ISSN: 0976-3031

Available Online at http://www.recentscientific.com

CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research
Vol. 8, Issue, 12, pp. 22209-22212, December, 2017

International Journal of Recent Scientific Research

DOI: 10.24327/IJRSR

Research Article

ISOLATION AND OPTIMIZATION STUDIES OF PHOSPHATE SOLUBILIZING RHIZOBIUM LEGUMINOSARUM

Gelli Veena Shravanthi[1] and Prabakaran Panchatcharam[1,2]

[1]Department of Microbiology, Marudhupandiyar College, Vallam, Thanjavur
[2]Department of Botany M.R .Government Arts College, Mannargudi, Tamilnadu

DOI: http://dx.doi.org/10.24327/ijrsr.2017.0812.1222

ARTICLE INFO

Article History:

Received 15th September, 2017
Received in revised form 25th October, 2017
Accepted 23rd November, 2017
Published online 28th December, 2017

Key Words:

Rhizobium, Phosphate solubilizer, Root nodule, Biofertilizer

ABSTRACT

The work was an effort to study the impact of Phosphate solubilizing bacteria in biofertilizer production, which in turn enhances the nutrient quality of soil. The study presents the detailed account of isolation of phosphate solubilizing bacteria from the root nodule of *Vicia faba* from different locations of Thanjavur district, Tamilnadu state. Phosphorous plays an important role in plant metabolism as most of the soils are deficient in phosphorous. Phosphate solubilizing bacteria utilized as biofertilizer, which actually enhance the nutrient quality of the soil. Thirteen *Rhizobium* colonies separated and analyzed based on confirmation tests .biochemical studies. In some strains showed excellent zone of diameter. The effect of different concentration of glucose as a carbon sources on the *Rhizobium* strains with phosphate solubilization efficiency were calculated.

INTRODUCTION

Agriculture and soil management activities such as fertilization, tillage and biomass alteration represent great challenge towards food and environment worldwide (McLauchlan 2006, Spiertz 2010, Gordon *et al.,* 2010). The extensive use of chemical fertilizers in agro systems for enhancing fertility and agronomic yield induce several issues including soils depletion and pollution (Gupta *et al.,*2014, Bohlool *et al.,*1992, Peoples and Craswell, 1992, Velthof *et al.,* 2009). Indeed, these chemical fertilizers are expensive and are known to be immobilized soon after their application in soils and become unavailable to plants nutrition (Dey,1988).

The phosphorus based chemical fertilizers derived from phosphate rocks, have several impacts especially on landscape transformation, and water alteration with radioactive compounds and heavy metals. Besides, phosphorus is one of the major macronutrients for biological development and growth along the plant cycle (Spiertz, 2010 and Enrilich, 1982). However, it represents a limiting factor to plant nutrition due to its low soluble forms in soil (varying from 0.001 mg./l^{-1} mg./l^{-1} in deficient soils to 1 mg./l^{-1} in heavily fertilized soils) (Hani, 2012 and Shekhar, 1999). Furthermore, phosphorus is involved in different cellular process including photosynthesis, respiration energy storage and transfer, cell division and early stages of seed formation. Already investigated Phosphate Solubilizing Rhizobia (PSR) as an biofertilizers, in order to reduce the cost of chemical fertilizers and to decrease soil degradation and pollution (Peix *et al.,* 2001, Tagore *et al.,* 2013, Pereira and Castro, 2014). The mechanism behind phosphate solubilization explained by the ability of some soil bacteria to produce organic acids and chelate oxoacids from carbonic compounds (Dadarwal *et al.,* 1989, Leyval and Barthelin, 1989).

Phosphorus is one of the major plant nutrients limiting plant growth hormones. Most of the essential plan nutrients, including phosphorus, remain in insoluble form in soil (Abd-Alla, 1994 and Jones and Darrah, 1994). A large portion of inorganic phosphates applied to soil as fertilizer rapidly immobilized after application and becomes unavailable to plants (Murphy and Riley, 1962 and Yadav and Dadarwal, 1997). The release of insoluble and the forms of phosphorus is an important aspect of increasing soil phosphorus availability. Seed or soil inoculation with phosphate-solubilizing bacteria known to improve solubilization of soil phosphorus .The applied phosphates resulting in higher crop yield. Phosphate

Corresponding author:* **Gelli Veena Shravanthi
Department of Microbiology, Marudhupandiyar College, Vallam, Thanjavur

solubilizing microorganisms routinely screened by a plate assay method using Pikovskaya (PVK) agar (Pikovskaya, 1948). The test of the relative efficiency of isolated strains is carried out by selecting the microorganisms which capable of producing a halo/clear zone on plate due to the production of organic acids into the surrounding medium (Katznelson *et al.,* 1962 and Hajjam *et al.,* 2016). However, the reliability of this halo-based technique is questioned as many isolates which did not produce any visible halo/zone on agar plates could solubilize various types of insoluble inorganic phosphates in liquid medium (Louw and Webley, 1959 and Gupta *et al.,* 1994).

MATERIALS AND METHODS

Root nodules collection and preservation (Beck et al., 1993)

Root nodules of *Vicia faba* was collected from different locations of Thanjavur district, Tamilnadu state. The sampling made by digging nearly 15 cm to either side of the faba bean (*Vicia faba* L.) plant to 25 cm depth. Then, root nodules of plants transferred to the laboratory for further nodules preservation. From each plant, a number of thirty nodules were collected and preserved in silica gel tubes at room temperature, in order to keep them dry and to inhibit any growth of fungi or other bacteria.

Isolation, purification and preservation of rhizobial isolates (Vincent 1970)

Fresh nodules were carefully surface sterilized by ethanol 70% for 60 seconds, then transferred and soak in 3% of calcium hypochlorite or chlorox ($CaCl_2$) solution for 5 to 6 minutes. Nodules washed immediately by distillated water, five to seven times. Each nodule covered by a drop of distillated water for further crush and isolation. The nodule suspension was streaked out on YEM medium (Yeast extract mannitol) supplemented with Congo red. As a confirmatory test of rhizobia, Gram staining was performed on the isolates and the observation was made by a microscope at 100x magnification using oil immersion. The selected pure cultures recognized by their white and creamy appearance, which is the morphological characteristic of rhizobia, comparing to the other cultures that can absorb Congo red. Finally, pure cultures were preserved in glycerol 50% (v/v) and kept in the freezer at (-80°C) for further analysis. Screening of Rhizobial strains was performed.

Bromothymol blue test (BTB) (Beck et al., 1993)

Five ml of BTB (0,016N) added to YEM medium before autoclaving. This indicator turns yellow at pH=6.0 and blue at pH=7.6 and is green between pH 6.0 and 7.6. The selection of isolates in terms of fast/ slow growth and production of acids/alkali based on the appearance of agar plates (yellow color; fast growing rhizobia with production of acids; blue color: slow growing rhizobia with production of alkali).

Effect of pH and temperature

The resistance of phosphate solubilizing rhizobia (PSR) to ranges 5 to 9 pH, temperature (between 20 and 50°C) was examined on agar plates by blotting technique. The reading of agar plates was made after 48 hours of incubation at 28 ±2°C.

Phosphate solubilization ability on agar plates assay (Edi-Premono et al., 1996)

Phosphate solubilizing PSR ability was assessed on Sperber's basal medium (glucose: 10g; yeast extract: 0.5 g; $MgSO_4.7H_2O$: 0.25g; $CaCl_2$:0.1g; agar: 15g) supplemented with 2.5g of tricalcium phosphate $Ca_3 (PO_4)_2$ in 1000 ml of distillated water; pH=7.2. The ability of solubilization was visualized by the appearance of a clear zone halo on Sperber's basal plates. The index and the efficiency of solubilization were calculated based on the colony diameter and halo zone diameter of each isolate.

Estimation of phosphate solubilizing ability by PSR in broth culture (Jackson, 1958)

Phosphate solubilizing ability of selected PSR was estimated in Sperber's basal medium broth culture.

Table 1 Isolation of Rhizobium sp from *Vicia faba* root nodules

S. No	Dilution	No. of colonies (CFU/ml)
1	10^{-3}	42
2	10^{-4}	34
3	10^{-5}	24
4	10^{-6}	09
Total colonies		109

Table 2 Identification of *Rhizobium* isolated from *Vicia faba* root nodules

S. No	Strain no	G	M	I	MR	VP	C	Gl	S	Ma	L
1	PSRV1	-	+	-	-	-	-	+	-	+	+
2	PSRV2	-	+	-	-	-	-	+	-	+	+
3	PSRV3	-	+	-	-	-	-	+	-	+	+
4	PSRV4	-	+	-	-	-	-	+	-	+	+
5	PSRV5	-	+	+	-	-	-	+	-	+	+
6	PSRV6	-	+	+	-	-	-	+	-	+	+
7	PSRV7	-	+	+	+	+	+	-	-	+	-
8	PSRV8	-	+	-	+	+	-	-	+	-	+
9	PSRV9	+	+	+	-	+	+	+	-	-	+
10	PSRV10	+	-	+	+	-	+	-	+	-	+
11	PSRV11	+	-	+	+	-	+	-	+	-	+
12	PSRV12	+	+	+	-	-	-	+	-	+	-
13	PSRV13	-	+	-	+	-	+	-	-	+	-

G- Gram stain, M- Motility, I-Indole, MR- Methyl red, VP- Voges proskeaur, C-Citrate, Gl- Glucose, S-Sucrose, Ma- Maltose, L-Lactose, + Present, - Absent

Table 3 Identification and biochemical characterization of *Rhizobium leguminosarum*

Strain no	BTB	GPA	HA	A
PSRV1	-	-	-	+
PSRV2	+	-	-	+
PSRV3	-	-	-	+
PSRV4	+	-	-	+
PSRV5	+	-	-	+
PSRV6	+	-	-	+
PSRV7	+	+	+	+
PSRV8	+	-	+	_
PSRV9	+	+	-	-
PSRV10	-	+	-	+
PSRV11	+	-	+	+
PSRV12	+	+	-	+
PSRV13	-	+	+	-

BTB-Bromothymol blue, GPA- Glucose peptone agar, HA-Hoffer's Alkaline agar, A- Agar

Each 1ml (1.108 CFU/ml) of selected PSR, according to their significant symbiotic effectiveness of inoculated plants, were transferred into 250 ml Erlenmeyer flask filled with liquid

Sperber's basal medium and incubated on a rotator shaker during 8 days at 28±2°C, (200 rev/min). Phosphate solubilizing ability was estimated after 24 hours.

Table 4 Screening of Phosphate solubilization with different strains of *Rhizobium leguminosarum* by plate method

S .No	Strain no	Zone of diameter (mm)
1	PSRV1	5.6
2	PSRV2	5.0
3	PSRV3	9.0
4	PSRV4	15.0
5	PSRV5	13.0
6	PSRV6	3.4
7	PSRV7	4.3
8	PSRV8	18.4
9	PSRV9	5.7
10	PSRV10	6.9
11	PSRV11	18.3
12	PSRV12	8.4
13	PSRV13	16.5

PSRV1- PSRV13 – Different strains of *Rhizobium leguminosarum*

Table 5 Environmental stress tolerance in pH and temperature of *Rhizobium leguminosarum.*

Isolates	pH					Temperature (°C)			
	5	6	7	8	9	20	30	40	50
PSRV1	+	+	+	+	+	+	+	+	+
PSRV2	-	-	+	+	-	+	+	+	-
PSRV3	-	-	+	+	-	+	+	+	-
PSRV4	-	+	+	+	-	+	+	+	-
PSRV5	-	-	+	+	-	+	+	+	+
PSRV6	-	-	+	+	-	+	+	+	-
PSRV7	+	+	-	-	+	-	+	-	+
PSRV8	+	+	-	-	+	-	+	-	+
PSRV9	-	-	-	+	+	+	-	+	-
PSRV10	+	-	+	-	+	+	-	+	+
PSRV11	+	-	+	-	+	+	-	+	-
PSRV12	+	+	-	+	-	+	-	+	-
PSRV13	+	+	-	+	-	-	+	-	+

(+) growth (-) no growth

Table 6 Effect of different concentrations of glucose as carbon on *Rhizobium leguminosarum.*

Different Strains	Glucose Concentration			
	2g		4g	
	PSI	PSE %	PSI	PSE %
PSRV1	2.4	60.14	2.5	50.00
PSRV2	2.2	48.00	NS	NS
PSRV3	2.3	60.15	2.2	20.00
PSRV4	2.42	66.65	2.56	56.25
PSRV5	2.52	67.27	2.55	56.40
PSRV6	2.32	32.07	1.73	36.36
PSRV7	2.25	25.00	NS	NS
PSRV8	2.41	65.45	2.41	66.87
PSRV9	2.25	25.00	2.25	25.00
PSRV10	NS	NS	1.47	32.34
PSRV11	2.47	66.23	2.65	62.54
PSRV12	2.38	37.82	2.16	20.34
PSRV13	2.36	36.66	2.43	65.49

NS – No significant solubilization
PSI – Phosphate solubilization index
PSE – Phosphate solubilization efficiency

RESULTS AND DISCUSSION

The current study suggested that the isolation of *Rhizobium leguminosarum* from *Vicia faba* root nodules from different areas of Thanjavur district, Tamilnadu. From serial dilutions 10^{-3} to 10^{-6} a total of 109 colonies were isolated and identified by biochemical tests. (Vishal Kumar Deshwal and Abhishek Chaubey, 2014). Out of which thirteen strains showed excellent growth from different dilutions, the biochemical characterization of those thirteen strains of *Rhizobium leguminosarum* are represented in Table 2 and 3.

Screening of phosphate solubilization test were performed from the thirteen strains. The maximum zone of diameters was 18.4 mm with PSRV8 strain and minimum zone of diameters was 3.4 mm with PSRV6 strain recorded respectively (Zephania *et al*.,2016). Some of the Rhizobium strain moderate zone was 15.0, 18.4 and 18.3 mm with PSRV4, PSRV8 and PSRV11 strains recorded respectively. (Table-4)

Effect of different concentration of glucose as carbon source on Rhizobium *leguminosarum* strains were performed. The strains with phosphate solubilization were analyzed with reference the phosphate solubilization efficiency. It was observed that phosphate solubilization efficiency of PSRV5 was 67.27% and 56.40% when 2g and 4g of glucose was added respectively. Followed by PSRV4, PSRV11, PSRV3 with 66.65%, 66.23%, 60.15% respectively when treated with 2g of glucose. But in higher concentrations i.e. 4g of glucose the phosphate solubilization efficiency was performed less (Table-6).

CONCLUSION

Thirteen strains of *Rhizobium leguminosarum* was isolated from *Vicia faba* bean root nodules from Thanjavur district, Tamilnadu. All isolates were performed by various biochemical tests and confirmed as *Rhizobium leguminosarum.* Some of the isolates were positive for phosphate solubilization activity and some specific strains showed excellent zone of diameter, which were analyzed further. Thus, the potential capacity of the present *Rhizobium leguminosarum* isolates with phosphate solubilization efficiency and can be employed as Biofertilizer and would have a significance role in achieving sustainable agricultural development.

Reference

Abd-Alla, M.H. (1994) Phosphatases and the utilization of organic phosphorus by Rhizobium leguminosarum biovar viceae. *Lett. Appl. Microbiol*. 18, 294 -296.

Beck D.P., Materon L.A., Afandi F., (1993) Practical Rhizobium-Legume Technology Manual No. 19. International Center for Agricultural Research in the Dry Areas (ICARDA) Box 5466, Aleppo, Syria.

Bohlool B. B., Ladha J. K., Garrity D. P. and George T., (1992) Biological nitrogen fixation for sustainable agriculture: A perspective *Plant and Soil.* 141 1-11.

Dadarwal K. R., Jawaharlal Nehru., (1989) Yadav K. S., Biological Nitrogen Fixation Research Status in India: In Commemoration of Birth Centenary of Pt. Jawaharlal Nehru. *Society of Plant Physiology and Biochemistry*, *New Delhi.* 11-86.

Dey KB. (1988) Phosphate solubilizing organisms in improving fertility status. *In Biofertilizers: Potentialities and Problems. Calcutta: Plant Physiology Forum*, Naya Prakash. 237-248

Edi-Premono M., Moawad, A.M. and Vlek, P.L.G., (1996) *Indonesian Journal of Crop Sciences.*11 13-23.

Enrilich H.L., (1982) Enhanced removal of Mn^{2+} from seawater by marine sediments and clay minerals in the presence of bacteria, *Can.J. Microbiol*. Vol. 28 1389-1395.

Gupta, D.K., S. Chatterjee b., S. Datta b., V. Veer b., C. Walther., (2014) Role of phosphate fertilizers in heavy metal uptake and detoxification of toxic metals *Chemosphere.* 108 134-144.

Gupta, R., Singal, R., Shankar, A., Kuhad, R.C. and Saxena, R.K. (1994) A modiced plate assay for screening phosphate solubilizing microorganisms. *J. Gen. Appl. Microbiol*. 40, 255-260.

Hajjam. Y., Alami I.T., Udupa S.M., Cherkaoui S. *(2016),* Isolation and evaluation of phosphate solubilizing rhizobia from root nodules of faba bean *(Vicia faba L.)* in Morocco, *J. Mater. Environ. Sci.* 7 (11) 4000-4010.

Hani Antoun., (2012) Beneficial Microorganisms for the Sustainable Use of Phosphates in Agriculture, *Procedia Engineering.* 46 62-67.

Jackson M. L. (1958) Soil chemical analysis*.,* Verlag*: Prentice Hall, Inc., Englewood Cliffs, NJ*. 498 S. DM 39-40.

Jones, D.L. and Darrah, P.R. (1994) Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. *Plant Soil.* 166, 247-257.

Katznelson, H., Peterson, E. and Rouatt, J.W. (1962) Phosphate dissolving microorganisms on seed and in the root zone of plants. *Can. J. Bot*. 40, 1181- 1186.

Leyval, C. and Barthelin, J. (1989) Interactions between Laccaria laccata, Agrobacterium radiobacter and beech roots: influence on P, K, Mg and Fe mobilization from mineral and plant growth. *Plant Soil*. 17, 103-110.

Louw, H.A. and Webley, D.M. (1959) A study of soil bacteria dissolving certain phosphate fertilizers and related compounds. *J. Appl. Bacteriol.* 22, 227-233.

McLauchlan K., (2006) The Nature and Longevity of Agricultural Impacts on Soil Carbon and Nutrients*: A Review Ecosystems*. 9 1364-1382.

Murphy J. and J. P. Riley., (1962) A modified single solution method for determination of phosphate in natural water., *Anal. Chim. Acta*. 27 31-36.

Peix, A. Rivas-Boyero, a. a. Mateos., P. F. Rodriguez-Barrueco., C. Martínez Molina., E. Velazquez, E., (2001) Growth promotion of chickpea and barley by a phosphate solubilizing strain of Mesorhizobium Mediterranean under growth chamber conditions, *Soil Biology & Biochemistry*. 33 103-110.

Peoples, M.B. & Craswell, E.T., (1992) Biological nitrogen fixation: investments, expectations and actual contributions to agriculture *Plant Soil* 141 13-39.

Pereira, S.I., and Castro, P.M., (2014) Biological nitrogen fixation: investments, expectations and actual contributions to agriculture *Ecological Engineering*. 73 526-535.

Phosphate solubilizing microorganisms FEMS *Microbiology Letters* 170 265-270.

Pikovskaya, R.I. (1948) Mobilization of phosphorus in soil in connection with the vital activity of some microbial species. *Microbiology* 17, 362-370.

Shekhar Nautiyal. C (1999) An efficient microbiological growth medium for screening

Spiertz JHJ., (2010) Nitrogen, sustainable agriculture and food security. A review A review. *Agron Sustain Dev*. 30 43-55.

Tagore, G. S. Namdeo., S. L. Sharma., S. Kumar., Narendra., (2013) Effect of Rhizobium and Phosphate Solubilizing Bacterial Inoculants on Symbiotic Traits, Nodule Leghemoglobin, and Yield of Chickpea Genotypes, Hindawi. 10 1-8.

Velthof, G.L., Oudendag, D., Witzke, H.P., (2009) Asman, W.A.H., Klimont, Z., Oenema, O., *Journal of Environmental Quality*. 38 402-417.

Vincent J. M. A Manual for the Practical Study of Root Nodule Bacteria. Oxford: Blackwell Scientific. (1970).

Vishal Kumar Deshwal and Abhishek Chaubey (2014), Isolation and Characterization of *Rhizobium leguminosarum* from Root nodule of *Pisum sativum* L. *Journal of Academia and Industrial Research (JAIR)* Volume 2, Issue 8.

Yadav, K.S. and Dadarwal, K.R. (1997) Phosphate solubilization and mobilization through soil microorganisms. In: Biotechnological Approaches in Soil Microorganisms for Sustainable Crop Production (Dadarwal, K.R., Ed.), pp. 293-308. *Scientific Publishers*, Jodhpur.

Zephania Simon, Kelvin Mtei, Amare Gessesse, Patrick A. Ndakidemi (2016) Isolation and purification of *Rhizobium* from French bean (*Phaseolus vulgaris* L.) root nodules *J. Biol. Today's World.*; 5 (2): 30-34.

How to cite this article:

Gelli Veena Shravanthi and Prabakaran Panchatcharam.2017, Isolation And Optimization Studies of Phosphate Solubilizing Rhizobium Leguminosarum. *Int J Recent Sci Res.* 8(12), pp. 22209-22212. DOI: http://dx.doi.org/10.24327/ijrsr.2017.0812.1222

****