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Diversity among the Salt Tolerant Rhizobacteria of Tungabhadra Command Area

Avinash D Patil[1], Tamilvendan, K[2], Mahadevaswamy[3]

Department of Agricultural Microbiology, College of Agriculture, Raichur
University of Agricultural Sciences, Raichur - 584104

Abstract: ***Pure cultures of 450 salt tolerant rhizobacterial isolates were prepared by isolating them from saline rhizospheric soils of Tungabhadra command area of north eastern Karnataka on nutrient agar medium supplemented with 1, 3, 6, 9 and 12 per cent salt and all the isolates could able to grow at 12 per cent salt. Among which, 20 isolates were selected and subjected to morphological, biochemical examinations which exhibited the presence of great diversity, as 14 isolates tested gram negative rods and rest found gram positive cocci, with the 14 isolate having motility. All the bacterium tested negative for starch hydrolysis, nitrate reduction and citrate utilization test and positive for catalase test, 11 were negative for urease test, 10 positive for gelatine liquefaction, 13 positive for H_2S production and 2 positive for protease test. The in vitro screening of these salt tolerant isolates for their plant growth promotional activity revealed the identification of GP-21 as efficient P- solubilizer, GS-36- nitrogen fixer (0.018 per cent) and MP-63 as IAA (21.2 µg/50 ml) producer.***

Keywords: Salt tolerant, nutrient agar, rhizospheric and diversity

1. Introduction

Soil salinity is a serious problem and steadily increasing in many parts world, particularly in arid and semi-arid areas. Over 2.1 million hectares of salt-affected land is located in the country's key bread basket in the North. Chloride and Sodium ion are the predominant ions in the soil and water in these areas. Salt affected soils, dry lands, flooded soils, high and low temperature exhibiting soils are considered as a an extreme environments for the survival of the specific microorganisms (1). Soil salinity is also a kind of extreme environment, where the habitation of salt tolerant or halobacteriums are witnessed. Many plant growth promoting rhizobacteria like *Azotobacter, Azospirillum, Rhizobium, Pseudomonas, Bradyrhizobium,* and *Bacillus* have been reported from saline areas of India (2). Previously salt tolerant bacteria were isolated from barren lands and rhizospheric soils (3). Their 16S rRNA gene sequencing analyses showed that they belonged to 10 different bacterial genera: *Bacillus, Brevibacterium, Planococcus, Zhihengliuella, Halomonas, Exiguobacterium, Corynebacterium, Arthrobacter,* and *Micrococcus*. Previously, rhizobacteria have been isolated from a salt affected soil of wheat rhizosphere and screened for plant growth promoting (PGP) traits at higher salt (NaCl) concentrations (2, 4, 6, and 8 per cent) (4). Novel P-solubilizing salt tolerant bacteria have been isolated from salt affected soils in China, which could grow at 20 per cent NaCl concentration and carryout P- solubilisation (5).

2. Materials and Methods

A total of 75 soil samples were collected from salt affected rhizospheric soils of sorghum, paddy and cotton of Tungabhadra command area of Manvi, Sindhanur and Gangavati regions of north eastern Karnataka. Salt tolerant bacteria were isolated on nutrient agar (NA) medium supplemented with 1, 3, 6, 9 and 12 per cent salt and all the isolates could able to grow at 12 per cent salt.

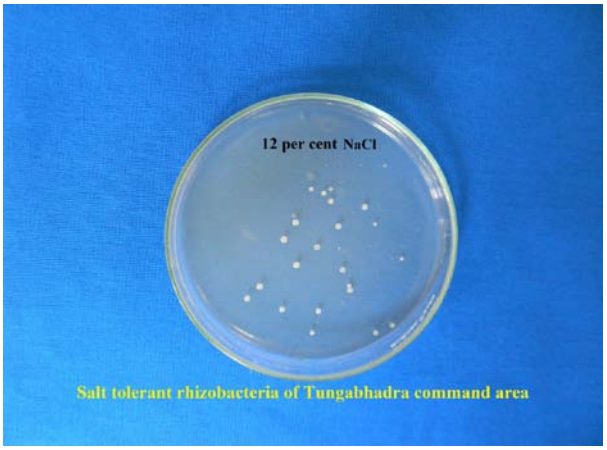

Figure 1: Salt tolerant rhizobacteria isolated on nutrient agar medium supplemented with 12 per cent NaCl.

Figure 2: Salt tolerant rhizobacterial pure cultures isolated on nutrient agar medium supplemented with 12 per cent NaCl.

The pure cultures were stored in refrigerator at 4°C. Subsequent sub culturing for morphological and biochemical studies were performed using nutrient broth and medium supplemented with 12 per cent NaCl. Of the 450 pure cultures of the isolates, 20 isolates were selected and

examined for shape, size, structure of colonies and pigmentation and Gram reactions. Biochemical tests of all the 20 isolates were recorded. Indole acetic acid (IAA) production by salt tolerant bacterial isolates was estimated by the method prescribed by Tien *et* al. (6), NH_3 production (7), *In vitro* N_2 fixation (8), HCN production (9), Siderophore production by using Chrome azurol assay (CAS) (10) and Phosphate solubilisation by using Pikovskaya's agar plates supplemented with 12 per cent salt (11).

3. Result and Discussion

Rigorous examination of all the 20 salt tolerant isolates established the existence of great diversity among them with respect to morphological, biochemical and plant growth promoting substances production potentials, has been elaborated here under.

3.1 Morphological Diversity

Among the 20 isolates, 14 isolates tested Gram negative rods and rest of them found Gram positive cocci (Table 1). Salt tolerant bacteria isolated from the saline soils exist in both Gram negative and Gram positive form (12). Fourteen isolate among the 20 showed to possess motility (Table 1). Salt tolerant bacteria such as *Bacillus megaterium* var. *Phosphaticum*, *Bacillus megaterium* ATCC 14581 and QW 1011 previously isolated were tested positive and negative, respectively for motility test (13).

3.2 Biochemical Diversity

No isolate tested positive for starch hydrolysis (Table 2). Similar result of being negative for starch hydrolysis in many salt tolerant bacteria such as *Sereratia* sp, *Rhizobium* sp, *Bacillus* sp, *Pseudomonas putida* and many *Pseudomonas* sp, have been reported (12).

Table 1: Morphological diversity among the salt tolerant rhizobacteria of Tungabhadra command area

Sl. No.	*Isolate code*	*Cell shape*	*Motility*	*Colour of the colonies*	*Gram reaction*
1.	GC-9	Rod	+	Dense creamy white	-
2.	GP-14	Rod	+	White	-
3.	GP-17	Rod	+	Pale white	-
4.	GP-18	Rod	-	Pale white	+
5.	GP-19	Rod	+	Colour less	+
6.	GP-20	Spherical	-	Pale white	+
7.	GP-21	Rod	+	Dense creamy white	-
8.	GP-25	Rod	-	Pale white	-
9.	GP-26	Spherical	-	Pale white	+
10.	GP-27	Spherical	+	Dense creamy white	+
11.	GP-28	Rod	+	Pale white	+
12.	GP-30	Rod	+	Pale white	-
13.	GP-34	Rod	+	Pale white	-
14.	GS-36	Rod	+	Dense creamy white	-
15.	GS-41	Rod	-	Pale white	-
16.	MP-61	Rod	+	Dense creamy white	-
17.	MP-62	Spherical	+	Pale white	+
18.	MP-63	Rod	+	Pale white	-
19.	MP-65	Rod	+	Dense creamy white	-
20.	MP-74	Rod	-	Pale white	-

+: Positive for the test - : Negative for the test

All the isolates showed positive for the catalase test. This result positively correlates with the finding where, salt tolerant bacteria (*Micrococcus* sp, *Azotobactor* and *Bacillus safencis*) previously isolated from saline soils were reported to be catalase positive (12). A total of 9 isolate out of 20 showed positive for the urease test. Salt tolerant bacteria were previously isolated, reported to exhibit both positive and negative for urease test (14) and (15). All the isolates tested negative for the indole production. *Serratia* sp., *Micrococcus* sp., *Bacillus safensis* previously isolated were tested negative (12). Thirteen out of the 20 isolates tested positive for H_2S production. Similar reports, where salt tolerant bacteria being both positive and negative for H_2S production have been mentioned (16). All the 20 isolates showed negative for citrate utilization test (Table 2).

Salt tolerant bacteria previously isolated from saline soil such as *Bravibacillus* sp., *Pseudomonas* sp. and *Pseudomonas putida* showed negative for citrate utilization (12) and (17). Among the twenty isolates, 10 isolates were positive for the gelatin liquefaction test. Previously isolated *Bacillus pumilus*, *Bacillus cereus*, were positive and *Azotobactor* sp., *Pseudomonas putida* were negative for gelatin liquefaction (14). All the 20 isolates tested negative for nitrate reduction. Similar reports of salt tolerant bacteria being negative for nitrate reduction have been depicted (13). Isolates *viz*., GP-21 and MP-61 tested positive for protease test (Fig-3). Similar convincing report involving salt tolerant bacteria isolated from saline soil (*Bacillus megaterium* var. *Phosphaticum*) have been mentioned to be positive for protease test (13)

Table 2: Biochemical diversity of salt tolerant rhizobacteria of Tungabhadra command area

Sl. No.	Isolate code	Starch hydrolysis	Catalase test	Indole test	Urease Test	Gelatine liquefaction	Citrate utilization	Nitrate reduction	Protease test	H_2S production
1.	GC-9	-	+	-	+	-	-	-	-	-
2.	GP-14	-	+	-	-	+	-	-	-	+
3.	GP-17	-	+	-	-	-	-	-	-	+
4.	GP-18	-	+	-	-	+	-	-	-	-
5.	GP-19	-	+	-	-	+	-	-	-	+
6.	GP-20	-	+	-	-	-	-	-	-	+
7.	GP-21	-	+	-	-	+	-	-	+	-
8.	GP-25	-	+	-	+	-	-	-	-	+
9.	GP-26	-	+	-	-	-	-	-	-	-
10.	GP-27	-	+	-	+	-	-	-	-	+
11.	GP-28	-	+	-	-	-	-	-	-	+
12.	GP-30	-	+	-	+	+	-	-	-	+
13.	GP-34	-	+	-	+	+	-	-	-	+
14.	GS-36	-	+	-	+	+	-	-	-	-
15.	GS-41	-	+	-	+	+	-	-	-	+
16.	MP-61	-	+	-	-	-	-	-	+	-
17.	MP-62	-	+	-	+	+	-	-	-	+
18.	MP-63	-	+	-	-	-	-	-	-	-
19.	MP-65	-	+	-	-	-	-	-	-	+
20.	MP-74	-	+	-	+	+	-	-	-	-

+ : Positive for the test - : Negative for the test

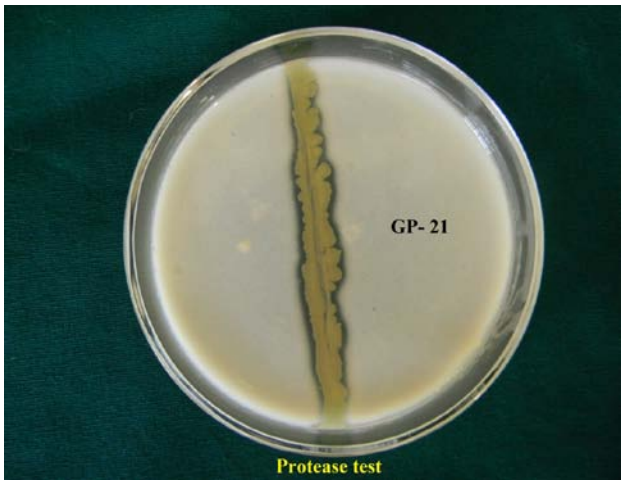

Figure 3: Salt tolerant rhizobacteria GP-21 showing positive for protease test (12 per cent NaCl)

3.3. Diversity with respect to plant growth promoting substance production potential

All the isolates could able to produce IAA at the higher salt concentration of 12 per cent (Table 3). IAA producing salt tolerant bacteria such as *Serratia* sp., *Pseudomonas* sp. have already been isolated (12) and (18). All the 20 isolates fixed nitrogen. Previously isolated *Azotobacter* sp, *Azospirillum* sp, and *Pseudomonas* sp were reported to fix nitrogen under salt stress condition (19) and (20). Only one isolate (GP-21) out of 20 isolates, tested positive for phosphate solubilisation (Fig-4). *Bacillus cereus*, *Bacillus subtilis*, *Pseudomonas putida*, *Pseudomonas* sp. have been previously isolated showed to exhibit phosphate solubilisation even under salt stress (12) and (15).

Table 3: Diversity of the isolates with respect to production of plant growth promoting substance

Sl. No	*Isolate Code*	*Plant growth promoting activity*		
		Phosphate solubilisation	*Nitrogen fixation*	*IAA production*
1.	GC-9	-	+	+
2.	GP-14	-	+	+
3.	GP-17	-	+	+
4.	GP-18	-	+	+
5.	GP-19	-	+	+
6.	GP-20	-	+	+
7.	GP-21	+	+	+
8.	GP-25	-	+	+
9.	GP-26	-	+	+
10.	GP-27	-	+	+
11.	GP-28	-	+	+
12.	GP-30	-	+	+
13.	GP-34	-	+	+
14.	GS-36	-	+	+
15.	GS-41	-	+	+
16.	MP-61	-	+	+
17.	MP-62	-	+	+
18.	MP-63	-	+	+
19.	MP-65	-	+	+
20.	MP-74	-	+	+

+ : Positive for the test - : Negative for the test

4. Selection of Efficient salt tolerant plant growth promoting rhizobacteria

Screening of the salt tolerant bacteria for their plant growth promotional attributes yielded the identification of three most efficient local salt tolerant plant growth promoting isolates *viz*., GP-21 as efficient P- solubilizer (Fig-4) , GS-36- efficient nitrogen fixer (0.018 per cent) and MP-63 as IAA producer (21.2 µg/50 ml).

Figure 4: GP-21 showing positive for phosphate solubilisation test (12 per cent NaCl)

4. Conclusion

Variability with respect to morphological, biochemical and plant growth promoting substance production potentials among the salt tolerant bacterium of Tungabhadra command area indicates the existence of great diversity among the miniature creatures. Many of the rhizospheric salt tolerant bacteria known to facilitate plant growth by modifying soil climate through nitrogen fixation, P- solubilisation, IAA production and release of unidentified substances. Still, many of the efficient salt tolerant plant growth promoting rhizospheric bacteria are unknown to the world. Identifying, characterising and utilizing such salt tolerant bacteria on commercial scale for the cultivation of crops in the salt affected soil would be boon for modern agriculture that could helps to sustain the land and food production for the future generation.

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Author Profile

Avinash D Patil (M.Sc. Agri), Department of Agricultural Microbiology, College of Agriculture, Raichur—584104