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Research Article

SCREENING OF ACTINOMYCETES FOR PHOSPHATE SOLUBILIZATION AND THEIR EFFECT ON PLANT GROWTH

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ABSTRACT

Improving soil nutrients status by supplementing featured bacterial flora which can add and/or modify the complex/bound compounds into simpler form and those could be utilized further by plants has been attempted in present study. Phosphate is one of the key macronutrient which can act as a limiting factor for plant growth. Available P assists in deeper rooting and promotes plant development. Out of thirty three actinomycetes strains isolated from rhizosphere of different plants, twenty two were showing phosphate solubilization. All these strains solubilized phosphorus, expressed phosphatase .Quantitative phosphate solubilization was checked by Murphy and Riley's method. When checked for the physiological parameters, P solubilization factor found to be best on 20th day of incubation with pH 7 at 28°C and recorded to be stable in all strains. In a pot assay, eight isolates showed to contribute in overall plant growth such as increase in root length and shoot length, few of them was even performed better than used positive control strain. In conclusion, candidate reported eight actinomycetes isolated from the rhizosphere of plants collected near Pune city found to posses important PGPR activity and strongly recommended as biofertilizer.

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INTRODUCTION

With the increasing global requirement of agricultural products and ever decreasing cultivable lands and resources, human has put a considerable burden on remaining agriculture land for its continuous productivity (Tilak, 2005). In the current era, we are generating our agricultural products mainly *via* chemical fertilizers. Advantage of this; there is fifty percent rise in the productivity but also leads to sever environmental pollution with associated health hazards (Gaur and Gaind, 1999). It has been reported that due to mineral phase re-precipitation, soil lose upto 75% of applied phosphate fertilizer (Gold stein, 1986; Sundara *et al*., 2002), where phosphate solubilizing bacteria (PSB) are able to convert insoluble phosphate to simple soluble forms (Illmer ans Schinner, 1995; Peix *et al*., 2001, Viverk and Singh, 2001; Sudhakara *et al*., 2002), and now been used to re-precipitated soil P for improvement in crop productivity (Young *et al*., 1986; Young *et al*., 1990; Shekhar *et al*., 2000).

Along with other macronutrients Phosphorus (P) is one of the major factors required for biological growth and development (Ehrlich, 1990). The common presence level of P recorded as 400-1200 mg/Kg of soil (Fernandez, 1988). But the soluble P in soil recorded on lower side as 1ppm or less (Goldstein, 1994). The cell takes up P in several forms but majorly been absorbed as phosphate (Beever and Burns, 1980) Generally a common soil is rich in phosphorus with percent content to about 0.05% (w/w) (Barber, 1984), but only 10% of it do remain available to the plant due to its insolubility form's dominance. This is the reason why plant cannot utilize available phosphorus for many instances, majorly due to less solubility and chemical fixation in soil (Gaurand and Gaind, 1999). To make phosphorus available for the plants, large amount of chemical fertilizer is applied on a regular basis. But with the use, a major fraction of the fertilizer phosphorus is quickly getting converted into insoluble forms. Resultant very low % fraction remain available for utilization and in requirement re-supplementation is the only option remain which makes it bio-concentrate in soil and not getting available further, even though it remains abundant (Abd alla, 1994). Soil microbiome is involved in number of biological mechanisms which certainly affect phosphate transformation and thus subsequently make it available to the roots of plant (Richardson, 2001). Even though presence of plant growth promoting bacteria recorded in the soil, most of the time their percent prevalence recorded to be on lower side to compete other common bacteria established around rhizosphere. In

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requirement, to enhance crop productivity external inoculation of these target microorganisms at a much higher concentration than those available in normal soil takes the advantage of plant yield enhancement (Igual, 2001). As information on phosphorous solubilizing actinomycetes is scanty, the present investigation was designed to study the phosphate solubilizing actinomycetes isolated from rhizosphere soil of plants near Pune region. Effect of these isolates on growth of wheat maize, chilly and Tomatoes is investigated by pot experiments.

MATERIALS AND METHODS

Determination of phosphate solubilization feature

Ability of solubilizing phosphate has been screened out as one of the PGPR feature when all 33 actinomycetes isolates were allowed to grow on Pikovaskaya's medium supplemented with 5g L^{-1} tri calcium phosphate [Ca_3 $(PO_4)_2$] as a sole phosphorus source. The capability to release inorganic phosphate from tri calcium phosphate has been evidenced after 73 hours of incubation time at 28±2°C when phosphate solubilizing actinomycetes appeared with clear zone around the colonies. Resultant phosphate solubilizing index (SI) was calculated as per formula:

SI = Colony diameter + Halo zone diameter/ Colony diameter

Effect of pH, incubation period and temperature on phosphate solubilization (G. Balkrishna *et al* 2012, Shilpi Bharadvaj *et al* 2012)

The study further investigated by aiming the increase of phosphate solubilization with alteration in the physiochemical features such as pH, incubation and temperature as given below:

Variable pH

The actinomycetes were spot inoculated on Pikovaskaya's agar medium plates set at given pH ranged between 7, 8, 9, 10, 11 and 12 and kept incubating at 28°C for seven days.

Variable Incubating Period

Further all actinomycetes isolates were allowed to grow on the Pikovaskaya's agar medium set as 28°C having pH 7 up to 20th days and every day the value was recorded to check for the optimum phosphate solubilization.

Variable Temperature

The ability of better phosphate solubilization was checked in relation to variable temperatures set as 25°C, 30°C, 40°C and 45°C. Isolates were inoculated on Pikovaskaya's agar media plates as the spot and observed for the clear zone around the colonies.

Quantitative estimation of phosphate solubilization ((Murphy &Riley, 1962)

Tubes (20 ml), containing 4 ml of Pikovaskaya's broth inoculated with each isolate (40µl inoculum with approximately 1x10^9 cfu ml^{-1}), are incubated at 30°C on rotary shaker for 7 days and centrifuged at 180 rev min^{-1}. The cultures are harvested by centrifugation at 13,500x g for 5 minutes and inorganic phosphate concentration is determined colorimetrically in the supernatant by the molybdenum-blue method (Murphy and Riley 1962). Obtained supernatant was used to determine the soluble phosphate produced by each isolate as per standard method given as: All the reagents for phosphate estimation were prepared fresh and 1:2:1 volume of 6N H_2SO_4, double distilled water and ammonium molybdate 1% was added to the one volume of 10% ascorbic acid and mixed thoroughly. This reagent about 4 mL was added to the tube containing an aliquot of test material and the final volume made up to 8 mL. Sample was further kept incubating at 37°C for one hour. The sample was analyzed by UV-VIS spectrophotometer (labtronics model no.290) at 829 nm along with blank control. The content of solubilise phosphate was determined as per standard curve prepared by using K_2HPO_4 and expressed as equivalent P.

Phosphatase Enzyme Detection

Phosphatase activity of the sediments was estimated by the method described by Kramer and Erdei (1958). The isolates were incubated with phenyl disodium orthophosphate in and the phosphatase activity was indirectly measured by the amount of phenol released .Phosphate solubilization occurs by organic acid production, inorganic acid production and by phosphatase enzyme production. The ability of isolates to produce phosphatase enzyme was determined by using CQC reagent. Phosphatase enzyme acts on disodium phenyl phosphate to liberate phenol. The indicator (CQC) 2, 6 dichloroquinone chloramide reacts with phenol to give blue color which is directly proportional to amount of phosphate present.

Pot Assay

Ability of each actinomycetes isolates to become candidate for PGPR agent was checked by pot assay. In which selected plant such as Wheat, Maize, Chilly and Tomato were allowed to grow in exposure of these actinomycetes as given below:

Seed sterilization

Commercially available Wheat, Maize, Chilly and Tomato seeds were used as the test plants. Surface sterilization of all seeds was carried out by soaking them in a solution of 0.4% sodium hypochlorite, followed by water wash and then with 0.1% Twin 80 for 5min, subsequently all seeds were rinsed extensively with sterile demonized water and soaked to dryness on sterile filter paper.

Treatment and Data recording

Dried seeds were then coated with particular bacterial suspension by using 1% carboxymethyl cellulose as an adhesive. After seed treatment, they were sowed in autoclaved soil kept in labeled plastic bags. In a positive control group, *Pseudomonas* species with known phosphate solubilizing capability was considered and in normal control group uninocualted plants were taken and given only water as supplement. Study was carried out in triplicate for each plant and the significant changes were recorded and statistically analyzed using one way ANOVA with $P<0.05$(Dunett's multiple comparison Test). The comparative data was recorded for the change in shoot length and root length compared to treatment and control.

RESULTS

Phosphate solubilization

Qualitative estimation of phosphate solubilization by Actinomycetes strain grown on Pikovaskaya's agar containing tri calcium phosphate showed the development of a clear solubilization zone around the colony as evidenced in Fig. 1 Based on the results, it has been observed that only 22 isolates were found to be capable of phosphate solubilization. Those isolates showcasing higher zone as >30 mm are preferable for further studies.

In the present study the optimum incubation period for the maximum phosphate solubilization was found to be 15 days of incubation which remain same on extension till 20th day .Our study states that the optimum temperature for phosphate solubilization was 28°C.

Quantitative analysis of P solubilization

During quantitative estimation it has been evidenced that 13 actinomycetes isolates profoundly detected positive for phosphate solubilization.

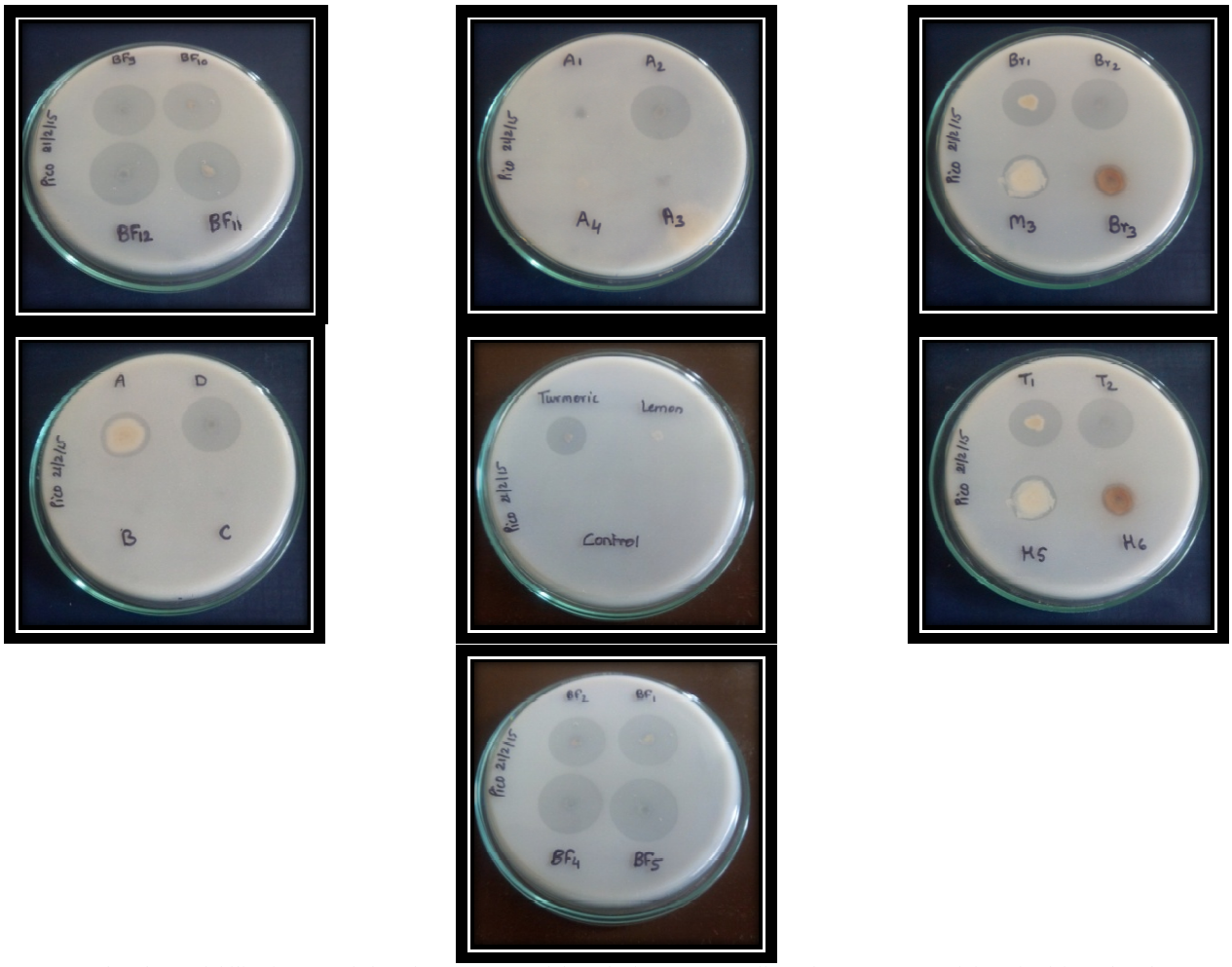


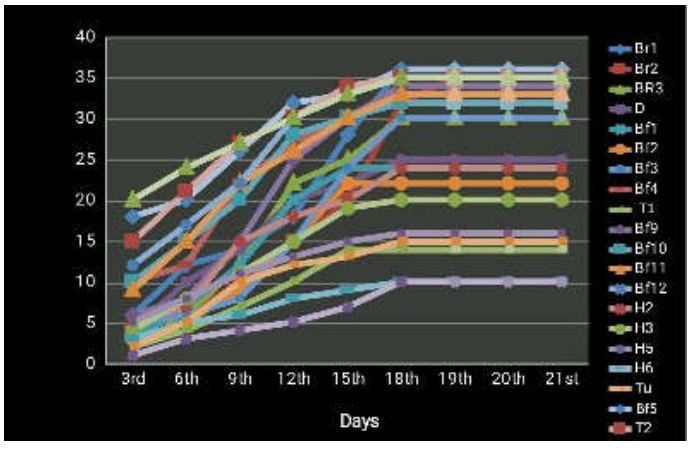
Fig 1 Phosphate solubilization recorded as clear zone around the colonies grown on Pikovaskaya's agar containing tri calcium phosphate.

Solubilization index

As per formula Actinomycetes isolates BF_3, BF_4, BF_9, BF_{10}, BF_{11}, BF_{12}, T_1, T_2 and A_2 showed highest SI, while the other isolate Br_1, Br_2, Br_3,BF_1,BF_2, BF_5,A, D, H_5, H_6, H_3, H_2 and Tu showed the lowest of SI. Among the isolates, lowest SI value was recorded as 8 and highest as 25 and the mean value as 17.04±1.32.

Effect of pH, temperature and Incubation period

To study the effect of pH, temperature and incubation time on the phosphate solubilization ability; isolates were inoculated in the Pikovaskaya's agar supplemented with 0.5% (w/w) tricalcium phosphate. Phosphate solubilization was observed at pH 7 with the maximum phosphate solubilization.



Graph 1 Effect of incubation period on zone diameter of phosphate solubilization

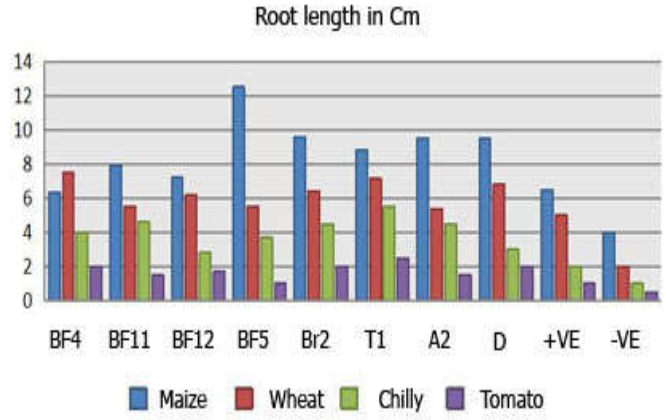
Among them decreasing order of solubilization was showcased as, BF_5 (180 µg/mL), T1(160 µg /mL), mL), Bf4(140ug/ml), Br2 (120 µg/mL), A2 (100 µg/mL), D (94 µg/mL), BF11 (72 µg/mL), BF3 (60 µg /mL), BF10 (54 µg /mL), BF9 (50 µg /mL), Br3 (30 µg /mL) and T2 (4 µg /mL). As per comparative study, huge difference was recorded among the isolates for the present phosphate solubilization property where minimum solubilization was recorded as 4 µg /mL and maximum as 180 µg /mL with the mean value recorded as 91.92±14.58 µg /mL .

Phosphatase Enzyme Detection

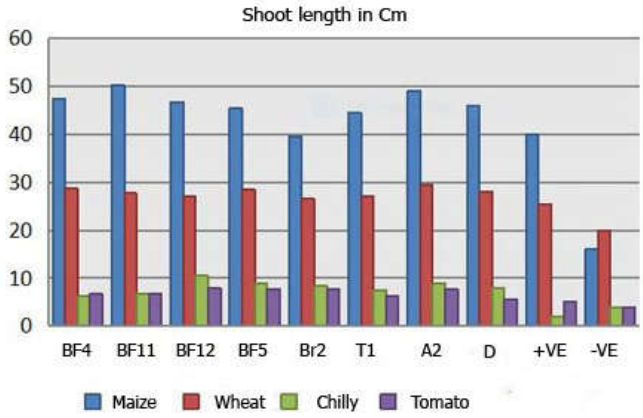
Presence of phosphatase activity by using indicator CQC again confirmed that the 12 isolates Br_1, Br_2, Br_3, BF_3, BF_5, BF_{10}, BF_{11},BF_{12}, T_1, T_2, A_2 and D isolates posses the feature when detected by the change in color to blue as compared to control which remained off light brown

Effect of selected isolates on growth of Wheat, Maize, chilly and Tomatoes

Overall it has been observed that the selected eight actinomycetes imparted positive regulation by increasing the root length of wheat, even in a better manner than positive control which signifies the features of these isolates. Pot trial reveals that actinomycetes isolates (BF5, A2 ,D, T1 , Br2) are showing significant effect on plant growth when the data was analyzed using Dunnett's Multiple Comparison Test with P Value set at <0.05 It has been observed that all bacteria inoculated plants showcased significant (P<0.05) increased in root and shoot length after 20 days of inoculation and even few strains for Ex: Isolate BF_5 treated plants of wheat outscored positive control plants (5.25±0.08cm) with root length (7.30±0.122cm) and shoot length (47.50±0.22 cm) as compared to positive control (41.08±0.39 cm) thus clearly indicated the promising features. Overall picture based on the results, highlighting that all eight Actinomycetes isolates recognized as better PGPR agents and even worked in define manner to allow promising growth of four plants tested. After field trial these isolates can be used for preparation of bioinoculant.



Graph 2 Effect of treatment of Actinomycetes isolate on root length of plants



Graph 3 Effect of treatment of Actinomycetes isolate on shoot length of plants

Fig 2 Planted Maize plants showing increased shoot length in treated groups

Fig 3 Planted chilly plants showing increased shoot length in treated groups compared

Fig 4 Planted Wheat plants showing increased shoot length in treated groups compared to control (extreme right)

Fig 5 Planted Tomato plants showing increased shoot length in treated groups compared to control (extreme right)

DISCUSSION

Phosphate solubilization has been observed in number of actinomycetes isolates on Pikovaskaya's agar containing tri calcium phosphate. Levels of zone of clearance remain varied as compared to each other. Among all, highest zone of clearance was recorded in isolates BF_5 and T_1 with zone measuring >30 mm. In number of reports actinomycetes and other bacterial species was reported to bring about P solubilization and indicative of plant growth promotion

activity. According to Dastager and Damare (2013) isolated actinobacteria from the sediments of Chrao island, Goa province, India posses phosphate solubilization feature. Out of 200 isolates, 30 isolates prominent in the activity and maximum solubilization was recorded to be 89.3±3.1 to 164.1± 4.1 μgmL^{-1} after 6 days of incubation in six of all isolates.

Sampling of actinomycetes also been reported from the agriculture soil and a rock phosphate processing unit and recorded promising for the mineral phosphate-solubilizing (MPS) ability. In total 30 isolates found to be positive for the said activity when grown on National Botanical Research Institute's phosphate broth. Among all, CTM396 and CTM397 strains showed highest MPS abilities and identified by 16S rRNA gene sequencing as members of the genus Streptomyces. They also recorded drop in pH due to secretion of gluconic acid (Farhat MB *et al*., 2015).

Isolation of actinomycetes with P solubilization feature from Sediment samples from different stations of the Vellar estuary, India has also been reported; where they recorded P solubilization remain affected by physiochemical parameters such as pH and incubation period. They reported that seven strains showcased optimum P solubilization at pH 7. In present study also it has been evidenced that the pH 7 brought about the highest P solubilization and in agreement with the reports published earlier. As per results in present study, maximum P solubilization minimum incubation period of 15 days is required so that maximum phosphorus leach out in the medium whereas, according to Sahu MK *et al*., (2007) 13 days is the maximum incubation period for actinomycetes to perform P solubilization (Sahu MK *et al*., 2007).

In current study for quantitative P solubilization, out of 33 isolates only 13 actinomycetes prominently detected for activity. Among them, highest solubilization was recorded for the isolate BF_5 (180 µg/ml) and followed by T1 (160 µg/ml). As per report published earlier maximum P solubilization of actinomycetes was 164.1µg/ml isolated from the Chorao Island, Goa India. Hence it is indicative that use of BF_5actinomycetes as PGPR agent may be best suited (Dastager SG and Damare S, 2013).

In phosphate solubilization phosphatase enzyme plays an important role. In the present study out of 33 isolates, only 12 isolates prominently expressed phosphatase activity and recognized to convert inorganic phosphate due to activity of phosphatase. In many reports soil fertility has been related with enzyme complex available in soil. This enzyme complex activity remains affected by factors such as pH and temperature. In one report, correlation analysis showed that fungi, actinomycetes and neutral phosphatase were significantly correlated with *P. notoginseng* agronomic characters. Also actinomycetes and neutral phosphatase correlated with the relative water content and soil pH (Liao PR *et al*., 2015). In one report effect of nitrogen, phosphorus and potassium in different fertilization levels on *Platycodon grandiflorum* soil microorganism and activities of soil enzyme using statistical design has been proposed. They found out that enzyme activities are getting affected due to concentration variation of macronutrients. Among them, increased phosphatase activity was recorded in N0P2K2, N2P2K0 and N3P1K3 except N1P3K3 (Wang WL *et al*., 2013). It has been reported earlier that added herbicides such as diflufenican, mesosulfuron-methyl and iodosulfuron-methyl-sodium when applied in soil in different doses, the phosphatase activity shows profound variations. It has been put forward that herbicides in recommended doses stimulated the activity of acid phosphatase, catalase, urease and had no effect on dehydrogenase and alkaline phosphatase. In contrast, highest dose of 36.480 mg Kg -1 significantly inhibited the activity of dehydrogenase, acid phosphatase and alkaline phosphatase of soil enzyme complex (Bacmaga M *et al*., 2015).

CONCLUSION

The present study clearly demonstrated that plant growth promoting rhizobacteria inoculation significantly improved the growth of four plants *viz*., Wheat, Maize, Chilly and Tomato. Based on biochemical and potency check of all isolates; finally selected eight isolates when applied as seed coating maintained their detected potential and overall plant growth was found to be improved compared to uninoculated control and recorded comparable to positive control group. This has suggested that addition of microbiome in close proximity of the plants definitely add up the required nutrition and protection which plants are always in requirement.

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References

1. Abd-Alla, M.H., 1994. Phosphatases and the utilization of organic phosphorus by Rhizobium leguminosarum biovarviceae. *Lett. Appl. Microbiol*. 18: 294-296.
2. Belimov, A. A., A. P., .Kojemiakov and C. V., Chuvarliyeva, 1995. Interaction between barley and mixed cultures of nitrogen fixing and phosphate-solubilizing bacteria. *Plant Soil*. 173:29-37.
3. Cakmak, I (2008) Zinc Deficiency in Wheat in Turkey. Chap. 7 In Alloway, B.J. (Ed.) Micronutrient Deficiencies in Global Crop Production, Springer, Dordrecht, pp181-200.
4. Cakmak I.," Enrichment of cereal grains with zinc: agronomic or genetic biofortification?" Plant and Soil, vol. 302, no. 1-2, pp. 1-17, 2008.
5. Chabot, R., C. J. Beauchamp, J. W. Kloepper and H. Antoun, 1998. Effect of phosphorous on root colonization and growth promotion of maize by bioluminescent mutants of phosphate solubilizing Rhizobium leguminosarum biovar. *Phaseoli. Soil Biol. Biochem*. 30:1615-1618.
6. Dastager SG, Damare S. Marine actinobacteria showing phosphate solubilizing efficiency in Chorao Island, Goa, India. *Curr Microbiol.* 2013 May;66(5):421-7.
7. Desai S., G. Praveen Kumar, U. Sultana *et al*., "Potential microbial candidate strains for management of nutrient requirements of crops," *African Journal of Microbiology Research*, vol. 6, no. 17, pp. 3924-3931, 2012.
8. Di Simine CD., J. A. Sayer, and G. M. Gadd, "Solubilization of zinc phosphate by a strain of Pseudomonas fluorescens isolated from a forest soil,"

Biology and Fertility of Soils, vol. 28, no. 1, pp. 87-94, 1998.

9. Ehrlich H. L., 1990. Geomicrobial processes. A physical and biochemical overview. In Geomicrobiology, 2nd Ed.Marcel Dekker.
10. Farhat MB, Boukhris I, Chouayekh H. Mineral phosphate solubilization by Streptomyces sp. CTM396 involves the excretion of gluconic acid and is stimulated by humic acids. *FEMS Microbiol Lett*. 2015 Mar; 362(5). pii: fnv008.
11. Fasim F., N. Ahmed, R. Parsons, and G. M. Gadd, "Solubilization of zinc salts by a bacterium isolated from the air environment of a tannery*," FEMS Microbiology Letters,* vol. 213, no. 1, pp. 1-6, 2002.
12. Gaur, A. C. and S. Gaind, 1999. Phosphate solubilizing microorganisms-An overview. Agromicrobes. Current trends in life sciences, Today and tomorrows publishers, New Delhi. India. 23:151-164.
13. Gaur, A. C. and S. Gaind, 1999. Phosphate solubilizing microorganisms-An overview. Agromicrobes. Current trends in life sciences, Today and tomorrows publishers, New Delhi. India. 23:151-164.
14. Goldstein, A. H., 1986. Bacterial solubilization of mineral phosphates: historical perspective and future prospects. *Am. J. Altern. Agric.* 1:51-57.
15. Goldstein, A. H., 1994. Involvement of the quinoprotein glucose dehydrogenase in the solubilization of exogenous mineral phosphates by gram negative bacteria. In: Torriani-Gorni, A., E. Yagil, S. Silver, eds. Phosphate in Microorganisms: Cellular and Molecular Biology. ASM Press, Washington, DC. 197-203.
16. Graham, R.D. (1983) Effects of nutrient stress on susceptibility of plants to disease with particular reference to the trace elements. In: Woolhouse, H. (ed.). Advances in Botanical Research, 10:221-276, Academic Press, London.
17. Grewal, H.S., R.D. Graham and Z. Rengel (1996) Genotypic variation in zinc efficiency and resistance to crown rot disease (Fusarium graminaearum Schw. Group 1) in wheat. Plant and Soil, 186:219-226.
18. Igual, J. M., A. Valverde, E. Cervantes, E. Velásquez, 2001.Phosphate solubilizing bacteria as inoculants for agriculture: use of updated molecular techniques in their study. *Agronomie*. 2:561-568.
19. Illmer P. and F. Schineer, 1995. Solubilization of inorganic calcium phosphate solubilization mechanisms. *Soil Biol Biochem*. 27:257-263.
20. Kucey, R. M. N., 1983. Phosphate solubilizing bacteria and fungi in various cultivated and virgin Alberta Soil. *Can. J. Soil Sci*. 63: 671-678.
21. Passari AK, Mishra VK, Gupta VK, Yadav MK, Saikia R, Singh BP. In Vitro and In Vivo Plant Growth Promoting Activities and DNA Fingerprinting of Antagonistic Endophytic Actinomycetes Associates with Medicinal Plants. PLoS One. 2015 Sep 30;10(9):e0139468.
22. Peix, A., A.A. Rivas-Boyero, P. F. Mateos, C. Rodirguez-Barrueco, E. Martinez-Molina and E. Velazquez, 2001a. Growth promotion of chickpea and barley by a phosphate solubilizing strain of Mesorhizobium mediterraneum under growth chamber conditions. *Soil Biol. Biochem*. 33: 103-110.
23. Peix, A., A.A. Rivas-Boyero, P. F. Mateos, C. Rodirguez-Barrueco, E. Martinez-Molina and E. Velazquez, 2001. Growth promotion of chickpea and barley by a phosphate solubilizing strain of Mesorhizobium mediterraneum under growth chamber conditions. *Soil Biol. Biochem*. 33: 103-110.
24. Poomthongdee N, Duangmal K, Pathom-aree W. Acidophilic actinomycetes from rhizosphere soil: diversity and properties beneficial to plants*. J Antibiot (Tokyo)*. 2015 Feb;68(2):106-14.
25. Puente ME, C. Y. Li, and Y. Bashan, "Microbial populations and activities in the rhizoplane of rock-weathering desert plants. II. Growth promotion of cactus seedlings," *Plant Biology*, vol. 6, no. 5, pp. 643-650, 2004.
26. Richardson AE. "Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants," *Australian Journal of Plant Physiology*, vol. 28, no. 9, pp. 897-906, 2001.Sahu MK, Sivakumar K, Thangaradjou T, Kannan L. Phosphate solubilizing actinomycetes in the estuarine environment: an inventory. *J Environ Biol*. 2007 Oct;28(4):795-8.
27. Selvakumar G, Bhatt RM, Upreti KK, Bindu GH, Shweta K. Citricoccus zhacaiensis B-4 (MTCC 12119) a novel osmotolerant plant growth promoting actinobacterium enhances onion (Allium cepa L.) seed germination under osmotic stress conditions. *World J Microbiol Biotechnol*. 2015 May;31(5):833-9.
28. Seshadre S., R. Muthukumarasamy, C. Lakshminarasimhan, and S. Ignaacimuthu, "Solubilization of inorganic phosphates by Azospirillum halopraeferans," *Current Science*, vol. 79, no. 5, pp. 565-567, 2002.
29. Shekhar, N. C., S. Bhaclauriay, P. Kumar, H. Lal, R. Mondal and D. Verma, 2000. Stress induced phosphate solubilization in bacteria isolated from alkaline soils. *FEMS Microbiol. Lett.* 182:291-296.
30. Sudhakara, R. M., S. Kumar, K. Babita and M. S. Reddy, 2002. Biosolubilization of poorly soluble rock phosphates by Aspergillus tubingensis and Aspergillus niger. *Bioresour. Technol*. 84: 187-189.
31. Sundara, B., V. Natarajan and K. Hari, 2002. Influence of phosphorus solubilizing bacteria on the changes in soil available phosphorus and sugarcane and sugar yields. *Field Crops Res*. 77: 43-49.
32. Tilak, K.V.B.R., N. Ranganayaki1, K. K. Pal, R. De, K. Saxena, C. S. Nautiyal, S. Mittal, A. K. Tripathi and N. Johri, 2005. Diversity of plant growth and soil health supporting bacteria. *Current Science*. 89: 136.
33. Toro, N., R. Azco´ n and J. M. Barea, 1997. Improvement of arbuscular mycorrhiza development by inoculation of soil with phosphate-solubilizing rhizobacteria to improve rock phosphate bioavailability (32P) and nutrient cycling. *Appl. Environ. Microbiol*. 63: 4408-4412.
34. Wang WL, Du JB, Xu FL, Zhang XH. [Effect of fertilization levels on soil microorganism amount and soil enzyme activities]. Zhongguo Zhong Yao Za Zhi. 2013 Nov; 38(22):3851-6.