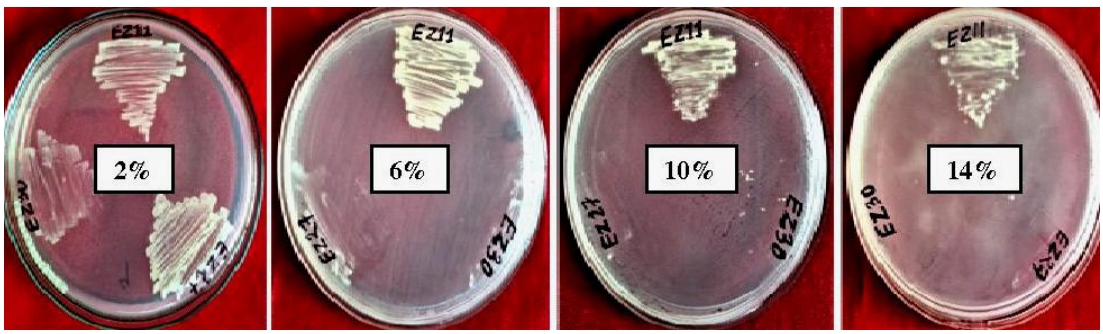

Figure 3. Salinity tolerance test (Conc. of NaCl, %) by *Pseudomonas putida* (EZ11), *Pseudomonas monteilii* (EZ30), and *Kosakonia arachidis* (EZ27)

kg/ha, whereas available phosphorus and available potassium were measured to be 9.63 kg/ha and 361.984 kg/ha, respectively after analysis of our soil sample (Table 1).

In the current study, 25 different bacterial isolates were taken from the mixed culture and purified before being tested for growth-promoting characteristics like phosphate solubilization, IAA generation, siderophore production, heavy metal tolerance, ammonia production, and salt tolerance. Bacterial isolates were also subjected to morphological and biochemical study. Bacterial isolates were grown on nutrient agar medium and three bacterial isolates (EZ30, EZ27 and EZ11)

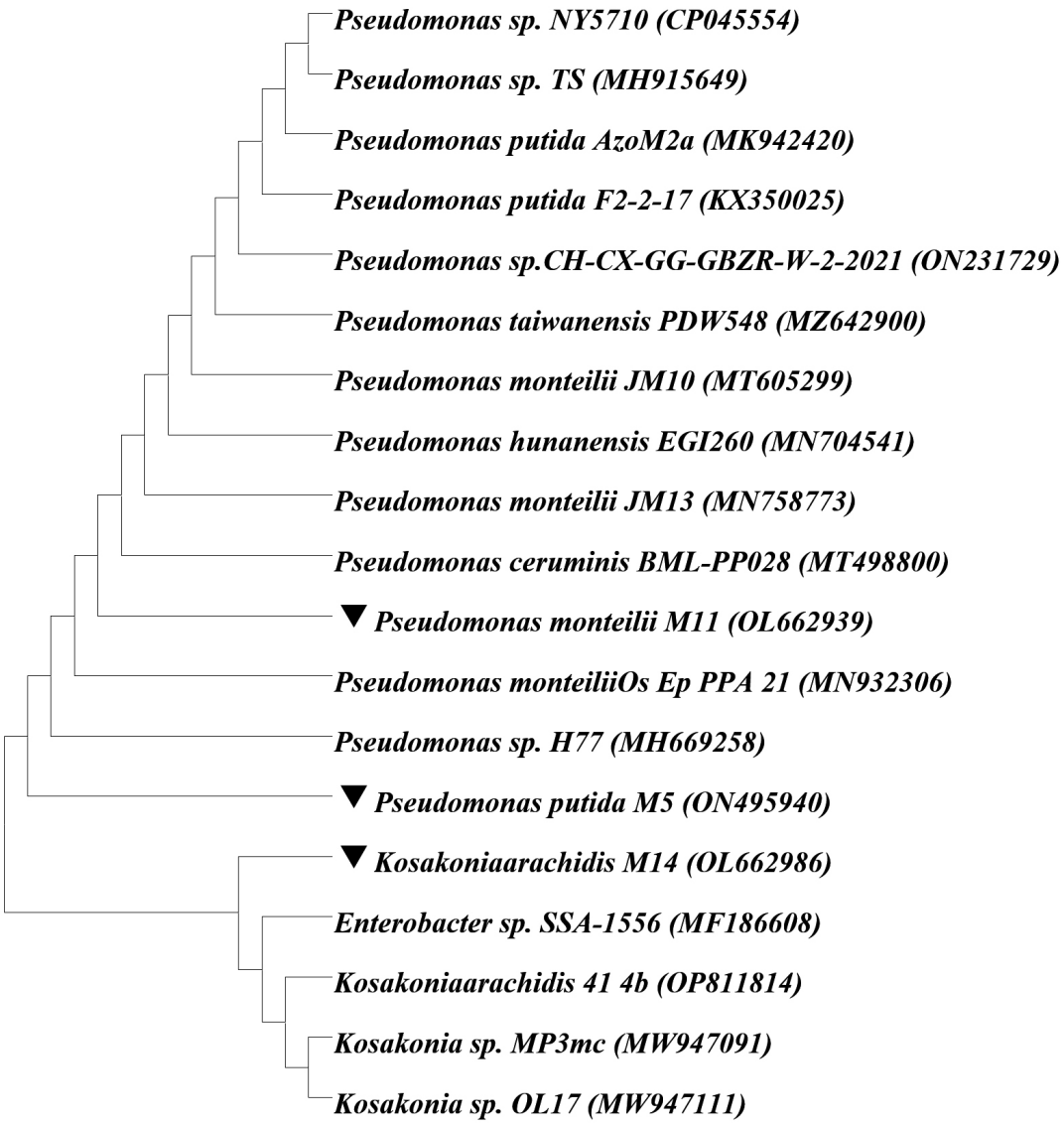

Figure 4. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. This analysis involved 21 nucleotide sequences. Codon positions included were $1^{st}+2^{nd}+3^{rd}$+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1131 positions in the final dataset. Evolutionary analyses were conducted in MEGA11

exhibiting highest phosphate solubilizing activity and at least three other growth promoting traits were selected for further study. The colony color and transparency of the bacterial isolates varied significantly. Isolates EZ11 and EZ27 colonies were off-white, while yellow color colony was observed with EZ30 isolate. Colony transparency of EZ11 and EZ30 was opaque and of EZ27 was fully transparent. Isolates EZ27 and EZ30 have raised colonies and EZ11 has flat colony. Margin was filamentous for both EZ11 and EZ30. EZ27 has entire margin. All isolates have round form of colony (Table 2). All the three isolates were gram negative, catalase test positive, citrate utilization positive, methyl red test negative and starch hydrolysis test negative. Isolates EZ11 and EZ27 were motile whereas, EZ30 was found to be non-motile. Different sources of carbohydrates (sucrose, maltose, dextrose, glucose, and mannitol) were tested for sugar fermentation (Table 3). Isolate EZ27 was able to utilize all five sugar forms given whereas EZ30 could utilize dextrose and glucose. EZ11 was only able to utilize dextrose, mannitol, and glucose.

Although soils typically contain large amounts of total phosphorus, the plant is rarely able to use it, which frequently restricts the growth of plants.[19] After one week of incubation on NBRIP agar plates with only tricalcium phosphate [$Ca_3(PO_4)_2$] as the only carbon source, a total of 14 bacterial isolates were found to be able to show phosphate solubilizing activity from which three isolates with highest PSI were selected for further analysis. The PSI was the highest for EZ27 (2.8), followed by EZ30 (2.7) and EZ11 (2.4) (Figure 1 a-c). Quantitative analysis of the isolates presented that EZ27 showed maximum P solubilization (272.89g/ml) followed by EZ30 (109.70g/ml) and EZ11 (89.12g/ml) (Table 4).

Among all three bacterial isolates only *Pseudomonas monteilii* was found to produce IAA. However, production of IAA *in vitro* depends on various factors such as hours of incubation, temperature, pH, and amount of the tryptophan added in the media (Figure 1 d-f and Table 4).

When there is a shortage of iron, microorganisms make siderophores, which are tiny organic molecules that increase the uptake of iron by the microorganism and is one of the mechanisms by which PGPR acts as biocontrol agents' and inhibits the growth of phytopathogens.[20] All three isolates were able to produce siderophore on CAS agar medium (Figure 1 g-i and Table 4). Positive isolates formed an orange halo zone around the colonies as siderophores produced by the bacterial isolates remove Fe from the Fe-CAS complex which is blue as described by Alexander et al.[21]

In plants, ammonia serves as a source of nitrogen and aids in the metabolic processes that produce amino acids. As a result, PGPR that produces ammonia promotes plant growth and biomass production.[22] In the present study, *Kosakonia arachidis* and *Pseudomonas putida* were found to produce more ammonia than *Pseudomonas monteilii* after four days of incubation (Table 4).

Figure 5. Pattern of plant growth and development of *Cicer arietinum* treated with PGPRs. a. Inoculated with *Kosakonia arachidis*; b. *Pseudomonas monteilii*; c. *Pseudomonas putida*; d. Control treatment

The presence of heavy metals limits the plant growth and development. Cadmium, a heavy metal is non-essential and considered one of the most toxic heavy metals because of its high mobility, persistent, bioaccumulation, and non-biodegradable properties.[17] When tested for the cadmium resistant, *Kosakonia arachidis* was able to grow up to 80µg/ml of cadmium supplemented in the medium whereas, *Pseudomonas monteilii* and *Pseudomonas putida* could tolerate up to 280µg/ml and 30µg/ml respectively (Table 4, Figure 2). The results of the current investigation indicate that *Pseudomonas putida*, *Pseudomonas monteilii* and *Kosakonia arachidis* can be a good candidate for biofertilizer in cadmium contaminated area and similar thought also shared by previous report by Manara et al.[23] about *P. putida*.

Salinity is regarded as the major hurdle in crop productivity in arid and semiarid regions.[16] In the present study *Pseudomonas putida* being able to grow in NaCl-supplemented agar medium up to 14% whereas *Kosakonia arachidis* and *Pseudomonas monteilii* could tolerate salinity up to 4% (Table 4, Figure 3) indicating these species can survive in high saline soil and improve plant growth. To the best of our knowledge, there are not many works on *Kosakonia arachidis* in the literature as PGPR. This the first ever report on salinity stress resistant characterization of *Kosakonia arachidis*. Through controlling ion homeostasis, photosynthetic molecules, redox potential, stress, and metabolites, other members of the genus *Kosakonia*, like *Kosakonia radicincitans,* have been proven to be beneficial in enhancing the salt tolerance of radish.[24]

Molecular characterization of isolates was done based on the 16S rRNA sequence and phylogenetic affiliation the three bacterial isolates was confirmed to be *pseudomonas putida* (ON495940), *Kosakonia arachidis* (OL662986), and *Pseudomonas monteilii* (OL662939) to which they exhibited 98.12, 99.62and 99.75% similarity respectively. The MEGA X software's Neighbour-Joining approach was used to create a phylogenetic tree that clustered the bacterial isolates with the appropriate known bacterial species (Figure 4). The morphological characterization of the isolates is consistent with the taxonomic and phylogenetic findings we have made. Eight validly described species of the *Kosakonia* genus have so far been identified, of which *K. radicincitans, K. sacchari, K. oryzae, K. cowanii* and *K. arachidis* are typically recognized as plant growth-promoting bacteria that increase the yield and quality of fruits like maize, radish, sugarcane, or cabbage.[25]

A sequence of experiments was conducted to evaluate the inoculation potential of all the three selected isolates with *Cicer arietinum* L. which showed a significant increase in all growth parameters of under controlled experiments (Figure 5, Table 5). An un-inoculated negative control was used. All three isolates promoted the plant growth compared to control treatment. Each bacterial isolate stimulated one or more plant experiment growth parameters. Shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight were the growth parameters that were tested. The plant inoculated with isolate *K. arachidis* was shown to have the highest shoot length, shoot fresh weight, root length, and root dry weight. *Cicer arietinum* L. when inoculated with *P. monteilii, K. arachidis,* and *P. putida*, the length of the shoot rose from 7.17 cm (under control conditions) to 17.27 cm (P=0.001), 17.90 cm (P=0.001), and 13.10 cm (P=0.001), respectively. Similarly, *K. arachidis* supported higher root length (18.87 cm) (P = 0.001) followed by *P. putida* (16.43 cm, P=0.001) and *P. monteilii* (12.00 cm, P=0.097) against 10.63 cm under controlled condition. Shoot fresh weight was highest with *K. arachidis* (1.25 gm) followed by *P. monteilii* (1.15 gm) and *P. putida* (0.84 gm) against 0.50 gm under controlled growth. Shoot dry weight was highest in *P. monteilii* (0.24 gm) followed by *K. arachidis* (0.20 gm) and *P. putida* (0.17 gm). Root fresh weight was highest in *P. putida* (1.11 gm) followed by *P. monteilii* (0.85 gm) and *K. arachidis* (0.73 gm). Finally, root dry weight was improved by inoculation with *K. arachidis*, *P. monteilii* and *P. putida* from 0.100 gm in control to 0.19 gm, 0.18 and 0.15 gm, respectively. Studies in the recent past on use of bacterial strains as biofertilizers in different crops such as corn,[26] sunflower,[27] tomato,[28] banana,[29] and groundnut has reported to promote growth and vigor.[30] In 3 years, *Arachis hypogaea* that received PGPR had higher pod yield, haulm yield, and nodule dry weight than the control.[2] Many studies on various crops have shown that combining multiple *Pseudomonas* strains and/or other microbes has advantages

over using single-strain inoculants.[31] *Pseudomonas* species supported a significant increase in growth, yield, oil contents and NP uptake against control in *Helianthus annuus*.[32] *Vigna unguiculata* seedlings when grown in presence of 50µg/mL of AuNPs, with *Pseudomonas monteilii* increased in the growth were observed.[33] Singh et al.[34] reported that the activity of nitrogen assimilation enzymes, chitinase, and endo-glucanase and the content of phytohormones significantly increased after the inoculation of *Kosakonia arachidis* in sugarcane. With the use of PGPR products in agricultural practices, the amount of synthetic agrochemicals can be reduced to a large extent.[35] In this study, the inoculation of PGPR increased the plant growth parameters. Many plant-growth promoting traits of the bacterial isolates identified and characterized in the current investigation may be responsible for the stimulation of the growth parameters of the plant species defined here.

CONCLUSION

The results of the current study suggested that *Musa itinerans* rhizospheric soil harbor rhizobacteria that can promote plant growth by solubilizing phosphate and generating indole-3-acetic acid and siderophores. The bacterial isolates also showed cadmium and salt tolerance to variable extent, suggesting its use in bioremediation of the heavy metal contaminated soil and can be effectively used in salinity stressed soil as biofertilizers. The inoculation of isolates on *Cicer arietinum* L. seeds promoted the growth metrics such as shoot and root length, fresh and dry weight of root and shoot and significantly outperformed the control treatment. With further study, a bacterial consortium can be developed from these isolates and used as a biofertilizer. This is the first-ever report to best of our knowledge to show that *Kosakonia arachidis* resides in wild Musa roots and potentially contributes to its growth and development.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

CRD designed the research proposal and experiments, arranged fund and supervised the research. CRD and MT performed data analysis. MT executed the research as part of her Doctoral Research and wrote the manuscript. CRD reviewed and edited the manuscript. Both authors read and approved the final manuscript for publication.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

This article does not contain any studies on human participants or animals performed by any of the authors.

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