Int.J.Curr.Microbiol.App.Sci (2018) *7*(6): 2749-2756

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 7 Number 06 (2018)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2018.706.323

Isolation and Characterization of Phosphate Solublizing Bacteria from Rhizosphere Soil of Rice in Jammu District, India

Renu Gupta[1]*, Harkirat Singh[1], Bharti Sharma[2] and Susheel Verma[2]

[1]Division of Soil Science and Agricultural Chemistry, Faculty of Agriculture, SKUAST-J, Chatha, Jammu, India
[2]Centre for Biodiversity Studies, School of Biosciences and Biotechnology, BGSB University, Rajouri-185234, J&K, India

**Corresponding author*

Keywords

Phosphorus, Phosphate-Solubilizing Bacteria (PSB), Isolation, Biochemical characterization, identification

Article Info

Accepted:
20 May 2018
Available Online:
10 June 2018

A B S T R A C T

Phosphorous is one of the important macronutrient along with nitrogen which limit the growth, development, and yield of the plant. The soluble forms of phosphorus when applied to soil become insoluble through chemical fixation. The Phosphate solublizing bacteria (PSB) plays a very vital role in making soluble phosphorous available to the plants. A study was undertaken in rice growing area of Jammu district, J&K, to screen the phosphate solubilizing microorganisms from the rhizosphere soil. The soil samples were collected from two rice growing blocks, R. S. Pura and Bishnah using Global Positioning System. The study revealed the presence of three dominant genera. They were identified following Gram staining method and some biochemical tests. Their phosphate solubilizing ability was screened by halozone test on solid agar plate after the incubation at 28 °C. The observations were recorded at 2nd,4th and 6th day interval. The microbes showed the formation of clear zones around their colonies on agar plate. The dominant genera were identified as *Bacillus*, *Pseudomonas* and *Enterobacter* on the basis of biochemical test performed on various isolates these were subjected to identification and placed in group I,VIII and IX by referring to the separation outlined in Bergeys manual of systematic bacteriology Thus, it was observed that PSB from the three genera have the ability to reduce the phosphate unavailability by making it available to plants and can be used as biofertilizers in soil because these microorganisms neutralize the soil and release acid in minute quantity during phosphate solubilization.

Introduction

Phosphorus (P) is an essential element next only to nitrogen that influence plant growth and production throughout the world (Vassileva *et al.*, 1998; Zahran, 1999; Sagervanshi *et al.*, 2012; Sungthongwises *et al.*, 2012). In comparison to macro nutrients, it is the least mobile and available to plants in most soil conditions (El-Azouni 2008). However, some soil microorganisms are able to mineralize and solubilize P from the organic and inorganic soil pools (Richardson, 2001). Phosphorous plays a major role in the process of photosynthesis, respiration energy storage and transfer, cell enlargement and

division and other life processes of a plant. Unlike nitrogen, this element is not acquired through biochemical fixation but comes from other sources including chemical fertilizers, animal manures, and plant residues and, native compounds of phosphorus, both organic and inorganic already present in soil to meet plant requirements (Subba Rao, 1982). Nitrogen fixation depends appreciably on the available forms of phosphorous. 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). Farmers apply phosphorus fertilizers in order to overcome this problem. Therefore, the release of insoluble and fixed forms of phosphorus is an important aspect of increasing soil phosphorus availability. Plant root-associated phosphate solubilizing bacteria (PSB) have been considered as one of the possible alternatives for inorganic phosphate fertilizers for promoting plant growth and yield (Thakuria *et al.,* 2004). In fact, PSB render more phosphates into the soluble form than required for their growth and metabolism by secreting organic acids and enzymes. 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)·

The farmers often fail to apply recommended doses of P fertilizers to the soil either due to non-availability or high cost of phosphatic fertilizers. The phosphate solubilizing bacteria are obiquotous with variation in forms and population in different soils. (Yahya and Al-Azawi, 1998). Use of PSB inoculants is one of the strategy to combat the deficiency of phosphorous and meet the demands of expensive fertilizer. The aim of the present research was to isolate and characterize PSB from the rhizosphere soil of rice and to study the microbial properties in rice growing areas of Jammu district.

Materials and Methods

Soil sampling

Soil samples were collected from rice rhizosphere at Panicle initiation stage from each rice growing village of R.S.Pura and Bishnah blocks of Jammu district by using GPS(MAGELLAN, MobileMapper). A total of twenty rice grown villages were selected and from each village two samples were collected, one from *Basmati-370* and other from *Ratna* variety through random sampling. Location of sites 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.

Isolation of PSBs

PSB were isolated on Pikovskaya medium (Pikovskaya, 1948) at panicle initiation stage in rhizosphere soil of two rice varieties, Basmati-370 and Ratna.

Microscopic examination and identification by gram staining

Bacteria were studied for colony and cell morphology following microscopic examination and further identification by gram staining.(Gram,1884).

Biochemical characterization of isolates

The bacterial isolates at panicle initiation stage in rhizosphere soil of two rice varieties,

Basmati-370 and Ratna were characterized using biochemical tests viz. Catalase test, Citrate utilization test, Nitrate reduction test and oxidase test.

Quantitative estimation of Phosphate solubilization

It was done by following the method given by Selvi *et al.*, 2011.

Results and Discussion

The study area was chosen after taking into consideration the fact that no such previous work was undertaken on the rice varieties being cultivated there. Since this area in Jammu Division of Jammu and Kashmir State is famous for best quality of Basmati Rice, therefore, the present study holds lot of significance.

Colony morphology of P-solubilising bacteria

Colonies of all the PSB isolates were round, small, had flat elevation, smooth surface and white in color (Table. 2).

Characteristics of P-soulbilizing bacteria

An appraisal of data presented in Table. 3 and 4 revealed that P-solubilising bacteria isolated from rhizosphere soils of rice were rod shaped, gram negative and gram positive types. Biochemical tests (Catalase, Nitrate reduction, Citrate utilization and Oxidase test) performed showed positive results except few of them were negative. Majority of P-solubilising bacteria isolated from rhizosphere soil of Basmati-370 were gram negative but the few mixed population isolates were having gram negative as well as gram positive bacteria (Table. 3).

Quantitative estimation of Phosphate solubilization

Maximum amount of phosphate solubilization occurred on sixth day of experiment accompanied with its responsible organisms of genus *Bacillus* (2.52 mg/litre) followed by *Pseudomonas* (2.34 mg/litre) and *Enterobacter* (2.02 mg/litre) in group IX. Besides this, fourth day experiment showed the phosphate solubilization efficiency range from 0.35 to 2.50 (mg/50 ml) in *Bacillus* as compared to uninoculated conditions respectively in rhizosphere soil. Similar effect was observed initially, 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 genus Bacillus as compared to bacteria from genus Pseudomonas. (Table. 5).

Table.5 Quantitative estimation of Phosphate solubilization in Pikovskaya;s medium

Microorganism	Available phosphate(mg/50ml)		
	2nd day	4th day	6th day
Uninoculated	0.36	0.35	0.35
Genus *Bacillus*	0.75	2.50	2.52
Genus *Pseudomonas*	0.50	2.35	2.34
Genus *Enterobacter*	0.42	1.86	2.02

Table.1 Collection sites of rhizosphere soil samples

S. No.	Village	Block	GPS Location
1.	Tinda	RS Pura	32°37'40.662"N 74°47'23.310"E
2.	Rattian	RS Pura	32°37'11.850"N 74°47'09.342"E
3.	Kir Pind	RS Pura	32°37'11.568"N 74°47'09.048"E
4.	Mahlawal	RS Pura	32°37'01.434"N 74°46'50.622"E
5.	Tanda	RS Pura	32°36'46.944"N 74°46'27.630"E
6.	Kotli Shah	RS Pura	32°36'56.796"N 74°46'06.198"E
7.	Mahlawal	RS Pura	32°37'03.300"N 74°45'52.020"E
8.	Tikrian	RS Pura	32°37'19.884"N 74°44'43.182"E
9.	Banota	RS Pura	32°37'19.734"N 74°44'41.988"E
10.	Langarwal	RS Pura	32°37'16.992"N 74°42'27.378"E
11.	Kulle	Bishnah	32°37'13.914"N 74°50'40.410"E
12.	Dhinda	Bishnah	32°37'06.498"N 74°50'48.810"E
13.	Bishnah Tehsil	Bishnah	32°36'58.716"N 74°50'57.354"E
14.	Ban Chak	Bishnah	32°36'24.256"N 74°52'09.522"E
15.	Pante Di Chubbian	Bishnah	32°36'05.634"N 74°53'13.776"E
16.	Sorer Tokor	Bishnah	32°35'47.604"N 74°53'34.026"E
17.	Chubbian	Bishnah	32°35'44.736"N 74°52'59.346"E
18.	Chorli	Bishnah	32°35'56.058"N 74°52'27.822"E
19.	Dabbar	Bishnah	32°35'39.756"N 74°51'46.266"E
20.	Daali	Bishnah	32°35'52.932"N 74°50'07.632"E

Table.2 Colony Morphology of P-solubilising bacteria in rhizosphere soils in different areas of Jammu District

Bacteria	Size	Shape	Elevation	Surface	Consistency	Pigmentation
PSB's	Small	Round	Flat	Smooth	Viscous	White

Table.3 Isolation and characterization of P-solubilising bacteria from rhizosphere soils of Basmati-370 rice in different areas of Jammu District

Sr. No.	Sample	Gram staining	Cell morphology	Catalase test	Nitrate reduction test	Citrate utilization test	Oxidase test
1	R1	_	Rod shaped	_	+	+	+
2	R2	_ & +	Rod shaped	+	+	_	+
3	R3	_ & +	Rod shaped	+	+	+	+
4	R4	_	Rod shaped	_	+	+	+
5	R5	_	Rod shaped	+	+	_	+
6	R6	_ & +	Rod shaped	+	+	+	+
7	R7	_	Rod shaped	+	+	+	_
8	R8	_	Rod shaped	+	+	+	+
9	R9	_	Rod shaped	+	+	+	+
10	R10	_	Rod shaped	+	_	+	+
11	B1	_	Rod shaped	_	+	+	+
12	B2	_	Rod shaped	+	+	_	_
13	B3	_ & +	Rod shaped	_	+	+	_
14	B4	_	Rod shaped	+	+	+	_
15	B5	_	Rod shaped	+	+	+	+
16	B6	_	Rod shaped	+	+	+	+
17	B7	_	Rod shaped	+	+	_	_
18	B8	_ & +	Rod shaped	+	+	+	+
19	B9	_ & +	Rod shaped	_	+	+	+
20	B10	_ & +	Rod shaped	+	+	+	+

Table.4 Isolation and characterization of P-solubilising bacteria from rhizosphere soils of Ratna rice in different areas of Jammu District

Sr. No.	Sample	Gram staining	Cell morphology	Catalase test	Nitrate reduction test	Citrate utilization test	Oxidase test
1	**R1**	_ & +	Rod shaped	+	+	+	+
2	**R2**	_ & +	Rod shaped	+	+	+	+
3	**R3**	_ & +	Rod shaped	_	+	+	+
4	**R4**	_ & +	Rod shaped	+	+	+	+
5	**R5**	_ & +	Rod shaped	+	+	_	+
6	**R6**	_ & +	Rod shaped	+	+	+	+
7	**R7**	_ & +	Rod shaped	+	_	_	+
8	**R8**	_ & +	Rod shaped	+	+	+	+
9	**R9**	_ & +	Rod shaped	+	+	+	_
10	**R10**	_ & +	Rod shaped	+	+	+	+
11	**B1**	_ & +	Rod shaped	+	+	+	+
12	**B2**	_ & +	Rod shaped	_	+	+	_
13	**B3**	_ & +	Rod shaped	+	+	+	+
14	**B4**	_ & +	Rod shaped	+	+	+	_
15	**B5**	_ & +	Rod shaped	+	+	_	+
16	**B6**	_ & +	Rod shaped	+	+	+	+
17	**B7**	_ & +	Rod shaped	+	+	+	+
18	**B8**	_ & +	Rod shaped	+	+	_	+
19	**B9**	_ & +	Rod shaped	_	+	+	+
20	**B10**	_ & +	Rod shaped	+	+	+	+

The present study revealed the existence of phosphate solubilizing bacteria in the rhizospheric soil of both varieties of rice, Basmati-370 and Ratna being cultivated in R. S. Pura and Bishnah Blocks of Jammu Division of J&K state. Similar studies have been carried by many other workers from rice as well as other rhizospheric soil of other plants. The PSBs have been isolated from Tea plant from Darjeleeng Hiis (Sharma *et al.*, 2012). They 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).

In conclusion, present study revealed the existence of Phosphate Solublizing Bacteria in the rhizosphere soils of both Basmati -370 and ratna varieties of rice cultivated in R. S. Pura and Bishna blocks of Jammu division of J&K. 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. The Isolates exhibited different phosphate solubilization activity as reflected by type of transparent halo zones. 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,VIII and IX by referring to the separation outlined in *Bergeys Manual of Systematic Bacteriology* and confirms the dominance of genus *Bacillus*, *Pseudomonodas*, and *Enterobacter*. The identification at species level will be further undertaken by using appropriate molecular marker.

Acknowledgements

Authors are thankful to the Head, Division of Soil Sciences and Agricultural Chemistry, SKUAST-J, Jammu and Director, Centre for Biodiversity Studies, BGSBU, Rajouri for providing necessary facilities to carry out this piece of research.

References

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.

Beck, M. A. and P. A. Sanchez. 1994. Soil phosphorus fraction dynamics during 18 years of cultivation on a Typic Paleudult. *Soil Science*. 34: 1424 – 14.

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.

Karunai Selvi, 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.

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.

Kieliszewska-Rokicka B. 2001. Enzymy glebowe i ich znaczenie w badaniach aktywności mikrobiologicznej gleby. In: Drobnoustroje środowiska glebowego; ed.: H. Dahm, A. Pokojska-Burdziej, Toruń, pp. 37-47.

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.

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.

Sagervanshi, A., Kumari, P. and Nagee, A. 2012Isolation and characterization of phosphate solublizing bacteria from anand agriculture soil. *International Journal of Life Science & Pharma Resarch* 2: L-256-L-266.

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.

Sharma B C, R Shubha, A Shaha,2012. Kurthia sps, a noval member of P-Solubilizing bacteria from rhizospheric tea soil of Darjeeling Hills, India. *IOSR Journal of Pharmacy and Biological Science* 3: 36-37.

Subba Rao, N. S. 1982. Advances in Agricultural Microbiology,**.** New Delhi: Oxford and IBH

Sungthongwises, K., Boonthaiiwai, C., Matsouka, M., Ohnishi, K. and Tanaka, S. 2012. Isolation of Phosphate Solubilizing Bactera from different filds crop productions. *International Journal of Environmental and Rural Development* 3: 150-154.

Tarafdar, J.C. and Claassen, N. 1988. Organic phosphorrus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. *Biology Fertility Soils*. 5: 308-312.

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.

Vassileva, M., Vassilev, N. and Azcon, R. 1998. Rock phosphate solubilization by *Aspergillus niger* on olivecake-based medium and its further application in soil-plant system. *World Journal of Microbiology and Biotechnology*. 14: 281-284.

Yadav K.S., Dadarwal K.R. 1997. Phosphate solubilization and mobilization through soil microorganism. In: Dadarwal K.R., editor. Biotechnological Approaches in Soil Microorganisms for Sustainable Crop Production. Jodhpur, India: Scientific Publishers, pp. 293–308.

Yahya, I. and Al-Azawi, S. K. 1989. Occurence of phosphate-solubilizing bacteria in some Iraqi soils. *Plant and Soil* 117:135-141.

Zahran, H. H. 1999. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Molecular Biology Review*. 63: 968-989.

How to cite this article:

Renu Gupta, Harkirat Singh, Bharti Sharma and Susheel Verma. 2018. Isolation and Characterization of Phosphate Solublizing Bacteria from Rhizosphere Soil of Rice in Jammu District, India. *Int.J.Curr.Microbiol.App.Sci.* 7(06): 2749-2756.
doi: https://doi.org/10.20546/ijcmas.2018.706.323