

Characterization of *Azospirillum* and Phospho-Solubilizing Bacterial Isolate from Salt-Affected Soil and their Effect on Rice (*Oryza sativa*) Crop

Pawan Kumar Srivastwa, Nishi Kumari, Ruby Chandra, Suman Kalyani, Radhey Shyam, S. K. Singh, Ranvir Kumar, Kanhaiyaji Verma

Received 29 December 2016; Accepted 31 January 2017; Published online 18 February 2017

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 *Azospirillum* (12) and phosphate Solubilizing bacteria (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 (RBD) with three replications. Results revealed that AzsMUZ₈ and PsbVA_{15B} an isolates of *Azospirillum* and phosphate solubilizing bacteria (PSB) significantly influenced the yield and yield attributes of rice over control in salt affected soils.

Keywords *Azospirillum*, Phosphate solubilizing bacteria, Microbial isolate, Rice.

Introduction

The use of microorganisms with the aim of improving nutrients availability for plants is an important practice and necessary for agriculture [1]. 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 earlier [2, 3]. Studies have also shown that the growth-promoting ability of some bacteria may be highly specific to certain plant species, cultivar and genotype. PGPR can affect plant growth by direct and indirect mechanisms [4]. The aim of this study was evaluate the effect of plant growth promoting rhizosphere (PGPR) in salt-affected soil.

Materials and Methods

Surface soil samples (0-15m) were collected from the rice growing salt affected area of AEZ-1 of Bihar. Fresh soil samples were used for the isolation of *Azospirillum* 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

Pawan Kumar Srivastwa^{1*}, Ruby Chandra¹, Kanhaiyaji Verma¹

¹University Department of Botany, JP University, Chapra, Bihar, India

Nishi Kumari², Suman Kalyani³, Radhey Shyam³, S. K. Singh³, Ranvir Kumar³

²JRF, ³Assistant Professor,

Bihar Agricultural University, Sabour, India

e-mail: Pawan.raj61@gmail.com

*Correspondence

Siderophore production by CAS assay, the physico-chemical properties like bulk density, water holding capacity, pH, EC, organic carbon, available N, available P and available K were determined from the processed soil sample. 12 isolate of *Azospirillum* 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 (var User Dhan-3) during *kharif* 2012. The pot experiment conducted with twenty one treatments having absolute control, full dose of NP, $\frac{3}{4}$ NP, $\frac{1}{2}$ NP. Isolates were uniformly tested with $\frac{1}{2}$ N, $\frac{1}{2}$ P₂O₅ and full K₂O. The pots were water saturation. Total amount of P₂O₅ and K₂O 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.

Results and Discussion

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 AzsMUZ₈ showed highest nitrogenase activity (1.72 μ Mol C₂H₄ formed/mg protein/h) and activity was detected lowest in PsbEC₆ (0.42 μ Mol C₂H₄ 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 can fix N₂ under reduced O₂ tensions but are aerobic N₂ fixers.

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

Table 1. Quantitative estimation of nitrogenase activity of the isolates. # Nitrogenase activity was tested in JNFb semi-solid (0.15%) medium. Cultures were pre-inoculated for 6 h with C₂H₂ for estimation of C₂H₄ formation, # Results are based on average of two experiments conducted under identical conditions.

Sl. No.	Isolate	Nitrogenase activity (μ mol C ₂ H ₄ /mg protein/h)
1	AzsSI ₄	1.25 \pm 0.19
2	AzsS ₁₈	0.98 \pm 0.13
3	AzsSI ₁₀	0.43 \pm 0.13
4	AzsSI _{10B}	0.86 \pm 0.08
5	AzsVA ₁	1.25 \pm 0.18
6	AzsVA _{1B}	0.73 \pm 0.13
7	AzsVA ₆	0.78 \pm 0.09
8	AzsVA ₁₅	1.32 \pm 2.24
9	AzsMUZ ₆	1.41 \pm 0.06
10	AzsMUZ ₇	1.53 \pm 0.14
11	AzsMUZ ₈	1.72 \pm 0.09
12	AzsMUZ _{8B}	1.71 \pm 0.09
13	AzsMUZ ₉	1.38 \pm 0.09
14	AzsMUZ ₁₁	0.83 \pm 0.11
15	AzsMUZ ₁₃	1.40 \pm 0.05
16	AzsMUZ _{13B}	1.52 \pm 0.13
17	AzsMUZ ₁₄	0.75 \pm 0.05
18	AzsEC ₃	0.71 \pm 0.05
19	AzsEC ₄	0.81 \pm 0.01
20	AzsEC _{4B}	0.71 \pm 0.09
21	AzsEC ₆	0.94 \pm 0.04
22	AzsEC ₇	0.88 \pm 0.08
23	AzsEC _{7B}	0.71 \pm 0.05
24	AzsEC ₈	0.95 \pm 0.14
25	AzsEC ₁₀	0.69 \pm 0.05
26	AzsEC ₁₁	1.70 \pm 0.09
27	AzsEC _{11B}	1.33 \pm 0.05
28	AzsWC ₁₃	0.98 \pm 0.12
29	AzsWC ₁₅	0.93 \pm 0.11
30	AzsWC _{15B}	0.82 \pm 0.15
31	PsbSI ₄	1.14 \pm 0.23
32	PsbSI _{4B}	0.69 \pm 0.05
33	PsbSI ₁₀	0.51 \pm 0.09
34	PsbVA ₁₅	0.66 \pm 0.12
35	PsbVA _{15B}	1.25 \pm 0.05
36	PsbMUZ ₈	0.69 \pm 0.06
37	PsbMUZ _{8B}	1.14 \pm 0.03
38	PsbMUZ ₁₃	0.87 \pm 0.08
39	PsbEC ₆	0.42 \pm 0.13
40	PsbEC ₈	0.77 \pm 0.14
41	PsbEC ₁₁	0.71 \pm 0.09
42	PsbEC _{11B}	1.16 \pm 0.03
43	PsbWC ₁₅	1.12 \pm 0.13

production. IAA production occurred solely in liquid JNFb- medium supplemented with tryptophan (100 μ g/mL), there was no production in medium lacking

Table 2. Test of IAA production, P solubilization and siderophore production in various isolates. # 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 FeCl₃ in place of Fe-EDTA (normal constituent of this medium), # Results are based on average ± standard deviation of two experiments conducted under identical conditions.

Sl. 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 _{7B}	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	PsbS ₁₄	7.35 ± 0.80	17.17 ± 4.80	18.07 ± 2.89
32	PsbS _{14B}	-	-	-
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

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. AzsMUZ₈ 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).

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

Treatments	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 ^{3/4} P ^{2/4} K)	23.6	14.6	66.0	15.3	12.3	22.9	13.0
T ₄ (N ^{1/2} P ^{1/2} K)	22.3	13.3	65.3	13.3	12.0	21.9	12.6
T ₅ (N ^{1/2} P ^{1/2} K + AzsSI ₄)	24.3	16.6	68.3	16.6	14.6	24.6	15.2
T ₆ (N ^{1/2} P ^{1/2} K + AzsVA ₁)	23.0	13.6	68.0	16.6	13.0	22.0	15.0
T ₇ (N ^{1/2} P ^{1/2} K + AzsVA ₁₅)	24.6	17.6	69.0	16.6	13.6	25.8	15.4
T ₈ (N ^{1/2} P ^{1/2} K + AzsMUZ ₆)	28.6	21.0	70.3	17.0	17.1	28.6	15.6
T ₉ (N ^{1/2} P ^{1/2} K + AzsMUZ ₇)	30.6	22.6	71.6	17.3	19.0	29.5	15.7
T ₁₀ (N ^{1/2} P ^{1/2} K + AzsMUZ ₈)	26.0	23.3	72.0	17.6	20.7	29.2	16.0
T ₁₁ (N ^{1/2} P ^{1/2} K + AzsMUZ _{8B})	30.3	23.3	71.6	17.3	18.8	30.2	15.9
T ₁₂ (N ^{1/2} P ^{1/2} K + AzsMUZ ₉)	24.6	17.6	69.0	16.6	13.6	25.8	15.4
T ₁₃ (N ^{1/2} P ^{1/2} K + AzsMUZ ₁₃)	25.6	18.0	69.3	17.0	15.0	27.2	15.6
T ₁₄ (N ^{1/2} P ^{1/2} K + AzsMUZ _{13B})	29.6	22.6	71.0	17.3	17.8	30.2	15.9
T ₁₅ (N ^{1/2} P ^{1/2} K + AzsEC ₁₁)	32.0	24.6	71.3	17.6	19.8	30.8	16.2
T ₁₆ (N ^{1/2} P ^{1/2} K + AzsEC _{11B})	25.6	17.3	68.6	16.3	13.0	25.2	15.4
T ₁₇ (N ^{1/2} P ^{1/2} K + PsbSI ₄)	23.3	14.3	68.3	16.0	13.0	25.6	14.3
T ₁₈ (N ^{1/2} P ^{1/2} K + PsbVA _{15B})	29.6	21.6	71.6	17.3	18.0	29.5	14.6
T ₁₉ (N ^{1/2} P ^{1/2} K + PsbMUZ _{8B})	23.6	17.6	69.6	15.6	13.6	25.8	14.4
T ₂₀ (N ^{1/2} P ^{1/2} K + PsbEC _{11B})	27.3	20.0	70.0	16.6	17.0	27.0	14.6
T ₂₁ (N ^{1/2} P ^{1/2} K + PsbWC ₁₅)	22.6	13.6	67.0	13.6	14.6	24.9	15.0
CD at (0.05)	1.79	1.13	1.47	0.64	1.09	1.77	0.45
CD at (0.01)	2.31	1.46	1.90	0.83	1.41	2.28	0.58
CV	6.60	5.93	2.03	3.78	6.75	6.44	2.88

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 ± 3.47 µg/mg dry weight).

Siderophore production

Siderophore production was detected by means of CAS agar plate assay where blue color of medium changed to yellow/orange around the growing colonies. On the basis of 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 AzsMUZ₈ whereas the lowest (8.92 ± 1.65 µg/mg dry weight) level is in AzsMUZ₁₄ (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 *Azospirillum* 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

No. of tillers/pot as affected by control, full dose, 3/4th dose and 1/2 dose, of fertilizer with different isolates of *Azospirillum* and PSB increased significantly. The highest increase in number of tillers was recorded with AzsEC₁₁ caused 49.49%, whereas PSB isolates PsvVA_{15B} caused 32.73% increase in number of tillers over without isolates.

Effective tillers/pot

No. of effective tillers/pot as affected by control, full dose, 3/4 dose and 1/2 dose, of fertilizer with different isolates of *Azospirillum* and PSB was found to have increased significantly. The highest increase in number of effective tillers was recorded with AzsEC₁₁ caused 84.96%, whereas PSB isolates PsvMUZ_{15B} caused 62.40% 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 *Azospirillum* and PSB was also observed to have increased significantly. The highest increase of plant height (10.26%) was recorded with AzsMUZ₈ whereas PSB isolates PsvVA_{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 *Azospirillum* and PSB over control was also observed to have increased significantly. The highest panicle length (32.33%) being recorded with AzsMUZ₈ whereas PSB isolates PsvVA_{15B} caused 30.07% 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

Azospirillum and PSB over control was observed to have increased significantly. The highest grain yield (72.5%) being recorded with AzsMUZ₈ whereas PSB isolates PsvVA_{15B} caused 50.0% 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 *Azospirillum* and PSB over control was studied. The straw yield increases significantly. The highest straw yield (40.63%) being recorded with AzsEC₁₁ whereas PSB isolates PsvVA_{15B} caused 34.70% 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 *Azospirillum* and PSB over control was studied. The test weight increase significantly. The highest test weight (28.57%) being recorded with AzsEC₁₁, whereas PSB isolates PsvVA_{15B}=PsvEC_{11B} caused 15.87% increase in test weight over without isolates.

Conclusion

Integrated use of *Azospirillum* and PSB bacteria isolates with or without NPK not only increases the yield of rice but also reduce the use of chemical fertilizer in salt affected soils. Conclusively our findings suggested that the use of *Azospirillum* and PSB may be a boon for the farmers which are destined to grow cereal in salt affected field.

References

1. Freitas ADS, Vieira CL, Santos CERS, Stamford NP, Lyra MCCC (2007) Caracterizaco de rizobios isolados de Jacatup cultivado em solo salino no Estado de Pernambuco, Brasil. *Bragantia* 66 : 497—504.
2. Silva VN, Silva LESF, Marti nez CR, Seldin L, Burity HA, Figueiredo MVB (2007) Estirpes de *Paenibacillus* promovem a nodulaco de soja na simbiose *Bradyrhizobium-caupi*. *Acta Sci Agron* 29 : 331—338.
3. Vessey JK (2003) Plant growth-promoting rhizobacteria as biofertilizers. *Plant Soil* 255 : 571—586.
4. Lucy M, Reed E, Glick BR (2004) Applications of free living plant growth-promoting rhizobacteria. *Biotechnol Adv* 16 : 729—770.