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Isolation and Partial Characterization of Phosphate Solubilizing Bacteria from Groundnut Rhizosphere

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ABSTRACT: *Phosphorus is an essential nutrient which is indispensible for proper growth and development of plants. Soil usually contains the inorganic phosphorus in the form of apatite, complexes of iron and aluminum phosphates, and phosphorus absorbed on clay particles. However, the solubility of these phosphorus compounds is extremely low and only very small amounts of this Phosphorus is actually available to the plants. So, Phosphorus is required as a fertiliser for all commercial plant production, including groundnut. However, this results into a higher cost per hectare and the risk of polluting fresh water resources. One of the possible solution to this problem is the use of bio fertilizers contain phosphate solubilizing microorganisms. Many soil microorganisms are able to solubilize this unavailable Phosphorus through their metabolic activities and make them available to the plants. In the present study, six soil samples were collected from rhizosphere of sugarcane plants growing in Punjab (Rupnagar, Anandpur sahib, Nurpur bedi, Nangal, Hoshiarpur and Ludhiana). A total of 35 phosphate solubilizing bacterial isolates were obtained by plating the soil samples on Pikovskaya's media plates. The isolates were subjected to primary and secondary screening and two isolates* viz. *PSB-H5 and PSB-NB3 showing the highest phosphate solubilizing efficiency were selected for optimization of conditions. The incubation period of 120 hrs and pH-7 were observed to be best suited for phosphate solubilization by both the isolates. Among the various nutrients, glucose and ammonium sulphate were found to be the best Carbon and Nitrogen source respectively. The isolates were then tested morphologically and biochemically. PSB-H5 seems to belong to the genus Bacillus and PSB-NB3 appears to be a member of the genus Pseudomonas.*

Key words*: Phosphate solubilizing bacteria, groundnut, rhizosphere*

INTRODUCTION

Phosphorus is an important and basic nutrient required for the key plant structure which mediates many biological reactions. Phosphorus is vital component of ATP and formed during photosynthesis and processes formed during beginning of seedling growth to the formation of grain. It is involved in strengthening of plant stalk and stem, root development as well as increasing nitrogen fixation capacity of legumes therefore deficiency of phosphorous leads to retardation in above said processes (Dey, 1988).The phosphorus in soil occurs in two forms i.e. organic and inorganic . Organic form is found in plant residues, manures and microbial wastes (3%- 50%) depending upon the soil type. Inorganic form or mineral form of phosphorus is represented in soil by insoluble minerals such as apatite, hydroxyapatite and oxyapatite (Anderson, 1980).However, the plants are not capable of utilizing the insoluble phosphates. The groundnut plant is a leguminous cash crop cultivated on approximately 23.95 million ha worldwide with the total production of 36.45 million tons (FAOSTAT, 2011). Phosphorus requirement for plant growth varies from 20 to 80 P_2O_5/ ha in case of legume plants. For groundnut plants it is 40 P_2O_5/ ha (Tomar *et al*, 1983). However, the total phosphorus content in most of surface soil is as low as 0.6 % as compared as compared to the nitrogen & potassium i.e. 0.14% and 0.83% respectively (Banger *et al* , 1990). Hence the farmers have to invest a substantial amount of money for the application of Phosphatic fertilizers. However, almost 75 to 90% of added P fertilizer in agricultural soils is precipitated by iron, aluminum and calcium complexes present in soils and thus become insoluble and unavailable to plants (Turan *et al*., 2006; Patel & Parmar, 2013). Furthermore, phosphatic fertilizers are expensive, and excessive use of rock phosphate (RP)

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is environmentally undesirable. The phosphates may be easily washed off and reach water bodies. The consequences of excess phosphorous in water are algae development, oxygen depletion and the potential massive mortality of fish. (Dey, 1988; Panhwar *et al*., 2011). Phosphate solubilising microorganisms can be a potential solution for these kinds of problems. These microorganisms include mycorrhizal fungi as well as many bacteria like *Rhizobium, Enterobacter, Bacillus, Pseudomonas* (Chen *et al.,* 2006; Park *et al.,* 2011) These microbes possess the capability to convert insoluble forms of phosphorus into bioavailable soluble form phosphorus *(*Whitelaw, 2000; Igual *et al*., 2001) They produce various acids (organic as well as mineral acids), siderophores protons, humic substances, CO_2 and H_2S.This results in acidification of the surrounding soil, releasing soluble orthophosphate ions ($H_2PO_4^{-1}$, HPO_4^{-2} and PO_4^{-3}) which can be readily taken up by plants (Kundu *et al*., 2009). Use of Phosphate solubilizing bacteria as inoculants may lead to the increase in the P uptake resulting in better plant growth (Guiñazú *et al.,* 2010; Mohamed and Ibrahim, 2011) and also lead to decrease the cost of farming.

MATERIALS AND METHODS

Isolation of Phosphate solubilizing Bacteria: A total of six soil samples were collected from rhizosphere of groundnut plant (*Arachis hypogaea*) from the following mentioned field areas of Punjab, India viz. Rupnagar, (Distt.- Ropar), Anandpur sahib (Distt. – Ropar), Nurpur bedi (Distt.- Ropar), Nangal (Distt.- Ropar), Hoshiarpur (Distt.- Hoshiarpur) and Ludhiana, (Distt- Ludhiana). Collection of soil samples was made at a depth of 15 cm from 6 different points within the area. Sample from per site was represented as single sample of that particular area. The rhizosphere soil samples were then air-dried, powdered and mixed well to represent a single sample. Ten grams of air-dried powdered soil was taken in to 90 ml sterilized distilled water and shaken for 15 minutes. Subsequent serial dilution was made up to 10-6.An inoculum of 0.1 ml of appropriate dilution was spread over plate having Pikovskaya's media of pH- 7.0 (Pikovskaya , 1948). Then the inoculated plates were incubated in an incubator at 30^{O}C for 48 hours. The composition of Pikovskaya's medium was as follows (g/L): Glucose- 10, Tricalcium Phosphate- 5, Ammonium sulphate- 0.5, sodium chloride- 0.2, Magnesium sulphate- 0.1, Potassium chloride- 0.2, yeast extract- 0.5, Manganous sulphate- 0.002, Ferrous sulphate- 0.002. The bacterial isolates obtained were purified by streaking on fresh Pikovskaya's media & purified isolates were transferred on to slants and stored in refrigerated conditions at 4^{o}C.

Screening of bacterial isolates: All the purified phosphate solubilising bacterial strains obtained were subjected to primary screening. For this the isolates were suspended in ten ml of distilled water and 0.01 ml of suspension was spotted over Pikovskaya's media having insoluble phosphorus source (Tri-Calcium Phosphate). The bacterial cultures were allowed for incubation for 7 days at 30 ^{0}C. After incubation period zone of hydrolysis and colony diameter was measured. After incubation period, the phosphate solubilization efficiency (PSE) was calculated on the basis of colony size and zone of hydrolysis as per the given formula (Kundu *et al*., 2009):

$$PSE\ (in\ \%) = (Z\text{-}C)/C \times 100$$

Z = Solubilization zone diameter

C = Diameter of bacterial colony

All the bacterial isolates were further subjected to Secondary screening by evaluation of solubilisation of insoluble phosphorus into soluble form in Pikovsakya's broth under agitated conditions. One loop-full bacterial culture of isolates was transferred from 24 hrs old slants to 10 ml Pikovskaya's broth and incubated at 37 °C for 48 hrs. One ml of bacterial culture (O.D = 0.5 $\lambda_{600\ nm}$) was transferred to 100 ml Erlenmeyer flask containing 25 ml of Pikovskaya's broth. Uninoculated flasks were taken as control. The flasks were incubated at 30 °C under shaking condition for 72 hrs. Subsequently, the bacterial culture was subjected to centrifugation at 10,000 rpm for 15min. The pellet was discarded and the quantity of solubilized phosphorus in the supernatant was assessed by John's (1970) method. The best two isolates were used for subsequent experiments.

Standardization of conditions: The conditions for maximum phosphate solubilisation were optimized by varying the cultural conditions like pH (5 to 9), carbon source (glucose, suscrose, lactose, starch and maltose), nitrogen source (ammonium sulphate, sodium nitrate, urea, casein, trypton). During optimization process one of the conditions was varied in each experiment keeping the other variables constant.

Partial Characterization of selected isolates: Selected strains were characterized for various morphological biochemical characteristics according

to bergey's manual (Kreig and Holt, 1994) different biochemical tests were carried out which includes Indole, Methyl red, Voges-Proskauer, Citrate utilisation, Hydrogen sulfide production, Urease, , Catalase, Motility, Starch hydrolysis, Casein hydrolysis, Nitrate reduction test and carbohydrate fermentation test, Triple sugar iron agar test.

RESULTS AND DISCUSSIONS

A total of 35 purified bacterial isolates were obtained from groundnut rhizosphere soil samples of six different sites in Punjab, out of which 13 were collected from Ropar, 6 from Hoshiarpur, 5 from Nangal, 3 from Ludhiana, 5 from Anandpur Sahib & 3 from Nurpur Bedi. PSB are ubiquitous in soil and have been found to be present in greater number and more metabolically active state in the plant rhizosphere (Vanquez *et al.*, 2000). Ponmurugan and Gopi (2006) made an extensive study on the PSB present in rhizospheres of different crops like chilli, ragi, groundnut, cotton, brinjal, paddy, sorghum, turmeric etc. and observed highest population density of PSB in groundnut rhizosphere. Similarly PSB by various workers from rhizospheres of different plants like potato and beet (Behbahani & Behbahani , 2009), betel vine plants

(Tallapragada and Seshachala, 2012) pea, spinach, lady's finger, french bean, cauliflower, turnip, brassica, cucumber, coriander, onion, potato, capsicum and field mint (Alia *et al.*, 2013).All purified bacterial isolates were then spotted over solidified Pikovskaya's media plates to check their insoluble phosphorus solubilizing efficiency (PSE) on the basis of the zone of phosphate solubilisation (Fig. 1). In case of the isolate PSB-NB3 maximum solubilization of 128.57% was observed and the least (5.88%) was shown by the isolate PSB-R10. All the strains were checked for P solubilization efficiency in liquid medium (Pikovskaya's broth) placed under shaking condition at 30 °C. Increase in P solubilization was observed in almost all strains as compared to the control (39.67±0.28). The PSB showed vast variation in their ability to solubilize the insoluble tri-calcium phosphate, with the isolate PSB-L2 showing the activity of 43.32 µg/ml and the isolates PSB-H5 and PSB-NB3 showing the maximum solubilization of 197.60 µg/ml and 183.11 µg/ml respectively. . On comparing the results of primary and secondary screening it was found that good correlation was not there between both the conditions. This may be due to the reason that PSB produce different type of organic acids which are may or may not able to show

Figure 1: Primary screening of bacterial isolates for phosphate solubilisation on Pikovskaya's medium plate

halo but strong enough to show solubilization in liquid media (Johnston,1952). For example, in case of PSB-H5 solubilization efficiency observed in primary screening was only 23.07% in primary screening. However it showed quite good P solubilization 105.67 µg/ml during the secondary screening. PSB-NB3 showed 80.00% P solubilization efficiency during primary screening its P solubilization was found to be 43.49%. This is in agreement with previous reports in which the workers have questioned the reliability of primary screening on solid media plates (Johnston, 1952;Yadav & Dadarwal, 1997; Balamurgan *et al.*, 2010).

The selected isolates PSB-H5 and PSB-NB3 were then followed up for the optimization process. The effect of incubation period was estimated on the basis of time duration of incubation of selected isolates (in hrs). This was done by checking the total insoluble phosphorus solubilized under liquid culture condition. It was observed that the phosphate solubilizing efficiency was increased up to 5th day (i.e. 120 hrs) (Fig. 2). In case of PSB-H5, maximum solubilization was observed in shaking condition up to 192.68 ±0.02 µg/ml at 120 hrs and afterwards no significant change was observed. In case of isolate PSB-NB3 also, it was found that maximum solubilization of 189.76 ± 0.05 µg/ml was there after incubation period of 120 hrs. Hu *et al*, (2006) found that 4 day's incubation period was optimum for maximum solubilization by the bacterial strains KNP413 and KNP414. Patel and Parmar (2013) used the incubation period of 5 days for assessment of

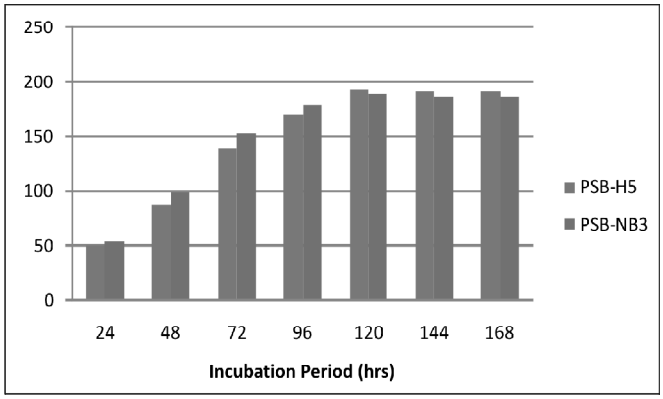

Figure 2: Effect of incubation period on phosphate solubilizing efficiency (µg/ml) of isolates PSB- H5 and PSB- NB3

phosphate solubilization by bacterial isolates obtained from sunflower rhizosphere. However some other workers have reported 10 to 15 days to be the optimum time for P solubilization by various bacterial isolates (Sheshadri *et al*, 2001; Pandey, 2006; Sahu *et al*., 2007; Sridevi & Mallaiah, 2009).

The medium pH is also one of the very crucial factors influencing the phosphate solubilization efficiency. Maximum solubilization resulted in case of PSB-H5 was 189.00 ± 0.12 µg/ml. On increasing pH above this level there was drastic decrease in the solubilization efficiency which was observed just 38.92± 0.02 µg/ml at pH 9. By decreasing the pH value up to 5 the solubilization reported was 69.98±0.10 µg/ml (Fig. 3). Also, in case of second isolate i.e. PSB-NB3 the optimum pH found to be was at 7, as maximum solubilization was observed here only 181.89 ± 0.01µg/ml and the minimum was at pH 5 which was up to 83.50 ±0.18 µg/ml. On the other hand, on increasing the pH up to 9 again there was comparative decrease in the solubilization which was 94.53 ±0.12 µg/ml. In some other studies similar results were found where highest phosphate solubilization was observed in the neutral pH range (Chen *et al*.,2005; Zhu *et al*., 2011; Sagervanshi *et al*., 2012).

To examine effect of carbon on the phosphate solubilizing efficiency, five different carbon sources viz. glucose, sucrose, starch, maltose and lactose were assessed at 1% (w/v). After the experiment it was found that sucrose was found to be the best carbon source in case of both the isolates was glucose (Fig. 4). In case of PSB-H5 sucrose was found to be the second best Carbon source, resulting in the activity of out of 115.40±0.02. The rest of the Carbon sources had a very negative impact on the bacterial phosphate solubilization efficiency (Fig. 4). However, in case of the isolate PSB-NB3, maltose resulted in good phosphate solubilization of 175.83 µg/ml. . Glucose has been reported to result into highest phosphate solubilisation by many workers (Gaur, 1990; Hameeda *et al*., 2006; Patel *et al*., 2008; Farhat *et al*., 2009; Kumari & Gupta,2013) .

The effect of different nitrogen sources on P solubilization efficiency was studied by using various nitrogen sources including inorganic nitrogen sources like ammonium sulphate, sodium nitrate, ammonium nitrate, urea, and organic nitrogen sources like trypton, peptone & casein were assessed at the level of 0.05 % (w/v). In the present study it was found that ammonium sulphate proved to be the best nitrogen source 192.68± 0.02 µg/ml followed by ammonium nitrate, casein urea, and sodium sulphate 57.87± µg/ml the last in case of PSB-H5 (Fig. 5). In case of PSB-NB3 it was found that ammonium sulphate (189.76± µg/ml) was followed up by ammonium nitrate (131.78± µg/ml), sodium sulphate (87.11 ±µg/ml) then urea (79.79 ±µg/ml). Thakker *et al*. (1993) tested many nitrogen sources like ammonium sulphate, ammonium chloride, potassium

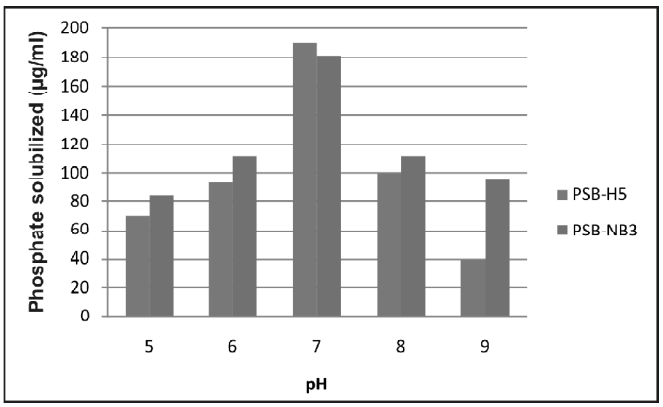

Figure 3: Effect of pH on phosphate solubilizing efficiency (µg/ml) of isolates PSB- H5 and PSB- NB3

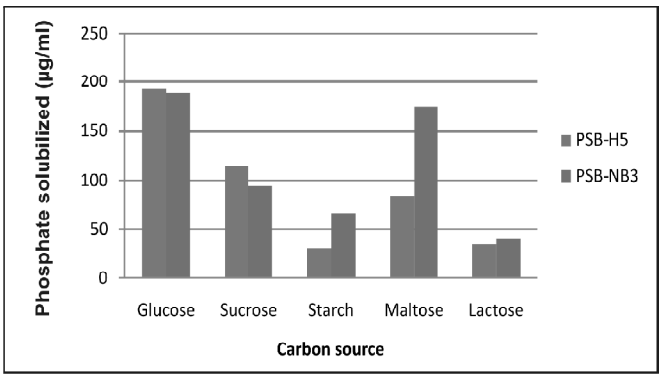

Figure 4: Effect of Carbon source on phosphate solubilizing efficiency (µg/ml) of isolates PSB- H5 and PSB- NB3

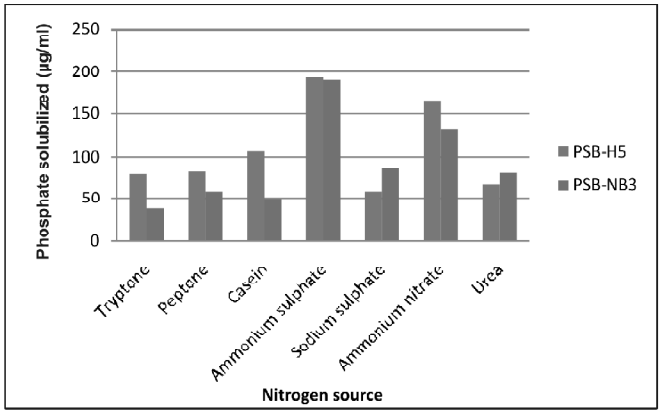

Figure 5: Effect of Nitrogen source on phosphate solubilizing efficiency (µg/ml) of isolates PSB- H5 and PSB- NB3

nitrate, sodium nitrate, and urea for the solubilization of Tri-Calcium Phosphate and Rock Phosphate with *Enterobacter aerogenes* and again reported ammonium sulphate to be most suitable for thepurpose. As per Kumari and Gupta (2013), Tri-Calcium Phosphate solubilization activity by *Pseudomonas lurida*decreased in the following order in presence of various nitogen sources: ammonium sulphate> Sodium nitrate > Potassium nitrate > ammonium chloride> urea.

The two isolates experimented for identification on the basis of various morphological and biochemical characteristics. The isolate PSB-H5 was found to be gram positive, rod shaped, spore forming,aerobic, motile, Indole negative, MR positive, VP negative, citrate positive, urease positive, catalase positive, starch hydrolyzing, non-H_2S producing and unable to hydrolyze casein. The isolate PSB-NB3 was found to be gram negative, rod shaped, non-spore forming, motile, Indole negative, MR negative, VP negative, aerobic, citrate positive, urease negative, catalase positive, starch hydrolyzing, non-H_2S producing, non-starch hydrolyzing and non-protease producing, Glucose and lactose non fermenting. By this result the PSB-H5 seems to belong to the genus *Bacillus* and PSB-NB3 to the genus *Pseudomonas.*

CONCLUSIONS

The Phosphate solubilizing bacteria are capable of converting insoluble Phosphorus in to soluble form, thus enhancing it's availability to plants. In the present investigation, a total of 35 isolates were obtained from rhizosphere of groundnut plant growing in Punjab, India. out of which 2 isolates PSB-H5 and PSB-NB3 showing highest phosphate solubilization were selected and conditions for *in vitro* phosphate solubilization were assessed. The two isolates seem to be good phosphate solubilizers & hence need to be assessed for phosphate solubilization efficiency in soil so that they can further be used as potential strains for biofertilizers.

REFERENCES

Dey, K. B., (1988), Phosphate solubilizing organisms in improving fertility status. In Biofertilizers: Potentialities and Problems (Sen S. P. and Palit P., eds.).Plant Physiology Forum, Naya Prokash, Calcutta, pp. 237-248.

Anderson, G. (1980), Assessing organic phosphorus in soils. In The Role of Phosphorus in Agriculture (Khasawneh, F.E., Sample E.C. and Kamprath E.J., eds) American Soceity of Agronomy, Madison, WI, pp. 411-432.

Tomar, S. P. S., Chauhan, Y. S., Jain, V. K. and Jain, P. C. (1983), Response of phosphorus on different varieties of groundnut (*Arachis hypogaea* L. *Madras Agric. J.* 70: 301-303.

Banger, K.C., Shankar, S. Kapoor, K. K., Kukreja, K. and Mishra, M. M. (1989), Preparation of nitrogen & phosphorus enriched paddy straw compost & its effect on yield & nutrient uptake by wheat. *Biol. Fertil. Soils.* 8:339-342.

Turan, M., Ataoglu, N. and Sahin, F. (2006), Evaluation of the capacity of phosphate solubilizing bacteria and fungi on different forms of phosphorus in liquid culture. *J. Sustainable Agri.* 28: 99-108.

Patel, D. and Parmar, P. (2013), Isolation and screening of phosphate solubilizing bacteria from sunflower rhizosphere. *Global J. Bio-sc. Biotechnol.* 2 (3) : 438-441.

Panhwar, QA. Radziah, O. Zaharah, AR. Sariah, M. and Razi, IM. (2011), Role of phosphate solubilizing bacteria on rock phosphate solubility and growth of aerobic rice. *J. Environ. Biol.* 32:607-12.

Chen, Y.P., Rekha, P.D., Arun, A.B., Shen, F.T., Lai, W.A., Young, C.C. (2006), Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl. Soil Ecol*. 34: 33-41.

Park, J.H., Bolan, N., Megharaj,M. and Naidu, R. (2011), Isolation of phosphate solubilizing bacteria and their potential for lead immobilization in soil. *J. Hazard. Mat.* 185:829-836.

Whitelaw, M. A. (2000), Growth promotion of plants inoculated with phosphate solubilizing fungi. *Adv. Agron*. 69: 99-151.

Igual, J. M., Valverde, A., Cervantes, E. and Velázquez, E. (2001), Phosphate-solubilizing bacteria as inoculants for agriculture: use of updated molecular techniques in their study. *Agronomie*., 21: 561-568.

Kundu, B.S. Nehra, K. Yadav, R. and Tomar, M. (2009), Biodiversity of phosphate solubilizing bacteria in rhizosphere of chickpea, mustard and wheat grown in

different regions of Haryana. *Indian J Microbiol.* 49:120–127.

Guiñazú , L.B., Andrés, J.A., Papa, M.F.D., Pistorio, M. and Rosas, S.B. (2010), Response of alfalfa (*Medicago sativa* L.) to single and mixed inoculation with phosphate-solubilizing bacteria and *Sinorhizobium meliloti. Biol Fertil Soils*. 46:185–190

Mohamed, H. M. and Ibrahim, E. M. A. (2011), Effect of inoculation with *Bacillus polymyxa* mutants on growth, phosphorus and iron uptake by tomato (*Lycopersicon esculentum* L.) in calcareous soils. *Inter. J. Soil Sci.* 6:176-187.

Pikovskaya, R.I. (1948), Mobilization of phosphorus in soil in connection with vital capacity of source microbial species, *Microbiologiya.*17: 362–370.

John, M.K. (1970), Colorimetric determination of phosphorus in soil and plant materials with ascorbic acid. *Soil Sci.* 109: 214–220.

Krieg N. R. and Holt G. (1994), Bergey's Manual of Determinative Bacteriology, 9th Edn, Williams and Wilkins, Baltimore.

Vazquez, P., Holguin,G., Puente, M. E., Lopez, C.A. and Bashan, J. (2000), Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. *Bio. Fert. Soils*. 30: 460–468.

Ponmurugan, P. and Gopi C. (2006), Distribution pattern and screening of phosphate solubilizing bacteria isolated from different food and forage crops. *J. Agron*. 5 : 600-604.

Tallapragada, P., and Seshachala, U. (2012), Phosphate-solubilizing microbes and their occurrence in the rhizospheres of Piper betel in Karnataka, India. *Turk. J. Biol*. 36: 25-38.

Alia, Afzal, A., Khokhar, S. N., Jabeen, B. and Asad, S. A. (2013), Phosphate Solubilizing Bacteria Associated With Vegetables Roots In Different Ecologies. *Pak. J. Bot*. 45: 535-544.

Behbahani,M. and Behbahani, M. (2009), Investigation of biological behavior of Iranian indigenous phosphate solubilizing bacteria and determinant of colonization ability of potato roots by these bacteria isolation. *J. Med. Plants Res*. 3: 1126-1133.

Johnston, H.W. (1952), The solubilization of phosphate: The action of various organic compounds on dicalcium and tricalcium phosphate. *N.Z.J.Sci.Technol*. 33: 436-444.

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.

Balamurgan, A., Princy, T., Vidhyapallavi, R., Nepolean, P., Jayanthi, R. and Premkumar, R. (2010), Isolation and Characterization of Phosphate Solubilizing Bacteria in Tea. *J. Biosci.* 1: 285-293.

Hu, X., Chen, J and Guo, J. (2006), Two phosphate- and potassium-solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. *World J. Microbiol. Biotechnol*. 22:983–990

Seshadri, S., Ignacimuthu S. and Lakshminarsimhan C. (2002), Variation in heterotrophic and phosphate solubilizing bacteria from Chennai, southeast coast of India. *Indian J. Mar. Sci*. 31: 69-72.

Pandey, A., Trivedi, P., Kumar, B. and Palni, L. M. S. (2006), Characterization of a phosphate solubilizing & antagnostic strain of *Pseudomonas putida* (BO) isolated from a sub-Alpine location in the Indian Central Himalaya, *Curr. Microbiol*. 53:102-107.

Sahu, M. K., Sivakumar, K. and Kannan, L. (2007), Phosphate solubilizing actinomycetes in the estuarine environment: An inventory, *J. Environ. Biol.* 28: 795-798.

Sridevi, M. and Mallaiah, K.V. (2009), Phosphate solubilization by *Rhizobium* strains *J. Microbiol. Biotechnol.* 49: 98-102.

Zhu, F., Qu, L., Hong, X. and Sun, X. (2011), Isolation and Characterization of a Phosphate-Solubilizing Halophilic Bacterium *Kushneria* sp. YCWA18 from Daqiao Saltern on the Coast of Yellow Sea of China. *Evidence-Based Complement. Alt. Med*. 2: 94-106.

Sagervanshi, A., Kumari, P., Nagee, A. and Kumar, A. (2012), Isolation and Characterization of Phosphate Solublizing Bacteria from Anand Agriculture Soil. *Inter. J. Life Sci. Pharma Res*. 23:256-266.

Gaur, A.C. (1990), Phosphate solubilizing microorganisms as biofertilizers. Omega scientific Publishers New Delhi. p. 176.

Hameeda B., Reddy Y. H. K., Rupela O. P., Kumar G. N. and Reddy, G. (2006), Effect of carbon substrates on rock phosphate solubilization by bacteria from composts and macrofauna, *Curr Microbiol.*,53: 298–302

Patel D. K., Archana G. and Kumar G. N. (2008), Variation in the Nature of Organic Acid Secretion and Mineral Phosphate Solubilization by *Citrobacter* sp. DHRSS in the Presence of Different Sugars. *Curr Microbiol*. 56: 168–174.

Farhat, M. B., Farhat, A., Bejar, W., Kammoun, R., Bouchaala, K., Fourati, A., Antoun, H., Bejar, S., Chouayekh H. (2009), Characterization of the mineral phosphate solubilizing activity of *Serratia marcescens* CTM 50650 isolated from the phosphate mine of Gafsa, *Arch Microbiol*. 191: 815–824.

Kumari, P. P. and Gupta, P. C. (2013), Effect of different carbon and nitrogen sources on solubilization of insoluble inorganic phosphate by psychrotolerant bacterial strains. *Bioscan*. 8: 1299-1302.

Thakker, J., Narsian, V., and Patel, H. H. (1993), Inorganic phosphate solubilization by certain soil bacteria. *Ind. J. Exp. Biol.* 31: 743-746.