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Plant Growth Promoting Potential of Bacteria from Wheat Rhizosphere of Saline Soil.

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ABSTRACT

In the present study total of 59 rhizobacteria was isolated from saline infested zone of Wheat rhizosphere, using different media. Nutrient agar was used 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 Rhizobiumspp., 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. All the isolates were identified using All the isolates were identified as per the Bergeys Mannual of Systematic bacteriology [1] and Micro IS software[2]. All the isolates was screened for plant growth promoting (PGP) activities at higher salt (NaCl) concentrations 2%,4%,6%,8%,10%. Results indicated that all the isolates grows up to 6 % NaCl concentrations, showed optimum activities at 4% NaCl concentration and tolerated 8% NaCl for 12 hours. Of all 59 isolates 22 produced Indole-3-acetic acid (IAA) 21 solublized phosphates, 17 fixed atmospheric nitrogen, and 8 produced Siderophores and 10 have not showed any plant growth promoting activity. All the isolates were identified up to genus level and most of up to species level using Bergeys manual of systematic bacteriology, and MICRO IS software. Amongst all the genera identified Bacillus was found to be dominant followed by Pseudomonas. Study indicated the importance of these organisms as bioinoculents for saline soils and can be explored for biofertilizers. 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.

Key words: PGP bacteria, Saline soils, Rhizosphere, Wheat rhizosphere., Biofertilizers

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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 [3].These bacteria can either directly or indirectly enhance the growth of the plant and increase crop yield, [4]. These bacteria enhance growth of the plant by phosphate solublization, Nitrogen fixation, phytohormones and exopolymer production [5,6,7].

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[8,9]. 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[10,11].

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, [12]. Rhizospheric bacterial communities however have efficient systems for uptake and catabolism of organic compounds present in root exudates [13].

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, Wheat rhizosphere is 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 wheat 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*s pp., 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 [1] and Micro IS software [2].

Screening of plant growth promoting bacteria:
a.Phosphate- solublization
Phosphate- solublization was detected qualitatively by spot inoculation of isolates on Pikovskaya medium [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 [15,16], 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 [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,[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 wheat 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	*Bacillus subtilis*	21	*Pseudomonas pinophilum*	41	*Aeromonas species*
2	*Bacillus brevis*	22	*Pseudomonas putida*	42	*Citrobacter species*
3	*Bacillus cereus*	23	*Pseudomonas stutzeri*	43	*Klebsiella species*
4	*Bacillus circulans*	24	*Serratia phosphaticum*	44	*Pseudomonas alcaligens*
5	*Rhizobium species*	25	*Azotobactor chroococcum*	45	*Pseudomonas pseudoalcaligens*
6	*Azospirillum lipoferum*	26	*Serratia marcescens*	46	*Bacillus pumilis*
7	*Azotobactor chroococcum*	27	*Pseudomonas fluorescens*	47	*Bacillus pulvifaciens*
8	*Methylobacterium species*	28	*Escherichia freundii*	48	*Azoarcus communis*
9	*Pseudomonas fluorescens*	29	*Bacillus mesentricuc*	49	*Flavobacterium species*
10	*Pseudomonas pseudomallei*	30	*Bacillus mycoides*	50	*Azospirillum caulinodans*
11	*Alcaligenes species*	31	*Bacillus pumilis*	51	*Paenibacillus polymyxa*
12	*Arthrobacter species*	32	*Azomonas species*	52	*Alcaligenes xylosoxidans*
13	*Azotobactor venelandii*	33	*Corynebacterium species*	53	*Pseudomonas striata*
14	*Azospirillum brasilens*	34	*Rhodospirillum species*	54	*Azotobactor chroococcum*
15	*Azospirillum halopraeferens*	35	*Rhodopseudomonas species*	55	*Azotobactor chroococcum*
16	*Bacillus circulans*	36	*Azotobacter beijerinkii*	56	*Pseudomonas fluorescens*
17	*Bacillus megaterium*	37	*Azotobacter nigricans*	57	*Pseudomonas fluorescens*
18	*Bacillus firmus*	38	*Azotobacter paspali*	58	*Sarcina species*
19	*Bacillus licheniformis*	39	*Acetobacter species*	59	*Micrococcus luteus*
20	*Pseudomonas cissicola*	40	*Pseudoxanthomonas species*		

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, [21]. Most of the researcher studied the maize PGPR and their role in plant growth promotion[22-26]. It was found that *Azotobacter chroococcum* and phosphate solublizer *Bacillus megaterium* as most dominant Nitrogen fixer and phosphate solublizer. I report *Pseudomonas fluorescens* as most dominant phosphate solublizer and *Azotobacter chroococcum* as dominant Nitrogen fixer.

It was also found the presence of genera Bacillus, Pseudomonas, *Azospirillum, Azotobacter, Herbaspirillum, Ideonella* in maize rhizosphere[27], however, my results indicated presence of above all except *Herbaspirillum* and *Ideonella*.

It was 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[28,29], However, *Rahenella aquatilis, Paenibacillus azofixans* were found to be absent in wheat rhizosphere of saline soils.

Table 2 indicates the isolates producing Indole acetic acid (IAA), Phosphate solublization,Nitrogen fixation, and Siderophore production.

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-21	6.2	-	-	+	N-41	5.3	-	-	-
N-2	-	+	-	-	N-22	-	-	-	+	N-42	7.2	+	-	+
N-3	-	+	-	-	N-23	20.4	-	-	+	N-43	-	-	-	-
N-4	-	+	-	-	N-24	-	-	-	-	N-44	6.4	-	-	-
N-5	-	+	-	-	N-25	-	-	+	-	N-45	-	+	-	-
N-6	12.3	-	-	-	N-26	-	-	-	-	N-46	8.3	-	-	-
N-7	24.5	+	-	-	N-27	-	-	-	+	N-47	5.4	+	-	+
N-8	-	-	-	-	N-28	-	-	-	-	N-48	-	-	-	-
N-9	6.3	-	-	+	N-29	-	+	-	-	N-49	-	-	-	-
N-10	28.2	+	-	-	N-30	-	+	-	-	N-50	-	+	-	-
N-11	-	-	+	-	N-31	-	+	-	-	N-51	-	+	-	-
N-12	-	-	+	-	N-32	-	+	-	-	N-52	-	-	+	-
N-13	17.9	-	+	-	N-33	9.4	-	-	-	N-53	-	-	+	-
N-14	-	-	+	-	N-34	-	-	+	-	N-54	-	-	-	-
N-15	31.2	-	-	-	N-35	12.3	-	-	-	N-55	-	-	+	-
N-16	4.7	-	+	-	N-36	24.4	-	-	-	N-56	6.8	+	-	
N-17	-	+	+	-	N-37	6.2	-	-	-	N-57	-	-	-	+
N-18	-	+	+	-	N-38	28.2	-	+	-	N-58	-	-	-	-
N-19	-	+	+	-	N-39	14.9	-	+	-	N-59	-	-	-	-
N-20	-	+	+	-	N-40	28.4	-	+	-					

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

Of all the 59 isolates 22 produced Indole acetic acid (IAA), 21 solublized phosphates, 17 fixed Nitrogen, 8 produced siderophores,

The overall results showed that only 10 isolates did not showed any of the four PGPR traits. Isolate N9,N21,N23 shared two PGPR traits i.e. produced IAA and siderophores. Isolate N42 and N47shared three PGPR traits i.e. produced IAA, solublized phosphates, and produced siderophores. The amount of IAA produced by some isolates was higher 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 two isolates N42 and N47 shared three PGPR traits, isolate N13,N16,N38,N39,N40 shared two PGPR traits and isolate N7,N18,N19,N20 solublized phosphates and fixed atmospheric Nitrogen 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. It was reported the 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[31].

The rhizosphere considered to be a hot spot of bacterial diversity, harbours 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 Wheat 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 Wheat rhizospheric microbiology of saline soil and potential of these microorganisms for commercially important bioinoculents for saline soils.
- As the isolate number N-42 and N47 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 Wheat rhizosphere of saline soils.

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