Int.J.Curr.Microbiol.App.Sci (2018) *7*(9): 1-8

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 09 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.709.001

Characterization of PSB Isolates in Rhizosphere Soil of Rice Varieties Grown at OFRC, SKUAST Jammu

Renu Gupta[1*] and J.P. Sharma[2]

[1]Division of Soil Sciences and Agricultural Chemistry, FoA, SKUAST-J, Chattha, Jammu, India
[2]Director Research, SKUAST-J, Chattha, Jammu, India

**Corresponding author*

Keywords

Rice, Rhizosphere, Phosphorus, Phosphate-solubilizing bacteria (PSB), Isolation, Biochemical tests, Characterization, Identification

Article Info

Accepted:
04 August 2018
Available Online:
10 September 2018

A B S T R A C T

Phosphate is the key macronutrient required for the growth of plants. The soluble forms of phosphorus when applied to soil are rendered insoluble by undergoing chemical fixation. The soil collected from organic farm, SKUAST- Jammu was investigated for screening the phosphate solubilizing bacteria. In the present study, soil samples were collected from rice rhizosphere of sixteen varieties grown in OFRC, SKUAST- Jammu. This study revealed the existence of Phosphate Solublizing bacteria in the rhizosphere soils of rice. These bacteria exhibited difference with respect to Gram property, nitrate reductase, oxidase, citrate utilization and catalase activities. Prominent halo zones were found in case of positive PSB isolates on Pikovskaya's agar. Phosphate solubilization effectively took on sixth day. Based on Gram property, bacterial morphology and biochemical tests performed on various isolates, these were subjected to identification and placed in group I and VIII by referring to the separation outlined in *Bergeys Manual of Systematic Bacteriology* and confirms the dominance of genus *Bacillus and Pseudomonas.* The identification at species level will be further undertaken using *16S rRNA technique.*

Introduction

It has been reported that phosphorus is the second most essential nutrient required by plants in adequate quantities for optimum growth. It has a very important role in affecting almost all major metabolic processes, including energy transfer, signal transduction, respiration, macromolecular biosynthesis, and photosynthesis (Anand *et al.*, 2016). It is difficult for plants to absorb the phosphorous as 95–99% of phosphorus present is in insoluble, immobilized, or precipitated forms in the soil. Therefore, plants absorb phosphate only as monobasic ($H_2PO_4^-$) and dibasic (HPO_4^{-2}) ions. However, a large portion of soluble inorganic phosphate applied to soil as chemical fertilizer is rapidly immobilized soon after application and becomes unavailable to plants (Yadav and Dadarwal, 1997). Therefore, the release of insoluble and fixed forms of phosphorus is an important aspect of increasing soil phosphorus availability. Phosphorous is the least mobile and available to plants in most soil conditions in comparison to other macro nutrients (El-

Azouni, 2008). Some soil microorganisms are able to mineralize and solubilize P from the organic and inorganic soil pools (Richardson, 2001). Soil bacteria mainly from genera *Bacillus* and *Pseudomonas* possess the ability to bring insoluble phosphates in the soluble forms by secreting organic acids. These acids lower the pH and bring about the dissolution of bound forms of phosphorous. The interest in PSB has increased due to the prospective use of efficient strains as bio-inoculants (biofertilizer) components in organic agriculture, which is emerging as an alternative to chemical inputs in intensive agriculture (Bashan and Holguin, 1998)·Use of PSB inoculants is one of the strategy to combat the deficiency of phosphorous and meet the demands of expensive fertilizer. The present research was aimed to isolate and characterize PSB from the rhizosphere of rice varieties grown in research farm at Organic farming research centre (OFRC).

Materials and Methods

Soil sampling

Soil samples were collected from rice rhizosphere at tillering stage from sixteen rice varieties grown at OFRC, SKUAST, Chatha is presented in Table 1.

Dilution of soil samples

The sampled rhizosphere soil was mixed thoroughly to make a composite soil sample. 10 gm of soil sample was diluted to 100 ml to make 10^{-1} dilution and further serial dilution were prepared to 10^{-8} dilution under aseptic conditions.

Enumeration of total biomass and PSB population

The total biomass was quantified in sixteen rice varieties grown at OFRC, SKUAST, Chatha and were replicated three times in Petri dishes for population count of total bacterial population using nutrient agar medium and phosphate solubilizers using Pikovskayas medium. The calculation for the total number of bacteria was done by plating soil dilution using plating enrichment and pour plate technique and incubating the plates for 7-10 days. The total count of the microorganisms presented in Table 2 was obtained by multiplying the number of cells per plate by the dilution factor viz. Bacteria/gm soil = Number of bacteria/ Wt. of soil x Dilution

Isolation of PSB

PSB were isolated on Pikovskaya medium (Pikovskaya, 1948) at tillering stage.

Characterization of isolates

Bacteria were studied for colony and cell morphology following microscopic examination and further identification by gram staining (Gram, 1884). The bacterial isolates were characterized using biochemical tests viz. Catalase test, Citrate utilization test, Nitrate reduction test and oxidase test (Table 3).

Quantitative estimation of Phosphate solubilization

It was done by following the method given by Selvi *et al.*, (2011) and is presented in Table 4.

Results and Discussion

The study was proposed after taking into consideration the fact that no such previous work was undertaken on the said rice varieties. The aim of the study was therefore to screen the better rice cultivars grown in research farm at OFRC centre. With respect to total biomass and phosphate solublizing rhizospheric bacteria

Enumeration of total biomass and P-solubilizers in rhizosphere soil

The total biomass and P-solubilizers estimated at 10^{-4} dilution was 31 and 8 numbers respectively. The percentage of P-solubilizers was 20.80 (Table 2).

Colony morphology of P-solubilising bacteria

The PSB isolates from sixteen rice varieties were small, circular in morphology and were white, yellow and, pink in color (Figs. 1a-c).

Characteristics of P-solubilizing bacteria

An appraisal of data presented in Table 3 revealed that P-solubilising bacteria isolated from rhizosphere soils of rice were rod shaped, gram positive and gram negative types (Figs. 2a-b). Biochemical tests (Catalase, Nitrate reduction, Citrate utilization and Oxidase test) performed showed positive results except few of them were negative (Table 3). Catalase production and activity was detected by adding substrate H_2O_2 to bacterial isolates. The enzyme was present in most cytochrome containing aerobic and facultative anaerobic bacteria. In the absence of enzyme, the toxic concentration cannot be degraded when these organisms are cultivated in the presence of oxygen (Srivastava, 2013).

In case of nitrate reduction test, in anaerobic conditions bacteria used NO_3^- as a source of oxygen and reduced to nitrite i.e. nitrate reduction positive as reported by Karpagam and Nagalaxmi, (2014). This suggests they are capable of breaking down nitrate containing fertilizers in case of anaerobic conditions in soil. Most bacteria utilize the available oxygen in the medium for their growth and rapidly produced anaerobic conditions for further reaction suggesting that aerobic condition also do prevail in soil sample tested.

Bacteria used citrate as sole source of carbon and nitrogen and give the blue color to medium denoted citrate utilization positive as reported by Panhwar *et al.*, (2012). This indicates that if the test is positive, the pH raises and there will be no acid in the end product as reported by Park *et al.*, (2005).

The oxidase test is used to determine if a bacterium produces cytochrome c oxidases. The cytochrome system usually is present in aerobic organisms that are capable of using oxygen as the final hydrogen acceptor. Maximum isolates of diazotrophic bacteria require aerobic conditions except few of them can grow even in anaerobic conditions as indicated by oxidase test. Similar results are also reported by Karpagam and Nagalakshmi (2014).

Quantitative estimation of phosphate solubilization

Maximum amount of phosphate solubilization occurred on sixth day of experiment accompanied with its responsible organisms of genera *Bacillus* (2.44 mg/litre) and pseudomonas (2.18 mg/litre). Besides this, fourth day experiment showed the phosphate solubilization efficiency range from 0.34 to 2.40 (mg/50 ml) in *Bacillus* as compared to uninoculated conditions respectively in rhizosphere soil.

Similar effect was observed initially in second day of experiment. The sets of experiment with two days interval denote the comparatively phosphate solubilization effectively takes place in sixth day with bacteria belonging to genera Bacillus as compared to bacteria from genera Pseudomonas (Table 4).

The present study revealed the existence of phosphate solubilizing bacteria in the rhizospheric soil of sixteen varieties of rice.

Table.1 Rice varieties selected for PSB isolation

S. No.	Variety
1	SJBR 95
2	SJBR 97
3	SJBR104
4	SJBR109
5	SJBR120
6	SJBR121
7	SJBR123
8	SJBR129
9	SJBR70
10	Pb. Basmati 03
11	Pusa 1121
12	Pusa 1612
13	Basmati370
14	Basmati 564
15	Basmati 1509
16	Basmati Pak

Table.2 Enumeration of total biomass and P-solubilizers in rhizosphere (composite soil) of rice varieties at OFRC, SKUAST Jammu

Dilution ($x10^{1-8}$)	Total Biomass	Phosphate solubilisers	% of P-solubilizers
1	98	16	
2	75	14	
3	48	11	20.80
4	31	8	
5	22	7	
6	12	4	
7	8	2	
8	4	-	
Grand total	298	62	

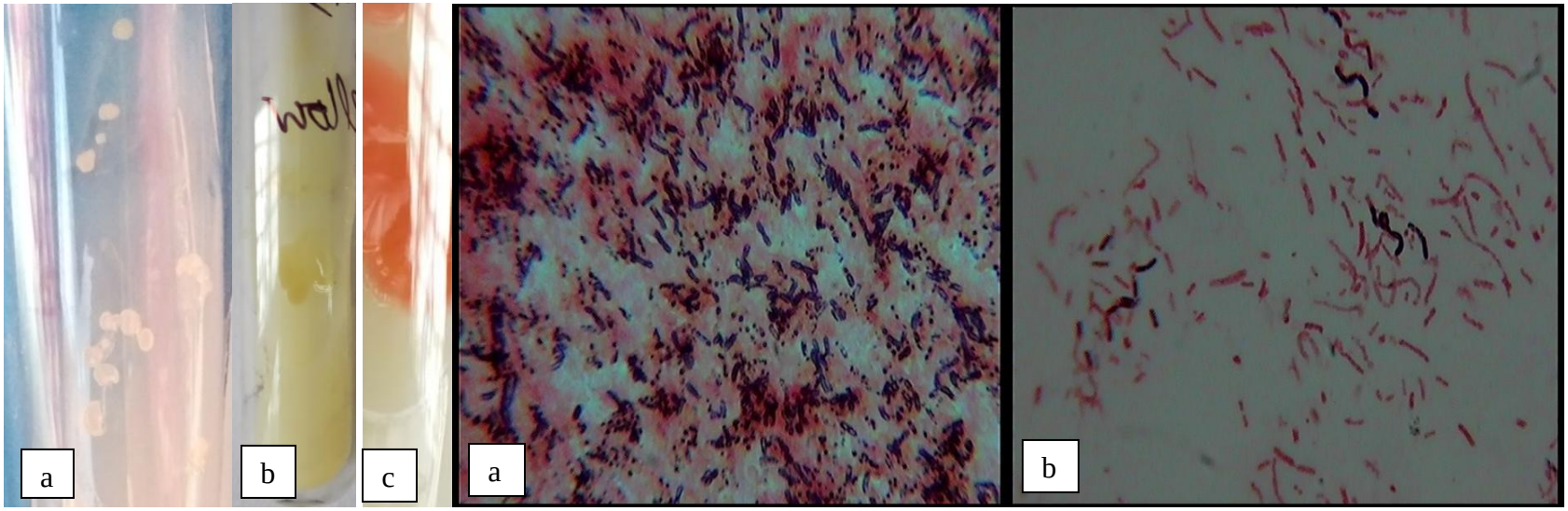
Table.3 Gram staining and characterization of P-solubilising bacteria at OFRC, SKUAST Jammu

S. No.	Sample No.	Gram staining	Colony Colour	Colony size	Bacterial Cell Shape	Isolate code	Catalase test	Citrate test	Nitrate test	Oxidase test
1	R1-1	+	White	Small	Rod shaped	P1	+	+	-	+
2	R1-2	+	White	Small	Rod shaped	P2	+	-	+	+
3	R1-3	-	White	Small	Rod shaped	P3	-	-	+	+
4	R1-4	-	White	Small	Rod shaped	P4	-	+	+	+
5	R1-5	+	White	Small	Rod shaped	P5	-	-	-	+
6	R1-6	+	Pink	Small	Rod Shaped Chain structure	P6	+	-	-	-
7	R1-7	-	White	Small	Rod shaped	P7	+	+	-	-
8	R1-8	+	White	Small	Rod shaped	P8	+	+	+	+
9	R1-9	-	White and Yellow	Medium	Rod shaped	P9	+	+	+	+
10	R1-10	+	Yellow and white	Medium	Rod shaped	P10	+	-	+	+
11	R1-11	\+	Pink	Small	Rod shaped	P11	-	+	-	-
12	R1-12	-	White	Small	Rod shaped	P12	-	-	-	-
13	R1-13	-	Pink,Yellow and white	Medium	Rod shaped	P14	-	-	+	+
14	R1-14	-	White	Small	Rod shaped	P15	+	+	+	+
15	R1-15	+	Purple which changes intopink	Medium	Rod shaped chain structure dispersed	P16	+	+	+	+
16	R1-16	-	White	Small	Rod Shaped	P16	+	+	+	+

Table.4 Quantitative estimation of phosphate solubilization in Pikovskaya's medium

Microorganism	Available phosphate(mg/50ml)		
	2nd day	4th day	6th day
Uninoculated	0.32	0.34	0.34
Genus *Bacillus*	0.70	2.40	2.44
Genus *Pseudomonas*	0.46	2.28	2.18

Fig.1 White (a) and yellow (b) **Fig.2** G +ve (a) and G-ve (b) bacteria isolated from rhizosphere soil of rice varieties and pink (c) colonies of PSB



Similar studies have been carried by many other workers on rice as well as other rhizospheric soil of other plants. The PSBs have been isolated from *Vigna mungo* L. (Qureshi *et al.,* 2012), *Festuca arundinacea* (Monk *et al.,* 2009), legume plants (Khan *et al.,* 2010) rice grown in acidic soil (Thakuria *et al.,* 2004), rice crop of eastern Uttar Pradesh (Shahi *et al.,* 2009).

Present study revealed the existence of Phosphate Solublizing Bacteria in the rhizosphere soils of rice. These bacteria exhibited difference with respect to Gram property, nitrate reductase, oxidase, citrate utilization and catalase activities. Prominent halo zones were found in case of positive PSB isolates on Pikovskaya's agar. Phosphate solubilization effectively took on sixth day with *Bacillus* than *Pseudomonas*. Based on Gram property, bacterial morphology and biochemical tests viz. nitrate reduction, oxidase, citrate utilization and catalase performed on various isolates these were subjected to identification and placed in group I and VIII by referring to the separation outlined in *Bergeys Manual of Systematic Bacteriology* and confirms the dominance of genera *Bacillus* and *Pseudomonas*. The identification at species level will be further undertaken by using 16SrRNA technique.

Acknowledgement

Authors are grateful to Heads, Division of Soil Sciences and Agricultural Chemistry, SKUAST- J, Chatha, and Department of Botany, BGSBU, Rajouri for providing the necessary facilities to undertake this work.

References

Anand, K., Kumari, B. and Mallick, M. A. 2016 Phosphate solubilizing microbes: An effective and alternative approach as biofertilizers. *International Journal of Pharmacy and Pharmaceutical Sciences* 8(2): 37-40.

Bashan, Y. and Holguin, G. 1998. Proposal for the division of plant growth-promoting rhizobacteria into two classifications: Biocontrol-PGPB (Plant Growth-Promoting Bacteria) and PGPB. *Soil Biology and Biochemistry* 30: 1225-1228.

EL-Azouni. 2008. Effect of phosphate solubilizing fungi on growth and nutrient uptake of soybean (*Glycine max* L.) plants. *Journal of Applied Science Research* 4: 592-598.

Gram, H.C. 1884. "Über die isolierte Färbung der Schizomyceten in Schnitt- und Trockenpräparaten". *Fortschritte der Medizin* (in German) 2: 185–189.

Karpagam, T. and Nagalaxmi, P. K. 2014. Isolation of phosphate solubilizing microbes from Agricultural soil. *International Journal of Current Microbiology and Applied Sciences* 3 (3): 601-614.

Khan, M.S., Zaidi, A., Ahmed, M., Oves, M. and Wani, P.A. 2010. Plant growth promotion by phosphate solubilizing fungi- Current Perspective. *Archives of Agronomy and* Soil Science 56: 73-98.

Monk, J., Gerard, E., Young, S., Widdup, K. and O'Callaghan, M. 2009. Isolation and identification of plant growth promoting bacteria associated with tall fescus. *Proceedings of the New Zealand Grassland Association* 71: 211-216.

Panhwar, Q. A., Othman, R., Rahman, Z. A., Meon, S. and Ismail, M. R. 2012. Isolation and characterization of phosphate-solubilizing bacteria from aerobic rice. *African Journal of Biotechnology* 11 (11): 2711-2719.

Park, M., Kim, C., Yang, J., Lee, H., Shin, W., Kim, S., and Sa, T. 2005. Isolation and characterization of diazotrophic growth promoting bacteria from rhizosphere of agricultural crops of

Korea. *Microbiological Research* 160(2), 127-33.

Pikovskaya, R. I. 1948. Mobilization of phosphorus in soil connection with the vital activity of some microbial species. *Microbiologiya* 17: 362–370.

Qureshi, M.A., Iqbal, A., Akhtar, N., Shakir, M.A. and Khan, A. 2012. Co-inoculation of phosphate solubilizing bacteria and rhizobia in presence of L-tryptophan for the promotion of mash bean (*Vigna mungo* L). *Soil and Environment* 31:47-54.

Richardson, A.E. 2001. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Australian Journal of Plant Physiology* 28: 897-906.

Selvi, K.B., John Paul, J.A., Ravindran, A.D. and Vijaya, V. 2011. Quantitative estimation of insoluble inorganic phosphate solubilization. *International Journal of Science and Nature* 2: 292-295.

Shahi S. K., A. K. Rai, M. B. Tyagi, R. P. Sinha and A. Kumar, 2009. Isolation and Characterization of Plant Growth Promoting Rhizobacterea from Rice Crops of Eastern Uttar Pradesh. *Journal of Mycology and Plant Pathology* 39: 556- 557.

Shrivastava, U. P. 2013. Isolation and characterization of diazotrophic plant growth promoting rhizobacteria. *International Journal of Pharmacy & Life Sciences* 4 (3): 2481-2488.

Thakuria, D., Talukdar, N. C., Goswami, C., Hazarika, S., Boro, R. C. and Khan, M. R. 2004. Characterization and screening of bacteria from the rhizosphere of rice grown in acidic soils of Assam. *Current Science* 86: 978- 85.

How to cite this article:

Renu Gupta and Sharma, J.P. 2018. Characterization of PSB Isolates in Rhizosphere Soil of Rice Varieties Grown at OFRC, SKUAST Jammu. *Int.J.Curr.Microbiol.App.Sci.* 7(09): 1-8. doi: https://doi.org/10.20546/ijcmas.2018.709.001