PLANT GROWTH PROMOTING POTENTIAL AND DIVERSITY OF BACTERIA FROM RICE RHIZOSPHERE OF SALINE SOIL

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Abstract:

Bacteria including PGPR plays a very important role in plant growth promotion and increase yield of crops. Most of the bacteria produce phytohormones, fixes atmospheric nitrogen, solublizes the phosphates and resist phytopathogens by production of siderophores. An understanding of microbial diversity perspectives in agricultural contest, is important and useful to know soil quality and also helpful for taking measures for soil management and increased plant productivity. It is also important to understand the relationship of soil and plants with the diversity of associated bacteria for their better exploitation. Therefore, it is important to know the microflora and their diversity. Most of the rhizospheric bacterial diversity from normal soil have been studied and organisms have been explored for their use as bioinoculents. However, saline soil rhizospheric microflora remain unexplored. By considering this, in the present study a total of sixty two bacterial isolates including PGPR have been isolated from rice rhizosphere of saline soil of Kolhapur district of southern Maharashtra, India. Isolates were identified up to genus and species level. All the isolates were studied for their nitrogen fixing ability, phosphate solublizing activity, Indole acetic acid production and Siderophore production at higher salt (NaCl) concentrations1%, 2%,3%,up to 15%.

.Results indicated that all the isolates grows up to 7 % NaCl concentrations, showed optimum activities at 4% NaCl concentration and tolerated 10% NaCl for 12 hours. Of all 62 isolates 21 produced Indole-3-acetic acid (IAA) 29 solublized phosphates, 21 fixed atmospheric nitrogen, and 10 produced Siderophores and 4 have not showed any plant growth promoting activity. All the isolates were identified up to genus level and most of them up to species level using Bergeys manual of systematic bacteriology, and MICRO IS software. Amongst all the genera identified *Pseudomonas* was found to be dominant followed by *Bacillus.*

Present study showed that amongst nitrogen fixing bacteria *Azotobacter spp.*, found to be most dominant and *Pseudomonas* was found to be most dominant phosphate solublizer. Study indicated the importance of these organism as bioinoculents for saline soil and can be explored for biofertilizer production.

Key words: Diversity, PGPR, Saline soils, Rhizosphere, Rice, Bioinoculents.

Introduction:

Plant growth-promoting bacteria are free-living, soil borne bacteria, present in the rhizosphere, which when applied to seeds or crops enhance the growth of the plant or reduce the damage from soil-borne plant pathogens Kloepper et al. [1].These bacteria can either directly or indirectly enhance the growth of the plant and increase crop yield, Argano [2]. These bacteria

enhance growth of the plant by phosphate solublization, Nitrogen fixation, phytohormones and exopolymer production Vessey [3];Fischer et al.[4]Cummings[5].

The soil gains importance, especially in saline agricultural soils, where high salts are present either naturally or through irrigated water or through excess use of chemical fertilizers. This effect is more pronounced in the rhizosphere as a result of increased water uptake by the plants due to transpiration, hence rhizobacteria in this region are adapted more to osmolarity, these adapted organism have the potential to be used as bioinoculents for saline soils. Investigations of bacterial diversity is an important step to access soil conditions due to its importance in nutrient cycling and crop productivity,Stocker et al. [6];Toro et al.,[7]. Microbial inoculants are promising components for integrated solutions to agro-environmental problems because inoculants possess the capacity to promote plant growth, enhance nutrient availability and uptake and support the health of the plants, Barea et al.,[8];Dobbelaere et al.,[9]

The indigenous species and strains of bacteria are very useful in production of bioinoculents for local crops because these organisms have already been adapted to local environmental conditions, hence they can be explored as bioinoculents for local crops. It is also important to study the organisms from saline rhizosphere habitats because these organisms have adapted to osmoregularity mechanisms which are still not well known. Studying diversity of such soil will contribute towards long term goal of improving plant-microbe interactions for salinity affected fields and crop productivity.

Plants play an important role in selecting and enriching the types of bacteria by the constituents of their root exudates. Thus, depending on the nature and concentrations of organic constituents of exudates, and the corresponding ability of bacteria to utilize these as sources of energy, the bacterial community develops in the rhizosphere, Curl and Truelove [10]. Rhizospheric bacterial communities however have efficient systems for uptake and catabolism of organic compounds present in root exudates, Barraquio et al.,[11].

Soil microorganisms also play an important role in soil processes that determine plant productivity. Therefore, it is necessary to determine the ability of these bacteria to enhance plant productivity, their diversity, distribution and behavior in indigenous soil habitats because these organisms have an potential to be used as bioinoculents for local soils.

By keeping in view this in the present study, Rice rhizosphere was explored for isolation identification and screening of plant growth promoting bacteria from saline soils of Kolhapur district of Maharashtra, India.

Material and Methods:

Collection of Samples

Soil adhered to roots of Rice plant from saline soils were collected from fourty different sites in sterile plastic bags from Kolhapur district of Maharashtra, India.

Isolation of Microorganisms:

One gram rhizospheric soil sample was dissolved in 100 ml of buffered saline and placed on shaker for 30 min, From this different dilutions viz 10^{-2},10^{-4} ,10^{-6},10^{-8},10^{-10} were prepared. From each dilutions 0.1 ml was spread Nutrient agar for isolation as well as enumeration of different bacteria,0.1ml was spread on Ashbys Mannitol agar for *Azotobacter* spp.,Congored yeast extract agar for *Rhizobium*spp., Nitrogen free agar for *Azospirillum* spp respectively. Individual colonies showing different morphology from respective medium were transferred on slants of respective media and further used for identification and other studies.Unless otherwise stated experiment was conducted in triplicates.

Identification of Microorganisms:

All the isolates were identified as per the Bergeys Mannual of Systematic bacteriology Williams *et al*., [12] Vol.I, II,III,IV,V,VI and Micro IS software as per Portyrata and Kricheosky [13].

Screening of plant growth promoting bacteria:

a.Phosphate- solublization

Phosphate- solublization was detected qualitatively by spot inoculation of isolates on Pikovskaya medium Subba Rao [14] , containing Glucose 10 g, Tribasic phosphate 5g, $(NH_4)2SO_4$-0.5g, KCl-0.2g, $MgSO_4.7H_2O$-0.1g, trace of $MnSO_4$ and $FeSO_4$, Yeast extract 0.5g, NaCl 4%, Agar Agar 15 g, Distilled water 1000 ml, pH-7.0. After incubation at room temperature for 48 hours a clear zone around colony was used as indicator for positive phosphate solublization.

b.Nitrogen fixation:

Nitrogen fixation was detected by Acetylene reduction assay as per Dobernier [15] and Hardy et al [16], using a chemically defined medium containing K2HPO40.60 g-l, KH2PO4 0.14 g-l,
MgSO4.7H2O 0.2 g-l, FeSO4.7H2O 0.44 g-l, ZnSO4.7H2O 0.00028 g-l, H2BO3 0.0032 g-l, Na2MoO4.2H2O 0.003 g-l,MnSO4.H2O 0.004 g-l, NaCl 4%, Sucrose 20 g-l using glass bottles with rubber stoppers. Isolates were grown in 100 ml above medium separately. Flask were incubated on rotary shaker for 48 hours to obtain full growth . From this 20 ml was transferred to a empty sterile glass bottle 30 ml capacity with rubber stopper. To this bottle 10 ml of acetylene gas was added and bottle was closed with rubber stopper and allowed to stand in shed for 1 hour for reaction time of enzyme nitrogenase on acetylene gas. From this bottle 1 ml of the gas was removed and ethylene percentage was determined using gas chromatography.

c.Indole acetic acid production:

Indole acetic acid produced by isolates was assayed colorimetrically using Ferric chloride-perchloric acid reagent as per Gordon and Weber [17]. For this isolates were grown in 50 ml modified nutrient broth inoculated with 4 % NaCl salt for 24

hours on rotary shaker at 150 rpm and room temperature and used as seed culture. From this 100 ul of was inoculated in 10 ml minimal salt (MS) medium containing KH_2PO_4-0.136, Na_2HPO_4-0.213 g, $MgSO_4.7H_2O$- 0.02 g , Trace element solution 0.001, Tryptophan 0.5mM, NaCl-4 g, Distilled water-100 ml, pH-7.0.,Frankenberger and Poth [18] . After incubation at room temperature for 48 hours, 1.5 ml broth culture was centrifuged at 12000 rpm for 5 minutes. One ml supernatant was added to 2 ml $FeCl_3$-$HClO_4$ reagent. After 25 minutes (once color density reaches maximum) the mixture was read in UV-spectrophotometer at 530 nm absorbance. The amount of IAA produced per ml culture was estimated using a standard curve.

d. Siderophore production:

It was assayed according to Schwyne and Neilands [19]. Isolates producing an orange halo zone around growth on Chromeazurol S agar (CAS) after 48-72 hours of incubation were considered as positive.

Result and Discussion:

Table 1 shows the different bacteria identified from Rice rhizosphere of saline soil.

Table 1 : List of Identified Bacterial isolates.

Isolate No.	Name of the bacterial Isolate	Isolate No. nNo	Name of the bacterial Isolate	Isolate No.	Name of the bacterial Isolate
1	*Paenibacillus polymyxa*	22	*Pseudomonas putida*	43	*Pseudomonas monteilii*
2	*Bacillus aerophilus*	23	*Pseudomonas stutzeri*	44	*Pseudomonas alcaligens*
3	*Bacillus cereus*	24	*Serratia phosphaticum*	45	*Pseudomonas pseudoalcaligens*
4	*Bacillus circulans*	25	*Azotobactor parvus*	46	*Bacillus pumilis*
5	*Ochrobactrum anthropi*	26	*Serratia marcescens*	47	*Bacillus pulvifaciens*
6	*Azospirillum lipoferum*	27	*Pseudomonas fluorescens*	48	*Azoarcus communis*
7	*Azotobactor chroococcum*	28	*Herbaspirillum aeropedicae*	49	*Flavobacterium species*
8	*Stenotrophomonas species*	29	*Bacillus mesentricucs*	50	*Azospirillum caulinodans*
9	*Pseudomonas fluorescens*	30	*Bacillus mycoides*	51	*Paenibacillus polymyxa*
10	*Pseudomonas pseudomallei*	31	*Brevibacterium antiquum*	52	*Alcaligenes xylosoxidans*
11	*Pantoea agglomerans*	32	*Gluconobacter azocaptans*	53	*Pseudomonas striata*
12	*Arthrobacter species*	33	*Corynebacterium species*	54	*Acetobacter diazotrophicus*
13	*Azotobactor venelandii*	34	*Rhodospirillum species*	55	*Gluconobacter johannae*
14	*Azospirillum brasilense*	35	*Rhodopseudomonas species*	56	*Pseudomonas aeruginosa*
15	*Azospirillum halopraeferens*	36	*Azotobacter beijerinkii*	57	*Pseudomonas fluorescens*
16	*Bacillus mesentricus*	37	*Azotobacter nigricans*	58	*Microbacterium pecies*
17	*Bacillus megaterium*	38	*Azotobacter paspali*	59	*Micrococcus luteus*
18	*Bacillus firmus*	39	*Stenotrophomonas maltophila*	60	*Bacillus stratosphaericus*
19	*Bacillus licheniformis*	40	*Xanthomonas oryzae*	61	*Pseudomonas vranovensis*
20	*Pseudomonas cissicola*	41	*Aeromonas species*	62	*Calothrix brauni*
21	*Pseudomonas pinophilum*	42	*Citrobacter diversus*		

Table 1 indicates the list of identified bacteria from wheat rhizosphere of saline soils. Amongst all the bacterial isolates genera *Bacillus* was found to be the most dominant followed by *Pseudomonas* which correlates with Gaur et al.,[20].

The strains from the genera *Bacillus ,Pseudomonas, Rhizobium* are amongst the most phosphate solublizers.Genera *Pseudomonas* was dominant, Koide [21]; Jetiyanon et al.,[22]; Vessy, [23]; Bashan et al.,[24]; Wu et al., [25]; Rodriguez and Fraga,[26] studied the maize PGPR and their role in plant growth promotion. They found that *Azotobacter chroococcum* and phosphate solublizer *Bacillus megaterium* as most dominant Nitrogen fixer and phosphate solublizer. I report *Pseudomonas spp.* as most dominant phosphate solublizer and *Azotobacter spp,* as dominant Nitrogen fixer.

Suman et al., [27] found the presence of genera *Bacillus, Pseudomonas, Azospirillum, Azotobacter, Herbaspirillum, Ideonella* in maize rhizosphere however, my results indicated presence of above all except *Ideonella*.

Garcia de Salamone et al.,[28] and Chelius Triplett,[29] found the presence of *Enterobacter* spp.,*Rahnella aquatilis, Paenibacillus azotofixans, Azospirillum species, Herbaspirillum species, Bacillus circulans and Klebsiella* species while my results indicated presence of *Azospirillum* species, *Herbaspirillum* species, *Bacillus circulans, Klebsiella* species, However, *Rahenella aquatilis, Paenibacillus azofixans* were found to be absent in Rice rhizosphere of saline soils.

Table 2 indicates the isolates producing PGPR traits.

Table 2. isolates producing (IAA), P- solublization,Nitrogen fixation, and Siderophore production.

Strain no.	(A)	(B)	(C)	(D)	Strain no.	(A)	(B)	(C)	(D)	Strain no.	(A)	(B)	(C)	(D)
N-1	-	+	-	-	N-22	6.2	-	-	+	N-43	5.3	-	-	-
N-2	-	+	-	-	N-23	-	-	-	+	N-44	7.2	+	-	+
N-3	-	+	-	-	N-24	20.4	-	-	+	N-45	-	-	-	-
N-4	-	+	-	-	N-25	-	-	-	-	N-46	6.4	-	-	-
N-5	-	+	-	-	N-26	-	-	+	-	N-47	-	+	-	-
N-6	12.3	-	-	-	N-27	-	-	-	-	N-48	8.3	-	-	-
N-7	24.5	+	-	-	N-28	-	-	-	+	N-49	5.4	+	-	+
N-8	-	-	-	-	N-29	-	-	-	-	N-50	-	-	-	-
N-9	6.3	-	-	+	N-30	-	+	-	-	N-51	-	-	-	-
N-10	28.2	+	-	-	N-31	-	+	-	-	N-52	-	+	-	-
N-11	-	-	+	-	N-32	-	+	-	-	N-53	-	+	-	-
N-12	-	-	+	-	N-33	-	+	-	-	N-54	-	-	+	-
N-13	17.9	-	+	-	N-34	9.4	-	-	-	N-55	-	-	+	-
N-14	-	-	+	-	N-35	-	-	+	-	N-56	-	-	-	-
N-15	31.2	-	-	-	N-36	12.3	-	-	-	N-57	-	-	+	-
N-16	4.7	-	+	-	N-37	24.4	-	-	-	N-58	6.8	+	-	
N-17	-	+	+	-	N-38	6.2	-	-	-	N-59	-	-	-	+
N-18	-	+	+	-	N-39	28.2	-	+	-	N-60	-	-	-	-
N-19	-	+	+	-	N-40	14.9	-	+	-	N-61	-	-	-	-
N-20	-	+	+	-	N-41	28.4	-	+	-	N-62				
N-21					N-42									

(A) IAA production(µmol $ml^{-1)}$,(B) P-solublization, (C)N_2-fixation, (D)Siderophore production, (+) positive, (-) negative

Of all the 62 isolates 21 produced Indole acetic acid (IAA), 29 solublized phosphates, 21 fixed Nitrogen, 10 produced siderophores,

The overall results showed that only 4 isolates did not showed any of the four PGPR traits. Isolate N9,N10,N13, N16,N18, N19, N22, N24, N39, N40, N41, N49, N58, N61, N62 shared two PGPR traits. Isolate N7 shared three PGPR traits i.e. produced IAA, solublized phosphates, fixed Nitrogen and isolates number N21 and N44 shared three PGPR traits i.e. produced IAA, solublized phosphates, and produced siderophores. The amount of IAA produced by iolate no. N-15 was higher (30.2) than that have been reported by De Freital et al.,[30], which range from 2.31 to 9.43 µmol ml^{-1}.Further study is required to utilize potential application for high IAA production.

As three isolates N7, N21, and N44 shared three PGPR traits, and other isolates shared two PGPR traits , these organisms have a potential to be used as bioinoculents for improving the plant growth in saline soils and can be explored for production of bioinoculents for saline soils. Tripathi et al., [31] reported accumulation of compatible solutes such as Glutamate, Proline, Glycine, Betaine and Trehalose in response to salinity/ osmolarity in *Azospirillum* and *Azotobacter* species which indicated that these strains can be used as bioinoculents for saline soils.

The rhizosphere considered to be a hot spot of bacterial diversity, harbors bacterial flora whose diversity is mainly expressed in terms of functions adapted to the root presence and in particular to favor plant growth. A continued exploration of the natural biodiversity of soil microorganisms and the optimization and manipulation of microbial interactions in the rhizosphere of crops is required to develop more efficient bioinoculents.

Conclusion:

- All the isolates tolerated 8 % NaCl concentration, grows optimally at 4 % NaCl, hence they have a potential to be used as bioinoculents for saline soils.
- There is a scope for use of nitrogen fixing *Azotobacter chrococcum* and *Azospirillum lipoferum*, *Azospirillum brasilense* as potential Nitrogen fixing biofertilizer and Bacillus subtilis as potential phosphate solublizer for reclamation of saline soils.On presenting this work, I am impressed with the ability of *Azotobacter chrococcum* and *Azospirillum lipoferum, Azospirillum brasilense* to grow in the presence of salts. Further there is lack of comparative results primarily due to difficulty in comparing results obtained, my work will encourage researcher to obtain comparative results. It may hope that my investigations may inspire others to carry out work on salt tolerant nitrogen fixing *Azotobacter chrococcum, Azospirillum lipoferum, Azospirillum brasilense and Bacillus subtilis*. other aspects which have not yet studied.
- Detail microbiological analysis of saline soil carried out with respect to PGPR Bacteria in Rice rhizosphere, which could serve as Basic data for further research.

- A survey of available literature, suggests that microbiology of saline soil and exploitation of microorganisms from these soil has not been dealt extensively. Considering this lacuna, investigations were focused on the Rice rhizospheric microbiology of saline soil and potential of these microorganisms for commercially important bioinoculents for saline soils.
- As the isolate number N-7 and N21 and N44 showed three PGPR traits i.e. produced IAA, dissolved phosphates, fixed nitrogen, and produced siderophores they can be commercially used for production of bioinoculents for saline soils.
- On completing this investigatation, I am impressed with the wide diversity of microorganisms present in Rice rhizosphere of saline soils.

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