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ISOLATION OF PHOSPHATE SOLUBILIZING BACTERIA FROM RHIZOSPHERE OF BT COTTON PLANT GROWN IN MEHSANA DISTRICT, NORTH GUJARAT

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ABSTRACT:

Phosphorus is one of the major nutrients which play an indispensable biochemical role in photosynthesis, respiration, energy storage and transfer, cell division, cell enlargement and several other processes in the living plant. Phosphate solubilizing bacteria (PSB) can solubilize different forms of inorganic phosphates. A total of twenty nine (29) phosphate solubilizing bacteria were isolated from the six (06) rhizospheric sample of BT cotton plant of Mehsana District, Gujarat. Out of 29 isolates; 14 isolates show remarkable zone of solubilization. The zone of solubilization was studied on pikovskaya's agar and quantitative phosphate solubilization was carried out by vanado- molybdate method. *Burkholderia latens* showed the maximum phosphate solubilization index 4.27 ± 0.094 in PVK agar plates along with phosphate solubilizing activity 328.7 ± 7.13 μg mL^{-1} in PVK broth and pH of the medium decreased upto 4.14 ± 0.067. However, the isolate PSB-3 shows the least solubilizing activity. Potent isolates show good phosphate solubilizing ability and thus were found potential further used as biofertilizers agents.

KEY WORD: *Phosphate Solubilizing bacteria, Burkholderia latens, Bt Cotton, Rhizosphere.*

INTRODUCTION:

Phosphorus is one of the major limiting factors of crop production in many tropical and subtropical soils. It is therefore necessary to identify and incorporate efficient strains of phosphate solubilizing microorganisms into cropping system (Fankem *et al.*, 2006). Most of it exist in soil insoluble metallic complex with iron and aluminium in acidic soil or with calcium carbonate in alkaline soil, as a result only a small fraction of phosphate is available for plant growth. Phosphate is highly insoluble and unavailable to plants in these forms (Maheswar and Sathiyavani, 2012). The insoluble phosphate, which is not directly available to plants or microorganisms, usually comprises 95-99 percent of the total phosphate (Hayman, 1975). A number of factors such as nature and content of clay, active sesquioxides, lime, pH and organic matter influence the availability of soil phosphate (Karunai *et al.*, 2011). Root development, stalk and stem strength, flower and seed formation, crop maturity and production, nitrogen fixation in legumes, crop quality and resistance to plant diseases are the attributes associated with phosphorus nutrition (Ahmad *et al.*, 2009). The phosphate solubilizing microorganisms convert the insoluble phosphate into soluble forms by acidification, chelation and exchange reaction (Chen *et al.*, 2006). The solubilization effect is generally due to the production of organic acids by these organisms (Ponmurugan and Gopi, 2006). Production of organic acids results in acidification of the microbial cell and its surrounding. These bacteria can play important role in supplement of phosphate to plants in a friendlier environmentally and in sustainable manner (Khan *et al.*, 2007). Due to the poor adaptability to change soil and agro climate condition, phosphate solubilizing microorganisms isolates elsewhere have not been very consistent in their performance everywhere (Alagawadi *et al.*, 1992). The aim of our investigation was to isolate the native phosphate solubilizing bacteria from rhizosphere of BT Cotton plant grown in Mehsana district, North Gujarat region.

MATERIALS AND METHODS:

Collection of soil sample

Soil samples were collected from rhizosphere of BT cotton plant grown in Mehsana district, North Gujarat. Collected soil samples were stored in polythene bags and maintained in the laboratory for further study.

Isolation of phosphate solubilizing bacteria

From each soil sample, 1g of soil was suspended in 9 ml blank sterile distilled water and serially diluted up to 10^{-6}. The dilutions were plated on pikovskaya's (PVK) agar medium in order to isolate the PSB. Colonies forming a halo zone were re-transferred to PVK agar medium for the purity of the culture. The bacterial cultures were stored at 4^0 C and regularly transferred on nutrient agar slants during study.

Analysis of phosphate solubilizing activity

The qualitative as well as quantitative analysis of phosphate solubilizing activity of 14 bacterial isolates were conducted by plate screening method and broth culture, respectively.

1). Qualitative measurement of phosphate solubilization

Bacterial isolates were screened for their tri-calcium phosphate (TCP) solubilizing activity on PVK plates. Isolates were spot inoculated on the center of agar plate aseptically. All the plates were incubated at $28^0 \pm 2^0$ C for 5 days. A clear zone around a growing colony indicates phosphate solubilization and was measured as phosphate solubilization index (SI). All the observations were recorded in triplicate. Solubilization index (SI) was measured by using following formula:

$$SI = \frac{\text{Colony diameter + Halo zone diameter}}{\text{Colony diameter}}$$

2). Quantitative measurement of phosphate solubilization

Bacterial isolates were inoculated (8 % v/v spore suspension) in pikovskaya's broth (100 mL) in 250 mL Erlenmeyer flask with 0.5 % TCP and incubated in shaker at 28^0C for six days at 100 rpm and then centrifuged at 10,000 rpm for 15 min. The dissolve phosphate concentration in supernatant was determined by vanado- molybdate method as described in APHA (1995).

IDENTIFICATION:

The isolated most potent bacterial culture PSB-6 was isolated and sent to Labreq bioscientific lab, Ahmadabad, Gujarat for 16s rRNA sequencing where sequencing was performed by using Big Dye terminator cycle sequencing kit (Applied Bio Systems, USA). This bacterial isolate on the basis of 16s rRNA sequencing was identified as *Burkholderia latens*.

RESULTS AND DISCUSSION:

Twenty nine (29) phosphate solubilizing bacteria were isolated from the total of six (06) rhizospheric samples from BT cotton plant of Mehsana district of North Gujarat. Among them, fourteen (14) bacterial isolates were selected for further qualitative and quantitative screening (Table 1).

It clearly appears from table 2 that in media with tri calcium phosphate, the values of solubilized phosphate were obtained from all the isolates were significantly different from those of control, showing that the tested isolates have effectively converted the insoluble phosphate into soluble form.

A decrease in pH values was also observed in the tested isolates compared to control. Phosphate solubilizing bacteria showed the solubilization index, ranging from 1.36 ± 0.078 to 4.27 ± 0.094 (Table 2). According to their efficiency, the most efficient phosphate solubilizer strain was PSB-6 (328.7 ± 7.13 µg mL^{-1}) while strain PSB-3 was the least solubilizer (30.2 ± 2.46 µg mL^{-1}) and also a significant drop in pH upto 4.14 ± 0.025 and 6.48 ± 0.036 from an initial pH of 7.0 after six days of incubation by PSB-6 and PSB-3.

Suliasih and Rahmat, (2007) stated that bacteria grown in solid pikovskaya's medium will solubilize P, characterized by clear zone around the colony as a result of solubilizing $Ca_3(PO_4)_2$. Bashan *et al.* (2013) confirmed that the P solubilization is due to the release of protons as H^+ where the mechanism is the same as tri calcium phosphate ($Ca_3(PO_4)_2$) solubilization, described as: $Ca_3(PO_4)_2 + 2H^+ \rightarrow 2CaHPO_4 + Ca^{2+}$. Phosphate solubilization in culture medium can be influenced by various factors, such as the composition of growth medium, pH changes in culture medium and the presence of phosphate solubilizing bacteria strains (Chen *et al.*, 2006; Park *et al.*, 2010; Karpagam and Nagalakshmi, 2014). Rodriguez and Fraga, (1999) observed that among 33 bacterial genera including *Pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Micrococcus, Aerobacter*, *Flavobacterium* and *Erwinia* showed significant phosphate solubilizing activity. Surapat *et al.*, (2013) found that in case of *Burkholderia* genus of soil bacterium inorganic phosphate solubilizing ability ranges between 126.36 to 488.55 µg mL^{-1} of phosphate. Nair *et al.*, (2016) reported that *Burkholderia cepacia* CC2 will effectively solubilized phosphate (374 ± 33 µg mL^{-1}). Nair and Vakil, (2015) stated that *Burkholderia tropica* (400 µg mL^{-1}) and *Burkholderia cepacia* (375 µg mL^{-1}) gave significant phosphate solubilization under shaking conditions and possessed solubilization index 4.3 and 4.9, respectively. Song *et al.*, (2008) reported that *Burkholderia cepacia* DA23 isolated from Gimhae soil area of Korea, it showed maximum phosphate solubilization of tri-calcium phosphate (345.9 µg mL^{-1}). Anandham *et al.*, (2007) stated that *Burkholderia sp.* strain CBPB-HIM showed the highest tri calcium phosphate solubilizing (363 µg mL^{-1}) activity. Walpola and Yoon (2013) reported that *Burkholderia anthina* showed efficient phosphate solubilizing ability (384.28 µg mL^{-1}). Gao *et al.*, (2016) observed that phosphate solubilizing bacteria isolated from rhizospheric soils of tobacco effectively solubilized phosphate and solubilization index ranging from 1.14 to 4.8 and concentration of solubilized phosphate ranged from 33.20 to 360.18 µg mL^{-1}. Muthaiyan and Ramalingam (2015) reported that phosphate solubilizing bacteria showed SI ranging from 2.9 to 4.5 on pikovskaya's agar medium. Omkar (2012) reported that phosphate solubilizing bacteria recorded high P solubilization potential having SI ranging from 2.11 to 3.40 and quantitatively solubilized 160 to 270 µg mL^{-1} P respectively after 7 day incubation. Most researcher reports showed phosphate solubilizing potentiality of PSB around 300-400 µg mL^{-1} under laboratory conditions (Chen *et al.,* 2006). Previous reports have also described few *Burkholderia* strains as efficient phosphate solubilizer (Peix *et al.,* 2001; Lin *et al.,* 2006; Khalimi *et al.,* 2012; Mursyida *et al.,* 2015). The drift in the pH of the medium was towards acidic side with phosphate solubilization. These studies also indicate drop in pH of the medium was usually accompanied by phosphate solubilization. This may indicate the possible mechanism of phosphate solubilization is organic acid production (Ramani and Patel, 2011).

Our data also indicate that *Burkholderia latens* isolates have more potential of solubilization of inorganic phosphates are in accordance to the results obtained by other authors.

CONCLUSION:

BT cotton rhizospheric soils are rich in phosphate solubilizing bacteria. The isolate *Burkholderia latens* strains on the basis of qualitative and quantitative estimation of phosphate solubilization showed maximum phosphate solubilizing activity. This strain can be used in the field as efficient biofertilizers. Biofertilizers are environment friendly, free from hazardous chemicals, possess no detrimental health effects and are cost effective.

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REFERENCES:

Ahmad, A.K., Ghilam, J., Mohammad, S.A., Syed, M.S.N. and Mohammad, R. (2009). Phosphorus solubilizing bacteria: occurance, mechanisms and their role in crop production. *Journal of Agricultural and Biological Science*, 1(1), 48-58.

Alagawadi, A.R., Sheelavantar, M.N., Patil, R.B. and Patil, S.V. (1992). India should take the best advantage of biofertilizers. In Some Aspects Agricultural and Rural Development. ISARD Publication. Dharwad. pp: 93-113.

Anandham R., Choi, K.H., Gandhi, P.I., Yim, W.J., Park, S.J., Kim, K.A., Madhaiyan, M. and Tong, M.S. (2007). Evaluation of shelf life and rock phosphate solubilization of *Burkholderia sp.* in nutrient amended clay, rice bran and rock phosphate-based granular formulation. *World Journal of Microbiology & Biotechnology*, 23(8), 1121–1129.

APHA (1992). Standard methods for the examination of water and waste water. American Public Health Association. Washington, D.C.

Bashan, Y., Kamnev, A.A., Luz, E. and Bashan, D. (2013), Tri calcium phosphate is inappropriate as a universal selection factor for isolating and testing phosphate solubilizing bacteria that enhance plant growth: a proposal for an alternative procedure. *Biology and Fertility of Soils*, 49, 465–479.

Chen, Y.P., Rekha, P.D., Arun, A.B., Shen, F.T., Lai, W.A. and Young, C.C. (2006). Phosphate solubilizing bacteria from subtropical soil and their tri calcium phosphate solubilizing abilities. *Applied Soil Ecology*, 34, 33–41.

Fankem, H., Dieudonne, N., Annette, D., Wolfgang, M. and Francois, X. (2006). Occurance and functioning of phosphate solubilizing microorganisms from oil palm tree (*Elaeis guinensis*) rhizosphere in Cameroon. *African Journal of Biotechnology*, 5(24), 2450-2460.

Gao, L., Kong, F., Feng, C., Wang, J., Gao, J., Shen, G. and Zhang C. (2016). Isolation, characterization and growth promotion of phosphate solubilizing bacteria associated with *Nicotiana Tabacum* (Tobacco). *Polish Journal of Environmental Studies*, 25(3), 993-1003.

Hayman, D.S. (1975). Phosphorus cycling by soil microorganisms and roots. In: Soil Microbiology. A critical review. N Walker (ed.). Butterworths, London and Boston. pp. 67-92.

Karpagam, T. and Nagalakshmi, P. (2014). Isolation and characterization of phosphate solubilizing microbes from agricultural soil. *International Journal of Current Microbiology and Applied Sciences*, 3(3), 601-614.

Karunai, S.B., Paul, J.A., Ravindran, A.D. and Vijaya, V. (2011). Quantitative estimation of insoluble inorganic phosphate solubilization. *International Journal of Science and Nature*, 2(2), 292-295.

Khalimi, K., Suprapta, D.N. and Nitta, Y. (2012). Effect of *Pantoea agglomerans* on growth promotion and yield of rice. *Agricultural Science Research Journals*, 2(5), 240-249.

Khan, M.S., Zaidi, A. and Wani, P.A. (2007). Role of phosphate solubilizing microorganisms in sustainable agriculture - A review. *Agronomy for Sustainable Development*, 27(1), 29-43.

Lin, T.F., Huang, H., Shen, F.T. and Young, C.C. (2006). The protons of gluconic acid are the major factor responsible for the dissolution of tricalcium phosphate by *Burkholderia cepacia* CC-A174. *Bioresource Technology*, 97(7), 957-960.

Maheswar, N. and Sathiyavani, G. (2012). Solubilization of phosphate by *Bacillus sp.* from groundnut rhizosphere (*Arachishypogaea L*). *Journal of Chemical and Pharmaceutical Research*, 4(8), 4007-4011.

Mursyida, E., Mubarak, N.R. and Leksono, A.T. (2015). Selection and identification of phosphate potassium solubilizing bacteria from the area around the limestone mining in Cirebon Quarry. *Research Journal of Microbiology*, 10(6), 270-279.

Muthaiyan, V. and Ramalingam, S. (2015). Determination of phosphate solubilization and plant growth promotion of bacterial isolates from paddy rhizosphere. *International Journal of Agronomy and Agricultural Research*, 6(3), 1-6.

Nair, S.K. and Vakil, B.V. (2015). Isolation and characterization of *Ralstonia pickettii* - A novel phosphate solubilizing bacterium from Pomegranate rhizosphere from Western India. *International Journal of Life Sciences Biotechnology and Pharma Research*, 4(1), 1-9.

Nair. S.K., Wagle, A.R. and Vakil, B.V. (2016). Shake flask optimization for some phosphate solubilizing bacteria. *International Journal of Applied Biology and Pharmaceutical Technology*, 7(3), 148-159.

Omkar, S. (2012). Isolation and characterization of PSB from rhizospheric soil samples. *Online International Interdisciplinary Research Journal*, 2(4), 28-39.

Park, K.H., Lee, O.M., Jung, H.I., Jeong, J.H., Jeon, Y.D., Hwang, D,Y., Lee, C.Y. and Son, H.J. (2010). Rapid solubilization of insoluble phosphate by a novel environmental stress-tolerant *Burkholderia vietnamiensis* M6 isolated from ginseng rhizospheric soil. *Applied Microbiology and Biotechnology*, 86, 947–955.

Peix, A., Mateos, P.F., Rodriguez, B.C., Martinez, M.E. and Velazquez, E. (2001). Growth promotion of common bean (*Phaseolus vulgaris L.*) by a strain of *Burkholderia cepacia* under growth chamber conditions. *Soil Biology and Biochemistry*, 33, 1927-1935.

Ponmurugan, P. and Gopi, C. (2006). In vitro production of growth regulators and phosphatase activity by phosphate solubilizing bacteria. *African Journal of Biotechnology*, 5(4), 348-350.

Ramani, V. and Patel, H.H. (2011). Phosphate solubilization by *Bacillus sphaericus* and *Burkholderia cepacia* in presence of pesticides. *Journal of Agricultural Technology*, 7(5), 1331-1337.

Rodriguez, H. and Fraga, R. (1999). Phosphate solubilizing bacteria and their role in plant growth promotion. *Biotechnology Advances*, 17(4-5), 319-339.

Song, O.R., Lee, S.J., Lee, S.C., Kim, K.K. and Choi, Y.L (2008). Solubilization of insoluble inorganic phosphate by *Burkholderia cepacia* DA23 isolated from cultivated soil. *Brazilian Journal of Microbiology*, 39, 151-156.

Suliasih and Rahmat (2007). Phosphatase activity and solubilization of calcium phosphate by phosphate solubilizing bacteria. *Biodiversitas*, 8(1), 23-26.

Surapat. W., Pukahuta, C., Kunsopon, P.R., Aimi, T. and Boonlue, S. (2013). Characteristics of phosphate solubilization by phosphate solubilizing bacteria isolated from agricultural chili soil and their efficiency on the growth of chili (*Capsicum frutescens* L. cv. Hua Rua). *Chiang Mai Journal of Science*, 40(1), 11-25.

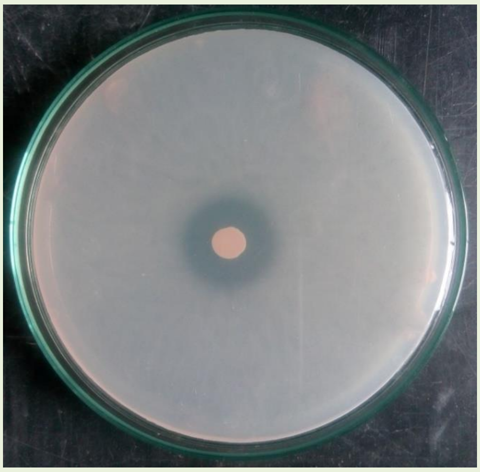
Walpola, B.C. and Yoon, M.H. (2013). Phosphate solubilizing bacteria: Assessment of their effect on growth promotion and phosphorus uptake of mung bean (*Vigna radiata* [L.] R. Wilczek). *Chilean Journal of Agricultural Research*, 73(3), 275-281.

Table-1. Isolation of Phosphate Solubilizing Bacteria

Sr. No.	Soil pH	No. of primary bacterial isolates	No. of selected PSB isolates based on SI
1	7.27	5	3
2	7.59	6	2
3	7.86	5	2
4	7.41	3	1
5	7.74	7	4
6	7.53	3	2
Total		29	14

Table-2. Qualitative and Quantitative Estimation of Phosphate Solubilizing Bacteria

Isolates	Solubilization index (SI)	Soluble P concentration (µg mL^{-1})	End pH
PSB-1	1.42 ± 0.085	060.9 ± 4.57	6.31 ± 0.025
PSB-2	1.53 ± 0.090	102.7 ± 6.72	6.29 ± 0.041
PSB-3	1.36 ± 0.078	030.2 ± 2.46	6.48 ± 0.036
PSB-4	2.39 ± 0.036	152.8 ± 8.81	5.26 ± 0.107
PSB-5	1.73 ± 0.063	136.1 ± 5.94	5.31 ± 0.046
PSB-6	4.27 ± 0.094	328.7 ± 7.13	4.14 ± 0.067
PSB-7	2.53 ± 0.059	157.3 ± 5.18	5.47 ± 0.053
PSB-8	1.52 ± 0.072	081.8 ± 1.32	6.08 ± 0.026
PSB-9	3.14 ± 0.048	180.5 ± 2.91	4.91 ± 0.039
PSB-10	2.94 ± 0.085	192.2 ± 4.82	4.29 ± 0.061
PSB-11	1.97 ± 0.061	124.1 ± 6.58	5.97 ± 0.058
PSB-12	3.53 ± 0.048	206.9 ± 5.69	4.45 ± 0.075
PSB-13	1.61 ± 0.050	085.3 ± 3.96	6.23 ± 0.083
PSB-14	1.47 ± 0.100	095.6 ± 1.05	6.18 ± 0.015



Burkholderia latens