Effects of Different Physical and Chemical Parameters on Phosphate Solubilization Activity of Plant Growth Promoting Bacteria Isolated from Indigenous Soil

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Abstract: The microorganisms play a vital role in fertility of soil and hence favors' large group of plants. In present study we have successfully isolated certain bacteria from soil including both Gram positive and Gram negative bacteria and access their ability in phosphate solubilization. Effects of temperature, pH and different carbon sources, on phosphate solubilization, by these isolates were also determined. Low temperature 25 °C, pH between 5-7, and glucose as carbon source were found to be best for phosphate solubilization by most of the isolates. Present study highlights the importance of these plants growth promoting bacteria and their uses for agriculture purposes.

Keywords: Phosphate solubilization, Plant growth promoting bacteria, Indigenous Soil, Temperature, pH and Carbon sources.

INTRODUCTION

Due to the advancements in science the approach towards use of pesticides and in organic fertilizers has changed and scientists prefer its use in a lesser quantity. This has in turn developed use of other sources which help in better agricultural yield in the prevailing conditions [1]. There is a varied population of population of microorganisms in our soil which may be harmful as well as beneficial. The plant growth promoting Rhizobacteria (RGPR) are beneficial organisms and play an important role in agricultural growth. These organisms take part in recycling of different elements such as nitrogen & phosphorus which help in soil fertility [2, 3] and increase plant growth by direct & indirect mechanisms [4]. Kloepper and Schroth in 1981 [5] used the term plant growth promoting Rhizobacteria for root colonizing bacteria that enhance growth of plant. About a century back, there was inoculation of beneficial bacteria onto plants. There were two major achievements in the history of plant growth promoting bacteria in late 70s, firstly, It was revealed that Azospirillum affects the plant metabolism directly and results promotion of plant growth [6, 7]. Secondly, In the recent days many of the bacterial isolates along with Serratia, Bacillus, Flavobacteria are considered for their plant growth promoting ability [8, 9] and few species of pseudomonas specially *P. putida* and *P. fluorescens* groups were particularly considered for their effectiveness in biocontrol [10, 11].

The inoculation of Rhizobium along with PGPR (plant growth promoting rhizobacteria) gave beneficial effects which have been reported in a number of papers [12-15]. It also increased yield of pigeon pea in rain field soil as they combined the rhizobium spp. along with rhizobacterial strain, it results in positive effect on fixing Nitrogen and physical growth of plant [16].

Nitrogen fixation can also be increased by addition of a wide verity of free living bacteria, but the amount of nitrogen fixed by these organisms is not sufficient for the bacterially associated plants. According to Verma, (1993) [17] with the use of phosphate solubilizing microorganisms (PSM) crops yield can be increased up to 70%. This is because of the ability of PSM to solubilized the fixed soil phosphate as well as applied phosphate which is given in the form of Agrochemical [18] and improves the growth of plant [19].

A component of Rhizosphere- *Micromonaspora enodolithica* can solubilize considerable amount phosphorus and which produces organic acid and acidic & alkaline phosphate but cannot synthesize growth enhancing compounds which have been reported to increase beans growth [20]. Species of Pseudomonas have been reported to increase the nodules number and its dry weight grain yield, availability and improved uptake of nutrient in soya bean crop [21].

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Excessive use of chemical fertilizers has very much disturbed the balance of ecology of soil which also results in removal of nutrients [22], which consequently results in considerable reduction in the use of chemicals as pesticides and fertilizers in agriculture throughout the world [23]. There is an immediate need of source that can maintain the quality & quantity of crops, which cannot contaminate the environment and which is not hazardous for human health which would be replaced the chemical fertilizers [1]. The use of microbes which are beneficial to plants can be used as biofertilizers which will improve health of plants and soil fertility. The soil microbes help to maintain health and quality of soil which is due to biogeochemical cycles of organic and inorganic nutrients [24]. The biofertilizers are in demand nowadays because they are not toxic, there is no hazard in their use and are friendly with the environment [22]. The need of the day is to select the best microorganism which is beneficial, inoculate it in a crop singly or in combination 25]. To improve the health of plant and have increased production there are free living bacteria which are beneficial and are in the form of Plant growth promoting Rhizobacter (RGPR) or yield increasing bacteria (YIB) yield [5, 26, 27]. Some bacteria are responsible for the resistance production against plant pathogens and increased plant growth [28].

The aim of present study was to isolate some environmental friendly bacteria from indigenous soil which can be used as biofertilizer

MATERIAL AND METHOD

Sample Collection

Seven (7) soil samples were collected in sterilized bottles from agricultural field of Malir and back yard garden of Staff Town, University of Karachi.

Isolation and identification of selected strains of bacteria was performed bacteria were identified by 16S rRNA [29], as well as phosphate solubilization ability of selected strains was also done [30].

Effect of Different Parameters on Phosphate Solubilizing Ability of Selected Strains

Effect of Temperature on Phosphate Solubilizing Activity of Selected Strains

The effect of various temperatures on phosphate solubilizing ability of selected phosphate solubilizing isolates was determined by the method of Shahab and Ahmad (2008) [31] with slight modification. Bacterial culture were grown in Tris minimal broth, approximately 10 µl of this over night (O/N) grown cultures were transferred to the tris minimal agar plates containing 1% glucose and 5 mm $ZnCl_2$ and incubated at 30°C and 37°C separately for 5 days and observed for clear zone after 48 hours.

Effect of pH on Phosphate Solubilizing Activity

The effect of pH on phosphate solubilizing ability of selected phosphate solubilizing isolates was determined by inoculating selected strains on TMM agar plates amended with 5mm $ZnPO_4$ and 1% glucose, pH was adjusted from 2-10. Bacterial cultures were grown for O/N in TMM broth and transferred 10 µl of these cultures to Tris minimal agar plates incubated for 48 hours at 28°C ± 2°C and were observed for the halo zone formation, the size of halo was also measured.

Effect of Different Carbon and Nitrogen Sources on Phosphate Solubilizing Activity

Effects of different carbon and nitrogen sources on phosphate solubilizing ability of selected phosphate solubilizing isolates was observed by adding 1% glucose, fructose, mannitol and lactose separately in TMM agar with 5mm ZnPO4 [32]. Bacterial cultures were grown in Tris minimal broth for 24 hours in {ris minimal broth}, nearly 10µl of culture was transferred to the Tris minimal agar plates amended with different source of carbon incubate at 28°C ± 2°C for 48 hours were observed for halo zone formation around bacterial colonies and halo zone were measured.

To determine the effect of different nitrogen sources on phosphate solubilization the ammonium chloride (NH_4Cl) from the medium was replaced by ammonium sulphate [$(NH4)_2SO_4$], ammonium nitrate (NH_4NO_3), sodium nitrate ($NaNO_3$) and calcium nitrate $Ca(NO_3)_2$

RESULTS

Isolation and Purification of Phosphate Solubilizing Bacteria (PSB) from Sea and Agricultural soil

Total of 32 bacterial isolates were isolated and purified from agricultural soil of Sindh.

Out of 32 isolated bacterial strains only 8 strains were gram positive whereas remaining were gram negative.

By 16S rRNA, CMG 7, CMG 13 and CMG 15 were identified as *Serratia marcescence*, whereas CMG 4 and CMG 22 were identified as *Pseudomonas aeruginosa*.

Strains CMG 5, CMG 24 and CMG 26 were Gram positive. Strain CMG 5 was Gram positive cocci and strain CMG 24 and CMG 26 were spore former Gram positive bacilli they have been identified up to genera as the kit for Gram positive organisms are not available. CMG 5 was identified as micrococcus whereas CMG 24 & 26 were identified as bacillus by 16S rRNA (CMG stand for center for Molecular Genetics).

Effects of Temperature on Phosphate Solubilization

Phosphate solubilization was found to be increased at low temperature 25°C, and it was observed that at high temperature although the bacterial growth was increased but the halo zone was decreased showed decreased in phosphate solubilization ability at high temperature (Table **1**).

Table 1: Effect of Temperature on Phosphate Solubilization

	Zone Size (in mm) at	
Strain code CMG	**Temp 37°C**	**Temp.30°C**
4	22	32
5	12	14
7	16	23
13	18	26
15	24	35
22	15	20
24	10	13
26	12	16

Effects of pH on Growth and Phosphate Solubilization

There was no significant growth and halo formation was observed between pH 1-5. Majority of the strains give maximum halo zone at pH 6 and pH 7 only. Three strains CMG4, CMG22 and CMG26 gave growth and halozones at pH 8 too (Table **2**).

Effects of Carbon Source on Phosphate solubilization

All strains showed largest halo when glucose was used as a carbon source indicating that all the strains gives maximum solubilization of phosphate in the presence of glucose. With the exception CMG 4 all the strains showed phosphate solubilization when fructose was added as carbon source. CMG7 and CMG13 did not show any solubilizing activity in the presence of mannitol whereas CMG7 and CMG24 did not show solubilization activity in the presence of lactose (Table **3** and Graph **1**).

Effect of Different Nitrogen Source on Phosphate solubilization

All the strains showed good phosphate solubilization activity in the presence of $(NH_4)_2SO_4$. Except CMG4, all isolates showed halo in the presence of $(NH_4)NO_3$ while no strains showed phosphate solubilization action in the presence of $NaNO_3$ and $Ca(No_3)_2$ (Table **4**).

DISCUSSION

The plant growth promoting rhizobacteria (PGPRB) have the ability to directly or indirectly promote plant growth. A number of bacteria act as PGPRB e.g. *Klebsella, Arthrobacter, Enterobacter, Pseudomonas* etc.

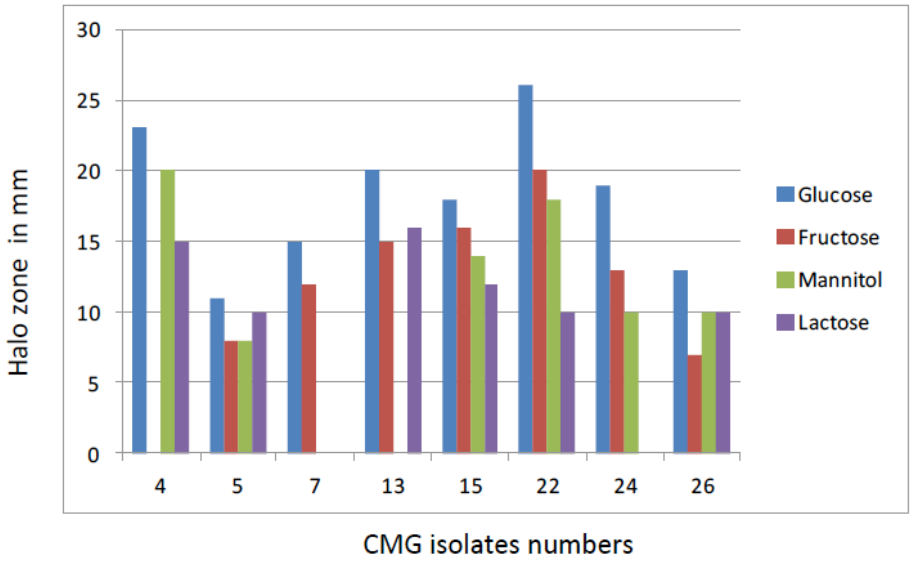
A bacteria may be called as PGPRB if it has the ability to solubilize the phosphate and make them available to plant for their growth, if they can stimulate plant growth, if they have aggressive colonization around root, if they can control Phytopathogens

Table 2: Effect of pH on Growth and Phosphate Solubilization

pH→	**2**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Source code CMG↓	**Size of hallow (in mm)**							
4	-	-	20	20	20	20	-	-
5	-	-	12	12	10	-	-	-
7	-	-	10	12	12	-	-	-
13	-	-	9	12	12	8	-	-
15	-	-	18	26	26	-	-	-
22	-	-	18	20	20	-	-	-
24	-	-	20	19	18	18	-	-
26	-	-	14	10	10	-	-	-

Table 3: Effect of Carbon Source on Phosphate Solubilization

Strain Code	Glucose	Fructose	Mannitol	Lactose
4	23mm	--------	20mm	15mm
5	11mm	8mm	8mm	10mm
7	15mm	12mm	--------	-----
13	20mm	15mm	--------	16mm
15	18mm	16mm	14mm	12mm
22	26mm	20mm	18mm	10mm
24	19mm	13mm	10mm	-------
26	13mm	7mm	10mm	10mm



Graph 1: Effect of Carbon source on Phosphate solubilization.

Table 4: Effect of Different Nitrogen Sources on Phosphate Solubilization Activity

Strain Code	$(NH_4)_2SO_4$	NH_4NO_3	$NaNO_3$	$Ca(NO_3)_2$
4	25mm		-	-
5	14mm	10mm	-	-
7	20mm	12mm	-	-
13	17mm	17mm	-	-
15	22mm	20mm	-	-
22	23mm	21mm	-	-
24	20mm	14mm	-	-
26	14mm	10mm	-	-

(Vessey 2003) [33] or at least have any two of these criteria.

Phosphate solubilizing microorganisms (PSM) which can solubilize phosphates and can demonstrate phosphate solubilizing ability phenotypically [34]. Present study was carried out in Karachi where 32 bacterial strains were isolated from the rhizosphere of different areas of Karachi. The common feature of gram negative bacteria is mineral phosphate

solubilization which has already been reported [35]. The present results are in agreement with [36], as reported that 90 % isolates of rhizosphere were gram negative.

Temperature, pH, carbon and nitrogen sources are different parameters and their effects were observes on phosphate solubilizing ability of PGPR. The isolates were grown at 37°C which is the best suited temperature for growth of mesophilic bacteria and 30°C to check effect of temperature on phosphate solubilizing isolates. As a result it was revealed that halozones were smaller at 37°C with large sized colonies but in case of 30°C, 100% isolates had maximum solubilization which is same as shown by researchers who worked on 30°C and declared 30°C as best temperature for phosphate solubilization [37, 38]. For optimum temperature there were differ the views of different researchers according to Shahab and Ahmed 2008 [31] 25^{0}C is the optimum temperature for phosphate solubilization. Kang (2002) [39] reported that in few studies 28°C was found as optimum temperature for phosphate solubilization. The solubilization can occurred at extreme temperatures i.e. 45^{0}C and 15-4°C respectively [23]. According to these studies, the bacteria can adapt themselves and show their activities according to conditions.

Conditions like type of organisms, pH., nitrogen source in media and carbon play an important role in solubilization of phosphates [40]. For best solubilization pH of strain should be 5-7 and there is no growth below 5 and above 8 pH. CMG 4 and CMG 22 exhibited best results at pH 8. Same was the result in case of CMG 13 but at a lower extent. These results match with the results of Farhat *et al.,* 2009 [38].

To check their effect on phosphate solubilizing ability different sources of carbon were added to minimal medium amended with $ZnPO_4$. The results of Narsian and patel (2000) [41] are also in agreement with the above results according to which *A. aculeatus* was able to show phosphate solubilization in the presence of different carbon sources in order of >glucose >fructose >mannitol>xylose >aranbinose >maltose >sorbitol>sucrose >glycerol >galactose>Lactose. CMG 4 did not show any solubilization on fructose whereas for all other isolates glucose supported the highest solubilization. All isolates showed solubilization on mannitol except CMG 7 and CMG 13 whereas CMG 15,CMG 7, CMG 13 & CMG 24 did not show any growth or solubilization on lactose also. These results are same as that of Nautiyal, (2000) [42] *Serratia marcesance* CTM 50650 can use different carbon sources with highest solubilization in presence of Glucose [38].

$(NH_4)_2SO_4$, NH_4NO_3, $NaNO_3$ and $Ca(NO_3)_2$ are four different nitrogen sources were used to determined the effect of nitrogen sources on phosphate solubilizing ability of isolates. All the isolates showed a big halo zone in the presence of $(NH_4)_2SO_4$ except CMG4 all showed halo zone in the plate amended with $(NH_4)NO_3$ or $CaNO_3$ as a source of nitrogen. According to several studies ammonia is a better source of Nitrogen as compared to nitrate [43, 44].

CONCLUSION

In the present research work we able to isolates such bacteria which can promote the growth of plants from indigenous soil samples. These bacteria were quite effective in phosphate solubilization and effect of various factors on growth were also studied, however, further research is needed to explore other potentials of these isolates and to find out genetic stability of these properties.

REFERENCES

[1] Ahmed S. Agriculture-fertilization interface in Asia issue of growth and sustainability. New Delhi: Oxford and IBH publishing Co. 1995.

[2] Woressa D, Assefa. Effect of Plant Growth Promoting Rhizobacteria on growth and yield of tef (Erogrostis tef zucc. Trotterunder greenhouse condition. Res J Microbiol 2011; 6: 343-355. http://dx.doi.org/10.3923/jm.2011.343.355

[3] Oyeyiola GP. Rhizosphere bacterial flora of Amaranthus hybridus. Res J Microbial 2010; 5: 137-143.

[4] Ahmed M, Khan MS. Assesment of plant growth promoting activities of Rhizobacterium pseudomonas Putida under insecticide stress. Microbiol J 2011; 2: 54-64.

[5] Kloepper JW, Schroth MN. Plant growth promoting rhizobacteria and plant growth under gnotobiotic conditions. Phytopathology 1981; 71: 642-644. http://dx.doi.org/10.1094/Phyto-71-642

[6] Dobereiner J, Day JM. Associative symbioses in tropicalgrasses; characterization of Microoganism and dinitrogen fixing sites. In proceeding of 1st International symposium on Nitrogen fixation. Newton WE, Nyman CJ, Eds. 1976; Vol. 2: pp. 518-538.

[7] Bashan Y, Holguin. Azospirillum-Plant relationships: environmental and physiological advances. Can J Microbiol G 1997; 43: 103-121. http://dx.doi.org/10.1139/m97-015

[8] Gulati A, Rahi P, Vyas P. Characterization of phosphate solubilizing florescent *Pseudomonas* from the rhizosphere of sea buckthorn growing in the cold desert of Himalayas. Curr Microbiol 2008; 56: 79-82. http://dx.doi.org/10.1007/s00284-007-9042-3

[9] Tang WH, Yang H. Research and application of biocontrol of plant diseases and PGPR in China. In plant growth promoting Rhizobacteria present status and future prospective 1997; pp. 4-9.

[10] Defago G, Berling CH, Burger U, Hoas D, Kahr G, Keel C, Voisard C, Wirthner P. Supression of black root rot of tobacco and other root diseases by strains of pseudomonas fluorescens; Potential applications and mechanisms. In biological control of soil borne plant pathogens. International Wallingford, Oxford, UK 1992; pp. 93-108.

[11] Glick BR. The enhancement of plant growth by free living bacteria. Can J Microbiol 1995; 41: 109-117. http://dx.doi.org/10.1139/m95-015

[12] Hicks PM, Loynachan TE. Bacteria of the soya bean rhizosphere and their effect on growth of Bradyrhizobium Japonicum. Soil Biol Biochem 1989; 21: 561-566. http://dx.doi.org/10.1016/0038-0717(89)90130-2

[13] Marcel D, Newyork RJ, Triplet EW. Toward more productive, efficient and competitive nitrogen fixing symbiotic bacteria. Crit Rev Plant Sci 1996; 15: 191-234. http://dx.doi.org/10.1080/07352689609701941

[14] Okon Y, Bloemberg GV, Lugtenberg JJ. Biotechnology of biofertilization and phyto stimulation. Agric Biotechnol 1999; 99: 327-349.

[15] Lata SAK, Tilak KVBR. Biofertilizer to augment soil fertility and crop production. Krishna KR, Ed. 2000; pp. 279-312.

[16] Tilak KVBR, Rangamayaki N, Manoharachari C. Synergistic effects of plant growth promoting rhizobacteria and Rhizobium on nodulation and nitrogen fixation by pigeon pea (Cajanus Cajan). Eur J Soil Sci 2006; 57: 67-71. http://dx.doi.org/10.1111/j.1365-2389.2006.00771.x

[17] Verma LN. Biofertiliser in agriculture. In: Thampan PK, Ed. Organics in soil health and crop production. Peekay Tree Crops Development Foundation, Cochin, India 1993; pp. 152-183.

[18] Zaidi A. Synergistic interactions of nitrogen fixing microorganisms with phosphate mobilizing microorganisms. Ph.D. Thesis, Aligarh Muslim University, Aligarh, India 1999; pp. 26-29.

[19] Ponmurugan P, Gopi C. Distribution pattern and screening of phosphate solubilizing bacteria isolated from different food and forage crops. J Agron 2006; 5: 600-604. http://dx.doi.org/10.3923/ja.2006.600.604

[20] Gamalero E, Lingua G, Berta G, Glick BR. Beneficial role of plant growth promoting bacteria and arbuscular mycorrhizal fungi on plant responses to heavy metal stress. Can J Microbiol 2009; 55: 501-514. http://dx.doi.org/10.1139/W09-010

[21] Son H, Park G, Cha M, Heo M. Solubilization of insoluble inorganic phosphates by a novel salt- and pH-tolerant Pantoea agglomerans R-42 isolated from soybean rhizosphere. Bioresour Technol 2006; 97: 204-210. http://dx.doi.org/10.1016/j.biortech.2005.02.021

[22] Sharma K, Dak G, Agrawal A, Bhatnagar M, Sharma R. Effect of phosphate solubilizing bacteria on the germination of *Cicer arietinum* seeds and seedling growth. J Herb Med Toxicol 2007; 1: 61-63.

[23] Selvakumar G, Kundu S, Joshi P, Nazim S, Gupta AD, Gupta HS. Growth promotion of wheat seedlings by *Exiguobacterium acetylicum* (MTCC 8707) a cold tolerant bacterial strain from the Uttarakhand Himalayas. Indian J Microbiol 2008; 43: 1-7.

[24] Jeffries P, Gianinazzi S, Perotto S, Turnau K. The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol Fert Soils 2003; 37: 1-16.

[25] Dey R, Pal KK, Bhatt DM, Chauhan SM. Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. Microbiol Res 2004; 159: 371-39. http://dx.doi.org/10.1016/j.micres.2004.08.004

[26] Suslow TV, Schroth MN. Rhizobacteria of sugarbeets: effects of seed application and root colonization on yield. Phytopathology 1982; 72: 199-204. http://dx.doi.org/10.1094/Phyto-72-199

[27] Tang WH. Yield increasing bacteria(YJB)and bio control of sheath blight of rice in improving plant productivity with Rhizosphere bacteria. Ryder MH, Stephens PM, Brown GD, Eds., 1994; pp. 267-278.

[28] Bloemberg GV, Lugtenberg BJ. Molecular basis of plant growth promotion and biocontrol by rhizobacteria. Curr Op in Plant Biol 2001; 4: 343-350. http://dx.doi.org/10.1016/S1369-5266(00)00183-7

[29] Mujahid TY, Siddiqui K, Ahmed R, Ahmed N. Isolation and partial characterization of Phosphate solubilizing bacteria isolated from soil and marine sample. Pak J Pharmaceut Sci 2014; 27(3): 1483-1490.

[30] Nguyen CW, Yan LT, Lapyire F. Genetic variability of phosphate solubilizing activity by monocaryotic and mycelia of the ectomycorrhizal fungus laccaria biocolor (Marine) PD. Orton Plant Soil 1992; 143: 193-199. http://dx.doi.org/10.1007/BF00007873

[31] Shahab S, Ahmed N. Effect of various parameters on the efficiency of zincphosphate solubilization by indigenous bacterial isolates. Afr J Biotechnol 2008; 7: 1543-1549.

[32] Karunaiselvi, Ravindar AD. Influence of different carbon and nitrogen sources on insoluble inorganic phosphate. Solubilization by bacillus subtillus. Int J Adv Biol Res 2012; 3: 441-445.

[33] Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 2003; 255: 571-586. http://dx.doi.org/10.1023/A:1026037216893

[34] Perez E, Sulbaran M, Ball M, Yarzabal LA. Isolation and characterization of mineral phosphate-solubilizing bacteria naturally colonizing a limonitic crust in the south-eastern Venezuelan region. Soil Biol Biochem 2007; 39: 2905-2914. http://dx.doi.org/10.1016/j.soilbio.2007.06.017

[35] Khan BS, Yeo TC, Martin WL, Duron MR, Rogers RD, Goldstein AH. Cloning of a mineral phosphate-solubilizing gene from Pseudomonas cepacia. Appl Environ Microbiol 1995; 61: 972-978.

[36] Melo MR, Flores AR, Murrieta S, Tovar AR, Zuniga AG, Hernandez Mendoza AP, Perez NA, Dorantes AR. Comperative Plant growth promoting traits and distribution of rhizobacteria associated with heavy metals in contaminated soil. Int J Environ Sci Tec 2011; 8: 807-816. http://dx.doi.org/10.1007/BF03326264

[37] Fasim F, Ahmed N, Parson R, Gadd GM. Solubilization of Zn salts by bacterium Isolated by the air environment of tannery. FEMS Microbial Lett 2002; 213: 1-6. http://dx.doi.org/10.1111/j.1574-6968.2002.tb11277.x

[38] Farhat MB, Farhat M, Bejor W, Kammoun R. Characterization of the mineral phosphate solubilizing activity of Serratia marcescens CTM 50650 isolated from the phosphate mineral of Gafsan. Arch Microbial 2009; 191: 815-824.

[39] Kang SC, Hat CG, Lee TG, Maheshwari DK. Solubilization of insolubleinorganic phosphates by a soil-inhabiting fungus *Fomitopsis sp.* PS 102. Curr Sci 2002; 82: 439-442.

[40] Nahas E. Factors determining rock phosphate solubilization by microorganism isolated from soil. World J Microb Biotechnol 1996; 12: 18-23. http://dx.doi.org/10.1007/BF00327716

[41] Narsian V, Patel HH. Aspergillus aculeatus as a rock phosphate solubilizer. Soil Biol Biochem 2000; 32: 559-565. http://dx.doi.org/10.1016/S0038-0717(99)00184-4

[42] Nautiyal BP, Vinay P, Nautiyal MC. Structure and diversity pattern along an altitudinal gradient in an alpine meadow of Madhyamaheshwar, Garhwal Himalaya, India. Indian J Environ Sci 2000; 4: 39-48.

[43] Illmer P, Schinner F. Solubilization of inorganic calcium phosphates-solubilization mechanisms. Soil Biol Biochem 1995; 27: 257-263. http://dx.doi.org/10.1016/0038-0717(94)00190-C

[44] Illmer P. Solubilization of hardly-soluble AlPO4 with P solubilizing microorganism. Soil Biol Biochem 1995; 27: 265-270. http://dx.doi.org/10.1016/0038-0717(94)00205-F

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