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Characterization of *Azospirillium* and phospho-solubilizing bacterial isolate from salt-affected soil and their effect on rice (*Oryza sativa*) crop

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ABSTRACT : The study was designed to isolate and characterize nutrient mobilizing soil microbes from salt affected soil of north Bihar. Out of 43 total 17 isolates of *Azospirillium* (12) and PSB (5) were selected in which, all the isolates produced one or the other different characteristics involved in plant growth promotion. They produced phytohormones like indole acetic acid, phospho-solubilization, siderophore and N_2 fixation. The observations were made with 21 treatments. The experiments on rice were carried in Randomized Block Design with three replications. In the present investigation an attempt has been made to ascertain the effect of PGPR in different plant parameter such as tillers, effective tillers, plant height, panicle length, grain yield, straw yield and test weight.

KEY WORDS : *Azospirillium*, Phospho-solubilizing, Bacterial isolate, Rice

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INTRODUCTION

The use of micro-organisms with the aim of improving nutrients availability for plants is an important practice and necessary for agriculture (Freitas *et al.*, 2007). During the past couple of decades, the use of plant growth promoting rhizobacteria (PGPR) for sustainable agriculture has increased tremendously in various parts of the world. Significant increases in growth and yield of agronomically important crops in response to inoculation with PGPR have been reported by (Kloepper *et al.*, 1980; Chen *et al.*, 1994; Zhang *et al.*, 1996; Chanway, 1998; Pan *et al.*, 1999; Bin *et al.*, 2000; Gupta *et al.*, 2000; Asghar *et al.*, 2002; Vessey, 2003 and Silva *et al.*, 2006). Studies have also shown that the growth-promoting ability of some bacteria may be highly specific to certain plant species, cultivar and genotype (Lucy *et al.*, 2004). PGPR can affect plant growth by direct and indirect mechanisms (Glick, 1995; Gupta *et al.*, 2000). The aim of this study was evaluate the effect of plant growth promoting rhizosphere (PGPR) in salt-affected soil.

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EXPERIMENTAL METHODS

Surface soil samples (0-15m) were collected from the rice growing salt affected area of AEZ-1of Bihar. Fresh soil samples were used for the isolation of *Azospirillium* and phosphate solubilizing bacteria. The pure culture was developed by reculturing of selected isolates and then further bio-chemical characterizations were done. For Nitrogenase activity was estimated by acetylene assay. The production of indole-3-acetic acid (IAA) was tested by colorimetric method. Test of phosphates solubilization capacity was performed as per the method of Goldstein (1986). Test of siderophore production by CAS assay the physico-chemical properties like bulk density, water holding capacity, pH, EC, organic carbon, available N, A P and A K were determined from the processed soil sample. 12 isolate of *Azospirillium* and 5 isolate of PSB shorted on the basis of physico-chemical and biochemical characterization and tested in pot culture with salt affected soil rice cop (user dhan-3) during *Kharif* 2012. The pot experiment conducted with twenty one treatments having absolute control, full dose of N P, ¾ N P, ½ N P. Isolates were uniformly tested with ½ N, ½ P_2O_5 and full K_2O. The pots were water saturation. Total amount of P_2O_5 and K_2O was applied as basal application through tricalcium phosphate (0.54g pot^{-1}) and muriate of potash (0.29g pot^{-1}), respectively. Thirty five days old tree seedling per hill and three hill per pot transplanted.

EXPERIMENTAL RESULTS AND ANALYSIS

The results obtained from the present study have been discussed in detail under following heads :

Nitrogenase activity :

Table 1 shows acetylene reduction assay (nitrogenase activity) of all the isolates. It is evident from the results that the rate of nitrogenase activity differs significantly among all the isolates. Among all the isolates $AzsMUZ_8$ showed highest nitrogenase activity (1.72 µMol C_2H_4 formed/mg protein/h) and activity was detected lowest in $PsbEC_6$ (0.42 µMol C_2H_4 formed/mg protein/h). It is interesting to note that the rate of nitrogenase varies markedly even in isolates obtained from the same location. However, results clearly show that all these isolates

Table 1 : Quantitative estimation of nitrogenase activity of the isolates

Sr. No.	Isolate	Nitrogenase activity (µmol C_2H_4/mg protein/h)	Sr. No.	Isolate	Nitrogenase activity (µmol C_2H_4/mg protein/h)
1.	$AzsSI_4$	1.25 ± 0.19	23.	$AzsEC_{7B}$	0.71 ± 0.05
2.	$AzsSI_8$	0.98 ± 0.13	24.	$AzsEC_8$	0.95 ± 0.14
3.	$AzsSI_{10}$	0.43 ± 0.13	25.	$AzsEC_{10}$	0.69 ± 0.05
4.	$AzsSI_{10B}$	0.86 ± 0.08	26.	$AzsEC_{11}$	1.70 ± 0.09
5.	$AzsVA_1$	1.25 ± 0.18	27.	$AzsEC_{11B}$	1.33 ± 0.05
6.	$AzsVA_{1B}$	0.73 ± 0.13	28.	$AzsWC_{13}$	0.98 ± 0.12
7.	$AzsVA_6$	0.78 ±0.09	29.	$AzsWC_{15}$	0.93 ± 0.11
8.	$AzsVA_{15}$	1.32 ± 2.24	30.	$AzsWC_{15B}$	0.82 ± 0.15
9.	$AzsMUZ_6$	1.41 ± 0.06	31.	$PsbSI_4$	1.14 ± 0.23
10.	$AzsMUZ_7$	1.53 ± 0.14	32.	$PsbSI_{4B}$	0.69 ± 0.05
11.	$AzsMUZ_8$	1.72 ± 0.09	33.	$PsbSI_{10}$	0.51 ± 0.09
12.	$AzsMUZ_{8B}$	1.71 ± 0.09	34.	$PsbVA_{15}$	0.66 ± 0.12
13.	$AzsMUZ_9$	1.38 ± 0.09	35.	$PsbVA_{15B}$	1.25 ± 0.05
14.	$AzsMUZ_{11}$	0.83 ± 0.11	36.	$PsbMUZ_8$	0.69 ± 0.06
15.	$AzsMUZ_{13}$	1.40 ± 0.05	37.	$PsbMUZ_{8B}$	1.14 ± 0.03
16.	$AzsMUZ_{13B}$	1.52 ± 0.13	38.	$PsbMUZ_{13}$	0.87 ± 0,08
17.	$AzsMUZ_{14}$	0.75 ± 0.05	39.	$PsbEC_6$	0.42 ± 0.13
18.	$AzsEC_3$	0.71 ± 0.05	40.	$PsbEC_8$	0.77 ± 0.14
19.	$AzsEC_4$	0.81 ± 0.01	41.	$PsbEC_{11}$	0.71 ± 0.09
20.	$AzsEC_{4B}$	0.71 ± 0.09	42.	$PsbEC_{11B}$	1.16 ± 0.03
21.	$AzsEC_6$	0.94 ± 0.05	43.	$PsbWC_{15}$	1.12 ± 0.13
22.	$AzsEC_7$	0.88 ± 0.08			

Nitrogenase activity was tested in JNFb$^-$semi-solid (0.15%) medium, Cultures were pre-inoculated for 6 h with C_2H_2 for estimation of C_2H_4 formation

Results are based on average of two experiments conducted under identical conditions

Table 2: Test of IAA production, P solubilization and siderophore production in various isolates

Sr. No.	Isolate	IAA production (µg/mg dry weight)	Phosphate solubilization (µg/mg dry weight)	Siderophore production (µg/mg dry weight)
1.	$AzsSI_4$	6.26 ± 0.50	8.23 ± 1.98	14.13 ± 2.15
2.	$AzsSI_8$	4.63 ± 0.60	------------	------------
3.	$AzsSI_{10}$	------------	7.84 ± 1.90	------------
4.	$AzsSI_{10B}$	6.60 ± 0.45	8.48 ± 1.84	------------
5.	$AzsVA_1$	5.70 ± 0.60	8.48 ± 1.84	11.38 ±2.45
6.	$AzsVA_{1B}$	2.30 ± 0.30	9.94 ± 1.48	1.45 ± 2.32
7.	$AzsVA_6$	5.70 ± 0.60	------------	9.66 ± 2.28
8.	$AzsVA_{15}$	6.35 ± 0.38	8.26 ±2.74	15.38 ± 2.92
9.	$AzsMUZ_6$	6.85 ± 0.70	7.84 ± 1.26	18.07 ± 2.89
10.	$AzsMUZ_7$	7.36 ± 0.80	7.24 ± 4.24	19.0 ± 2.38
11.	$AzsMUZ_8$	17.36 ± 0.80	6.26 ± 3.47	23.22 ± 3.45
12.	$AzsMUZ_{8B}$	16.36 ± 0.30	6.10 ± 9.94	22.22 ± 1.38
13.	$AzsMUZ_9$	6.60 ± 0.45	12.47 ± 3.50	17.78 ± 2.44
14.	$AzsMUZ_{11}$	6.35 ± 0.38	------------	------------
15.	$AzsMUZ_{13}$	6.69 ± 0.60	7.84 ± 1.90	17.9 ± 2.86
16.	$AzsMUZ_{13B}$	7.35 ± 0.80	7.48 ± 3.24	18.9 ± 2.38
17.	$AzsMUZ_{14}$	------------	------------	8.92 ± 2.95
18.	$AzsEC_3$	3.11 ± 0.35	------------	------------
19.	$AzsEC_4$	------------	11.52 ± 4.5	------------
20.	$AzsEC_{4B}$	4.99 ± 0.5	------------	11.38 ± 2.45
21.	$AzsEC_6$	------------	16.04 ± 4.2	------------
22.	$AzsEC_7$	5.35 ± 0.42	------------	------------
23.	$AzsEC_{7B}$	1.75 ± 0.25	5.26 ± 3.47	------------
24.	$AzsEC_8$	16.36 ± 0.30	------------	17.9 ± 2.86
25.	$AzsEC_{10}$	3.99 ± 0.60	------------	------------
26.	$AzsEC_{11}$	14.87 ± 0.75	6.12 ± 2.60	20.0 ± 3.57
27.	$AzsEC_{11B}$	6.26 ± 0.80	8.14 ± 2.67	15.38 ± 3.92
28.	$AzsWC_{13}$	2.34 ± 0.55	------------	------------
29.	$AzsWC_{15}$	------------	6.10 ± 4.94	------------
30.	$AzsWC_{15B}$	------------	------------	------------
31.	$PsbSI_4$	7.35 ± 0.80	17.17 ± 4.80	18.07 ± 2.89
32.	$PsbSI_{4B}$	------------	------------	------------
33.	$PsbSI_{10}$	------------	9.47 ± 3.50	15.38 ± 2.92
34.	$PsbVA_{15}$	------------	------------	------------
35.	$PsbVA_{15B}$	5.35 ± 0.42	92.13 ±3.50	11.38 ± 2.45
36.	$PsbMUZ_8$	------------	------------	------------
37.	$PsbMUZ_{8B}$	4.67 ± 0.60	20.13 ± 2.50	9.66 ± 2.28
38.	$PsbMUZ_{13}$	------------	------------	------------
39.	$PsbEC_6$	------------	------------	------------
40.	$PsbEC_8$	------------	------------	------------
41.	$PsbEC_{11}$	------------	------------	------------
42.	$PsbEC_{11B}$	4.99 ± 0.50	40.00 ± 10.25	9.73 ± 2.36
43.	$PsbWC_{15}$	4.50 ± 0.40	16.04 ± 4.20	8.95 ±2.59

For IIA production, cultures were grown in $JNFb^-$ liquid medium containing 100 µg/mL tryptophan for three days and thereafter IIA estimation was made

P-solubilization was tested after 3 days of growth in Nautiyal liquid medium.

Siderophore was estimated after 3 days growth of culture in $JNFb^-$ liquid medium containing 2 µM of $FeCl_3$ in place of Fe-EDTA (normal constituent of this medium).

Results are based on average ± standard deviation of two experiments conducted under identical conditions.

can fix N_2 under reduced O_2 tensions but are aerobic N_2 fixers (Table 1).

Test of plants growth promoting activities :

IAA production, phosphate solubilization and siderophore production are considered to be major plant growth promoting features of any bacterium. Accordingly, tests for these characters were made in all the isolates. Results are described under separate headings.

IAA production :

It is evident from the data of Table 2 that out of 43 isolates, 29 (67.44%) showed positive test for IAA production. IAA production occurred solely in liquid JNFb$^-$ medium supplemented with tryptophan (100 µg/mL), there was no production in medium lacking tryptophan. With a view to optimize the concentration of tryptophan for IAA production, varying concentrations ranging from 50 to 500 µg/mL were used in the medium and it was observed that optimum IAA production was achieved at 100 µg/mL among all the isolates AzsMUZ$_8$ showed highest IAA production (17.36 ± 0.80 µg/mg dry weights) and AzsEC$_{7B}$ showed the lowest level (1.75 ± 0.25 µg/mg dry weight).

Phosphate solubilization :

Out of 43 isolates, 25 (58.13%) produced halo-zones around the growing colony on goldstein solid medium, whereas other 18 (41.86%) either showed growth without any halo-zone formation or failed to grow. Efficiency of phosphate solubilization was further confirmed by measuring the level of solubilized P in the liquid Nautiyal medium (Table 2). It is evident that PsbVA$_{15B}$, an isolate from Vaishali showed highest P solubilization (92.13 ± 3.50 µg/mg dry weight) and AzsEC$_{7B}$, an isolate from East Champaran showed lowest P solubilization (5.26 ± 3.47µg/mg dry weight).

Siderophore production :

Siderophore production was detected by means of CAS agar plate assay where blue colour of medium changed to yellow/orange around the growing colonies. On the basis of

Table 3 : Effect of *Azospirillum* and phosphate solubilizing bacteria on yield and yield attributes of rice

Treatment	Tillers/pot	Effective tillers/pot	Plant height (cm)	Panicle length (cm)	Grain yield (g/pot)	Straw yield (g/pot)	Test weight (g)
T_1(N_0 P_0 K_0) (control)	21.0	11.6	61.3	12.6	8.6	20.3	12.6
T_2 (N P K) (full dose)	24.3	16.6	66.9	15.9	12.6	22.6	13.9
T_3 ($N_{¾}$ $P_{¾}$ K)	23.6	14.6	66.0	15.3	12.3	22.9	13.0
T_4 ($N_{½}$ $P_{½}$ K)	22.3	13.3	65.3	13.3	12.0	21.9	12.6
T_5 ($N_{½}$ $P_{½}$ K + AzsSI$_4$)	24.3	16.6	68.3	16.6	14.6	24.6	15.2
T_6 ($N_{½}$ $P_{½}$ K + AzsVA$_1$)	23.0	13.6	68.0	16.3	13.0	22.0	15.0
T_7 ($N_{½}$ $P_{½}$ K + AzsVA$_{15}$)	24.6	17.6	69.0	16.6	13.6	25.8	15.4
T_8 ($N_{½}$ $P_{½}$ K + AzsMUZ$_6$)	28.6	21.0	70.3	17.0	17.1	28.6	15.6
T_9 ($N_{½}$ $P_{½}$ K + AzsMUZ$_7$)	30.6	22.6	71.6	17.3	19.0	29.5	15.7
T_{10} ($N_{½}$ $P_{½}$ K + AzsMUZ$_8$)	26.0	23.3	72.0	17.6	20.7	29.2	16.0
T_{11} ($N_{½}$ $P_{½}$ K + AzsMUZ$_{8B}$)	30.3	23.3	71.6	17.3	18.8	30.2	15.9
T_{12} ($N_{½}$ $P_{½}$ K + AzsMUZ$_9$)	24.6	17.6	69.0	16.6	13.6	25.8	15.4
T_{13} ($N_{½}$ $P_{½}$ K + AzsMUZ$_{13}$)	25.6	18.0	69.3	17.0	15.0	27.2	15.6
T_{14} ($N_{½}$ $P_{½}$ K + AzsMUZ$_{13B}$)	29.6	22.6	71.0	17.3	17.8	30.2	15.9
T_{15} ($N_{½}$ $P_{½}$ K + AzsEC$_{11}$)	32.0	24.6	71.3	17.6	19.8	30.8	16.2
T_{16} ($N_{½}$ $P_{½}$ K + AzsEC$_{11B}$)	25.6	17.3	68.6	16.3	13.0	25.2	15.4
T_{17} ($N_{½}$ $P_{½}$ K + PsbSI$_4$)	23.3	14.3	68.3	16.0	13.0	25.6	14.3
T_{18} ($N_{½}$ $P_{½}$ K + PsbVA$_{15B}$)	29.6	21.6	71.6	17.3	18.0	29.5	14.6
T_{19} ($N_{½}$ $P_{½}$ K + PsbMUZ$_{8B}$)	23.6	17.6	69.6	15.6	13.6	25.8	14.4
T_{20} ($N_{½}$ $P_{½}$ K + PsbEC$_{11B}$)	27.3	20.0	70.0	16.6	17.0	27.0	14.6
T_{21} ($N_{½}$ $P_{½}$ K + PsbWC$_{15}$)	22.6	13.6	67.0	13.6	14.6	24.9	15.0
C.D. (P= 0.05)	1.79	1.13	1.47	0.64	1.09	1.77	0.45
C.D. (P=0.01)	2.31	1.46	1.90	0.83	1.41	2.28	0.58
C.V.	6.60	5.93	2.03	3.78	6.75	6.44	2.88

yellow orange halo zone formation, 23 isolates (53.48%) showed positive test for siderophore production. Quantitative estimation showed that the highest siderophore (23.22 ± 3.45 µg/mg dry weight) production occurs in isolate $AzsMUZ_8$ whereas the lowest (8.92 ± 1.65 µg/mg dry weight) level is in $AzsMUZ_{14}$ (Table 2).

Selection of efficient PGPR isolates :

Plant growth promoting tests of all these 43 isolates revealed that 35 isolates showed at least one character or three character *i.e.* IAA production, phosphate solubilization and siderophore production but 8 isolates failed to show any one of these three characters. Accordingly, out of 43 isolates, 17 isolates, on the basis of high value of nitrogen activity were selected for further study (Table 3).

Effect of *Azospirillium* and phosphate solubilizing bacteria on yield and yield attributes of rice :

The data regarding the effect of use of different microbial isolates with recommended fertilizer dose on tillers, effective tillers, plant height, panicle length, grain yield, straw yield and test weight are given in Table 3 and discussed under following heads.

Tillers :

Number of tillers/pot as affected by control, full dose, 3/4th dose and 1/2 dose, of fertilizer with different isolates of *Azospirillium* and PSB increased significantly. The highest increase in number of tillers was recorded with $AzsEC_{11}$ caused 49.49 per cent, whereas PSB isolates $PsbVA_{15B}$ caused 32.73 per cent increase in number of tillers over without isolates.

Effective tillers/pot :

Number of effective tillers/pot as affected by control, full dose, 3/4 dose and 1/2 dose, of fertilizer with different isolates of *Azospirillium* and PSB was found to have increased significantly. The highest increase in number of effective tillers was recorded with $AzsEC_{11}$ caused 84.96 per cent, whereas PSB isolates $PsbMUZ_{15B}$ caused 62.40 per cent increase in number of tillers over without isolates.

Plant height :

Plant height/pot as affected by control, full dose, 3/4 dose and 1/2 dose, of fertilizer with different isolates of *Azospirillium* and PSB was also observed to have increased significantly. The highest increase of plant height (10.26%) was recorded with $AzsMUZ_8$ whereas PSB isolates $PsbVA_{15B}$ caused increase (9.64%) in plant height over without isolates.

Panicle length :

Panicle length/pot affected by full dose, 3/4 dose and 1/2 dose of fertilizer with different isolates of *Azospirillium* and PSB over control was also observed to have increased significantly. The highest panicle length (32.33%) being recorded with $AzsMUZ_8$, whereas PSB isolates $PsbVA_{15B}$ caused 30.07 per cent increase in panicle length over without isolates.

Grain yield :

Grain yield/pot affected by full dose, 3/4 dose and 1/2 dose of fertilizer with different isolates of *Azospirillium* and PSB over control was observed to have increased significantly. The highest grain yield (72.5%) being recorded with $AzsMUZ_8$ whereas PSB isolates $PsbVA_{15B}$ caused 50.0 per cent increase in grain yield over without isolates.

Straw yield :

Straw yield/pot affected by full dose, 3/4 dose, and 1/2 dose of fertilizer with different isolates of *Azospirillium* and PSB over control was studied. The straw yield increases significantly. The highest straw yield (40.63%) being recorded with $AzsEC_{11}$, whereas PSB isolates $PsbVA_{15B}$ caused 34.70 per cent increase in straw yield over without isolates.

Test weight :

Test weight/pot affected by full dose, 3/4 dose and 1/2 dose of fertilizer with different isolates of *Azospirillium* and PSB over control was studied. The test weight increase significantly. The highest test weight (28.57%) being recorded with $AzsEC_{11}$, whereas PSB isolates $PsbVA_{15B} = PbsEC_{11B}$ caused 15.87 per cent increase in test weight over without isolates.

Conclusion :

Integrate management different dose of NPK with or without bacterial strain (*Azospirillium* and PSB) to salt affected soil not only increase the yield of rice also reduce the use of chemical fertilizer also. Conclusively our findings give us the confidence to suggest that the use of *Azospirillium* and PSB may be a boon for the farmers which are destined to grow cereal in salt affected field.

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