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Phosphate Solubilization and Plant Growth Promoting Actinobacteria from Rhizosphere Soil

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ABSTRACT

Background: Phosphorus has been considered as the key element as it is directly involved in most of the life processes including in plants. Soil contains both organic and inorganic forms of phosphorus. Phosphate solubilization is the important property of soil bacteria to develop them as plant growth promoting bacteria in the agricultural field. The present study report the phosphate solubilizing and plant growth promoting properties of selected actinobacteria isolated from rhizosphere soil.

Methods: Actinobacterial strains were isolated from rhizosphere soil and screened for *in vitro* phosphate solubilizing properties using agar plate method. The efficiency of phosphate solubilization and phosphatase activity of isolated actinobacterial strains were tested using Pikovskaya broth.

Result: In the present study phosphate solubilization and phosphatase activity of isolated actinobacteria, *Streptomyces* sp. CTD-2 was comparatively higher in lab conditions. In pot trial experiment strain CTD-2 showed higher growth when compared to the control plant. The chlorophyll content of leaves in the experimental plants were found maximum with actinobacteria strain CTD-2 treated plant. Plant growth measurements such as root length, shoot length, leaf length, total plant growth measurements, quick yield production were also observed.

Key words: Actinobacteria, Biodiversity, Biofertilizer, Plant growth, Phosphate, *Streptomyces*.

INTRODUCTION

Phosphorus (P) is one of the essential elements that are necessary for plant development and growth. Nitrogen among mineral nutrients most commonly limiting the growth of crops (Azziz *et al*., 2012). On average, the phosphorus content of soil is about 0.05% (w/w) however, only 0.1% of this phosphorus is available for plant use (Zhu *et al*., 2011). Soil microorganisms enhance plant nutrient acquisition.

They are involved in a wide range of biological processes including the transformation of insoluble soil nutrients (Babalola and Glick, 2012). Microbial P-solubilization and mineralization is the only possible way to increase plant available phosphorus. In the natural environment numerous microorganisms in the soil and rhizosphere are effective at releasing phosphorus from total soil phosphorus through solubilization and mineralization (Bhattacharyya and Jha, 2012).

Actinobacteria are of special interest since these filamentous sporulating bacteria can develop in extremely different soils (Pathom-Aree *et al*., 2006) and produce various substances (anti-fungal, insecticides, anthelminthics, phytohormone-like compounds *etc*.) that could benefit plant growth (Manulis *et al*., 1994). Additionally, the extracellular metabolites produced by actinomycetes may inhibit phytopathogens and, sometimes such metabolic compounds may also act as plant growth regulators (Vurukonda *et al*., 2018). These qualities, among others, make actinobacteria an ideal candidate for developing as microbial inoculants for ultimate use in agriculture production system (Saif, *et al*., 2014). In the present study, the actinobacterial strains were attempted to improve the phosphate solubilizing capacity by the production of phosphatase and the growth of *Vigna radiata* was studied by pot culture method.

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MATERIALS AND METHODS

Isolation, characterization and selection of actinobacteria

Soil samples were collected from agriculture field. All the soil samples were air dried at room temperature for 3-5 days. The dried samples were pretreated under dry heat at 55°C for 10 minutes (Pisano *et al*., 1986). Soil samples from 10^{-3}, 10^{-4} and 10^{-5} dilutions were plated on SCA plates were incubated at 28°C for 7 days. The individual colonies with actinobacterial morphology were selected and inoculated onto yeast extract malt extract agar (ISP2 medium) plates (Shiriling and Gottlieb, 1966).

Screening of phosphate solubilizing actinobacteria

All the selected actinobacterial strain were screened for phosphate solubilizing efficiency using Pikovskaya agar medium (Pikovskaya 1948). Actinobacterial colonies were selected based on the halo zone around the colonies in the Pikovskaya medium.

Phosphate solubilization in liquid broth inoculation

The pure actinobacterial strains were inoculated into yeast extract malt extract (ISP-2) broth. After 18 hrs of incubation, 0.5 ml of yeast extract malt extract broth culture with optical density of 1.0 was inoculated into 100 ml Pikovskaya broth containing 1 g of tricalcium phosphate. The flask was incubated at 37°C for 7days of incubation (Sudhansupal, 1999).

Phosphate estimation

Growth medium was withdrawn aseptically at three days interval from each flask and centrifuged at 10,000 rpm for 20 min. The supernatant was analyzed for phosphate content by chlorostannous reduced molybdophosphoric blue color method. (Atridave and Patel, 1999). The clear 10 ml of supernatant was collected in a 50 ml volumetric flask and 10 ml of chloromolybdic acid reagent was added along the sides to the flask. The content was diluted to 40 ml and 5 drops of chlorostannous acid reagent was added. After thorough mixing, the volume was made up to 50 ml quickly. The optical density was measured at 600 nm and the value was obtained with the help of the standard curve.

Acid phosphatase production and inoculation

The actinobacterial strains were inoculated on yeast extract malt extract broth. After 18 hours of incubation, 0.5 ml of yeast extract malt extract broth culture (1 O.D) was inoculated in 100 ml Pikovskaya broth containing 1 g of Tricalcium phosphate. The flask was incubated at 37°C (Sudhansupal, 1999).

Acid phosphatase assay

The enzyme acid phosphatase was assayed using para-nitrophenyl phosphate (PNP-P) as substrate. The reaction mixture contained 2.5 ml (0.1 M) sodium acetate buffer (pH 5.8), 1.0 ml (1 mm) magnesium chloride, 0.5 ml 1% PNP-P and 0.5 m1 of a suitable dilution of enzyme preparation. One ml of the reaction mixture was transferred to 2 m1 of 0.2 M NaOH before and after 15 min incubation at 37°C to stop the reaction. The NaOH solution added before incubation act as a control sample for each analysis. The amount of Para- nitro phenol (PNP) liberated was measured by recording the absorbance at 420 nm using an appropriate calibration curve. Activity is expressed as micro mol PNP liberated coin min^{-1}. The blank was run in a similar manner using distilled water (Eileen Ingham *et al*., 1979).

Seed treatment and PGP activity

Green gram (*Vigna radiata*) seeds were used for the seed germination to evaluate the growth promotion potential of the selected strains of bioassay. Ten days old cultures of the selected actinobacterial strains were taken and resuspended in sterile distilled water to get the spore suspensions. The suspensions were diluted with sterile distilled water and adjusted to 10^{-5} CFU, thereafter the surface sterilized (sequential dipping in to NaCl 5% to 3 min followed by ethanol 70%, 1 min) (Kumar *et al*., 2015) green gram seeds were soaked in the spore suspension for about 30 min and it was planted into the sterile soil, cow dung soil mixture and un inoculated seeds with sterile soil were served as control. Further leaf growth and chlorophyll contents were examined after 21st day of plant growth (Yang and Chen, 2003).

Estimation of chlorophyll content by Arnon method (1949)

A known amount of *Vigna radiata* leaf tissue (100 mg) was suspended in 10 ml of 80% acetone, mixed well and kept at 4°C overnight in dark. Supernatant was withdrawn after centrifugation (5000 rpm) and absorbance was recorded at 663 and 645 nm in Spectrophotometer. The amount of chlorophyll was calculated according to Arnon (1949).

Calculations

Using Arnon's equation (below) to convert absorbance measurements to mg Chl g1 leaf tissue

Chl a (mg g^{-1}) =

$[(12.7 \times A_{663}) - (2.6 \times A_{645})] \times$ ml acetone / mg leaf tissue

Chl b (mg g^{-1} =

$[(22.9 \times A_{645}) - (4.68 \times A_{663})] \times$ ml acetone / mg leaf tissue

Total Chl = Chl a + Chl b

Cultural characteristics

Cultural characteristics were studied by inoculating the growth of actinobacterial strain CTD-2 into different ISP (International Streptomyces Project) medium such as tryptone agar (ISP1), yeast extract-malt extract agar (ISP2), oatmeal agar (ISP3), inorganic salts-starch agar (ISP4), glycerol-asparagine agar (ISP5), peptone-yeast extract-iron agar (ISP6) and tyrosine agar (ISP7). All the media were prepared by following the guidelines described by Shirilling and Gottlieb (1966). All the plates were incubated for 10 days at 28°C and cultural characteristics were recorded.

Pot culture technique

In this experiment, *Vigna radiata* seeds were sown in sterilized pots containing soil. Pots were placed in the net house under ambient light and temperature. The inoculum for the pot trials were prepared by culturing the selected actinobacterial strains on ISP-2 liquid medium. A single colony was transferred from pure solid medium to 250 ml flasks containing ISP-2 and grown aerobically in flasks on a rotating shaker (125 rpm) for 7 days at 28°C. The actinobacterial suspension was diluted in sterile distilled water to a final concentration of 10^8 CFU ml^{-1}.1.0 ml of log culture (10^8 cells) of each actinobacterial isolates was transferred as inoculum in the corresponding treatments. (Parth Vinodrai Bhatt *et al*., 2014). At maturity stage, data were collected and analyzed statistically.

RESULTS AND DISCUSSION

Isolation, characterization and selection of actinobacteria

In the present study, totally 58 actinobacteria strains from four collection sites were isolated (Fig 1). The isolates were selected based on the colony characteristics such as growth, consistency, mycelial colour, reverse side pigment, soluble pigment, aerial mycelium and substrate mycelium.

Efficacy of phosphate solubilization of actinobacteria

Actinobacterial strain CTD-2 were found higher phosphate solubilization activity with 5.00 ± 0.06 ppm and 21.92% solubility of phosphate followed by actinobacterial strain PUB-1 whereas 4.75± 0.05 ppm and 20.75% solubility were recorded (Fig 2, Table 1). The earlier of reports were available for screening of phosphate solubilizing microorganisms, using Pikovskaya agar. (Hardy *et al*., 1998; Puente *et al*., 2004). Among the actinobacterial strains isolated in the present study, the strain CTD-2 was found to be predominant phosphate solubilizer followed by, PUB-1 and KSR-2. These strains have strong phosphate solubilization activity.

Efficacy of phosphate enzyme production of actinobacteria

Actinobacterial strain CTD-2 were found higher phosphate solubilization activity with 0.424 ±0.06 µmol min^{-1} and 39.93% production of phosphatase followed by actinobacterial strain PUB-1 where as 0.399±0.05 µmol min^{-1} and 31.68% production of phosphatase were recorded (Table 2). The maximum efficacy phosphatase activity of actinobacteria strain CTD-2 on 12th day recorded (0.424 µmol min^{-1}) with 39.93% followed by PUB-(0.399 µmol min^{-1}) 31.68% in the 12th day of incubation. It is well documented that expression of phosphatase in microorganisms is strongly affected by medium composition (Pedregosa *et al*., 1991).

Plant growth measurement of total height and root length, leaf length, shoot length in (cm

Under field conditions, actinobacteria strains significantly enhanced plant height (cm $plant^{-1}$), highest growth was observed in actinobacteria strain CTD-2 treated with a height of 7.1 cm $plant^{-1}$ on 30th day, followed by actinobacteria strain PUB-1 whereas 6.9 cm $plant^{-1}$ were recorded. Whereas 6.3 and 5.2 cm $plant^{-1}$ were recorded and compared with control (Table 3). Actinobacteria strain inoculated plants showed considerable improvement in root length of plant, maximum root length was recorded with actinobacteria strain CTD-2 treated seeds of about 5.1 cm $plant^{-1}$ on 30th day (Fig 3, Table 4). Actinobacteria strain inoculated plants showed

Table 1: Efficacy of phosphate solubilization of actinobacteria.

Name of the strain	*'P' ppm of filtrate (Day)			Increased % of phosphate solubilization (Day)		
	6th	9th	12th	6th (%)	9th (%)	12th (%)
KSR-2	2.65±0.07	3.95±0.09	4.45±0.06	8.70	10.96	18.45
CTD-2	3.35±0.05	4.05±0.08	5.00±0.05	13.56	18.46	21.92
PUB-1	3.75±0.06	4.00±0.08	4.75±0.05	13.64	14.29	20.75

*Mean value± Standard deviation of triplicates.

Table 2: Efficacy of phosphate solubilization of actinobacteria.

Name of the strain	*Phosphatase activity µmol min^{-1} (Day)			Increased % of phosphate activity (Day)		
	6th	9th	12th	6th (%)	9th (%)	12th (%)
KSR-2	0.103±0.02	0.218±0.04	0.341±0.03	6.19	13.54	12.54
CTD-2	0.165±0.02	0.275±0.05	0.424±0.06	70.10	43.23	39.93
PUB-1	0.134±0.03	0.260±0.05	0.399±0.05	38.14	35.42	31.68

*Mean value± Standard deviation of triplicates.

Table 3: Plant growth measurement height in (cm).

Number of days	Actinobacteria strain KSR-2 treated	Actinobacteria strain CTD-2 treated	Actinobacteria strain PUB-1 treated	Cow dung treated	Control
3	1.3	1.4	1.4	1.2	0.90
6	2.1	2.2	2.2	1.9	1.4
9	2.7	2.8	2.7	2.5	1.8
12	3.4	3.5	3.4	3.1	2.3
15	3.8	3.9	3.9	3.7	2.9
18	4.4	4.7	4.5	4.3	3.4
21	5.0	5.3	5.0	4.8	3.7
24	5.5	5.7	5.6	5.3	4.1
27	6.0	6.2	6.2	5.7	4.6
30	6.8	7.1	6.9	6.3	5.2

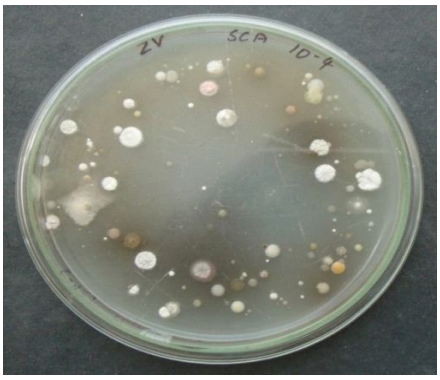


Fig 1: Isolation of actinobacteria (10^4 dilution).

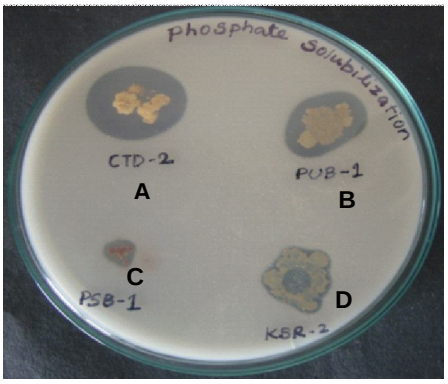


Fig 2: Phosphate solubilization of actinobacterial strains A-(CTD-2) B-(PUB-1) C -(PSB-1) D -(KSR-2).



Fig 3: Plant growth measurement root length **(A)** CTD- 2 Treated plant root **(B)** control.

considerable improvement in leaf length of plant, maximum leaf length was recorded with actinobacteria strain CTD-2 treated seeds about 3.3 cm plant^{-1} on 30th day (Table 4). Actinobacteria strain inoculated plants showed considerable improvement in shoot length of plant, maximum shoot length was recorded with actinobacteria strain CTD-2 treated seeds about 5.2 cm plant^{-1} on 30th day. (Table 4).

Estimation of chlorophyll content in the experimental Plants

The chlorophyll content of leaves in the experimental plants were found higher with actinobacteria strain CTD-2 treated seeds with 13.18 mg per leaves in which Chl a and Chl b was found to be 1.19 and 1.17 mg respectively on 30th day, followed by control with 8.12 mg per leaves in with Chl a and Chl b was found to be 0.73 and 0.67 mg respectively on 30th day as shown in (Table 5). In the present study, actinobacteria CTD-2 treated plants were having maximum chlorophyll content in their leaves (13.18 mg) when compared to the control (6.8 mg). The results obtained in this study are confirmed with those obtained by Bekhit *et al*., (2005), Salem *et al*., (2010) on potato plants.

Cultural characteristics of potential actinobacterial strain

Actinomycete strain CTD-2 showed good growth on ISP 1, ISP 2, ISP 3 and ISP 5 medium and moderate growth was observed on ISP 4, ISP 6 and ISP 7 medium (Table 6).

Pot experiment

The results of pot study showed that inoculation of *Vigna radiata* seeds with actinobacterial CTD-2 strain showed significantly increased plant growth compared to the control. Height of the treated *Vigna radiata* plants increased up to 93% in comparison to control. As same CTD-2 inoculation also had the positive effect on number of leaves in plant, which increased up to 76% in comparison to control. Enhancement of numbers of pot bearing branches per plant is up to 70% and number of pots per plant increased up to 45%. The number of nodules per plant increased up to 50% whereas the weight of seeds increased up to 27%. (Fig 4). The highest increase in growth parameters of *Vigna radiata* plant was recorded from CTD-2 inoculated plant.

Table 4: Plant root length, leaf length and shoot length measurement height in (cm).

Plant	Actinobacteria strain KSR-2 treated	Actinobacteria strain CTD-2 treated	Actinobacteria strain PUB-1 treated	Cow dung treated	Control
Root length	4.6	5.1	4.7	4.7	4.3
Leaf length	2.7	3.3	2.9	2.7	2.6
Shoot length	4.3	5.2	4.7	4.3	4.1

Table 5: Estimation of chlorophyll content in the experimental plants.

No of days	Actinobacteria strain CTD-2 treated			Cow dung treated			Control		
	OD_{664}	OD_{630}	Chl. (mg)	OD_{664}	OD_{630}	Chl. (mg)	OD_{664}	OD_{630}	Chl. (mg)
30th	1.19	1.17	13.18	0.73	0.67	8.12	0.62	0.61	6.867

Table 6: Cultural characteristics of potential actinobacterial strain CTD-2.

Medium	Colony consistency	Growth	Reverse side pigment	Soluble pigment
ISP 1 (tryptone agar)	Powdery	Good	Brown	Brown
ISP 2 (yeast extract - malt extract agar)	Powdery	Good	Brown	Brown
ISP 3 (oatmeal agar)	Powdery	Good	Brown	Brown
ISP 4 (inorganic salt- starch agar)	Powdery	Moderate	-	-
ISP 5 (glycerol- asparagine agar)	Powdery	Good	Brown	Brown
ISP 6 (peptone- yeast extract - iron agar)	Powdery	Moderate	-	-
ISP 7 (tyrosine agar)	Powdery	Moderate	-	-

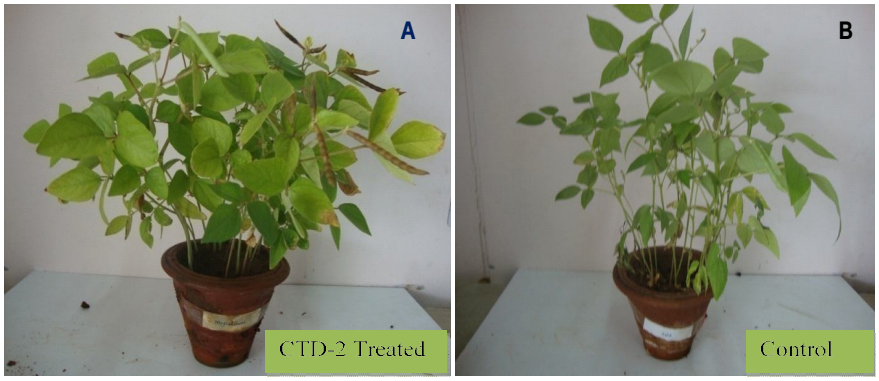


Fig 4: Pot experiment (A) CTD- 2 treated plant (B) control.

The CTD-2 showed better crop yield in a shorter period of time and was found much better than the control.

CONCLUSION

Actinomycetes are one of the predominant members of soil microbial communities and they have beneficial role in soil nutrients cycling and agricultural productivity. In addition, the knowledge on the participation of actinomycetes in hydrolysis of organic phosphorylated compounds is extremely limited. It is clear that production of phosphatases is strongly dependent on the actinomycetes strain as well as media composition. Furthermore, production of acid phosphatases and alkaline phosphatases was shown to be regulated independently.

Hence the present studies come up with strain of actinobacteria CTD-2, with capability of promoting plant growth and fitness in seed treatment. This could be related to the ability of these strains to produce phosphatase and solubilize phosphate that promote root and shoot growth and seed germination together. These strains are thus especially suited for amendments in formulations of bio-fertilizers products for cultivation. So, this can prevent pollution of soil, water and excessive accumulation of phosphorus. Therefore, it is suggested that use of the isolated *Streptomyces* sp. CTD-2 strain could be an effective biofertilizer beneficial for agricultural field.

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REFERENCES

Arnon, DI. (1949). Copper enzymes in isolated chloroplasts. Polyphenol oxidase in Beta vulgaris. Plant Physiology. (24): 1-15.

Artidaue and Patel, R.H. (1999). Inorganic phosphate solubilizing soil Pseudomonas. Indian Journal of Microbiology. 39: 161-164.

Azziz, G., Bajsa, N., Haghjou, T., Taulé, C., Valverde, Á., Igual, J. M. and Arias, A. (2012). Abundance, diversity and prospecting of culturable phosphate solubilizing bacteria on soils under crop-pasture rotations in a no-tillage regime in Uruguay. Applied Soil Ecology. 61: 320-326.

Babalola, O.O. and Glick, B.R. (2012). Indigenous African agriculture and plant associated microbes: current practice and future transgenic prospects. Sci. Res. Essays. 28: 2431-2439.

Bekhit, SR., Hassan, AH., Ramadan, IVIH., AlAnany, AMA. (2005). Effect of different levels and sources of nitrogen on growth, yield and quality of potatoes grown under sandy soil conditions. Annals of Agricultural Science, Moshtohor Journal. 43: 381-394.

Bhattacharyya, P.N. and Jha, D.K. (2012). Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World Journal of Microbiology and Biotechnology. 28: 1327-1350.

Dadarwal, L.R.L.S., Yadav and S.S., simdhu, (1997). Biofertilizer production technology prospects. In Bio Technological Approaches in Soil Microorganisms for Sustainable Crop Production. Scientific Publishers, Jodhpur, India. 323-337.

Eileen Ingham, K.T., Holland, G., Gowland and W.J., Cunliffe. (1979). Purification and partial characterization of an acid

phosphatase produced by Propionibacterium acnes. Journal of General Microbiology. 118: 59-65.

Hardy, J.E., Karamushka, V.I., Gruzina, T.G., Nikovska, G.N., Sayer, J.A. and Gadd, G.M. (1998). Influence of the carbon, nitrogen and phosphorus source on the solubilization of insoluble metal compounds by Aspergillus niger. Mycological Research. 102: 1050-1054.

Kumar, G.P., Desai, S., Amalraj, E.L.D. and Pinisetty, S. (2015). Impact of seed bacterization with PGPR on growth and nutrient uptake in different cultivable varieties of green gram. Asian Journal of Agricultural Research. 9: 113-122.

Manulis Shulamit, Kogan Nina, Valinsky Lea, Levi Michal, Wieman Liora, (1996). Detection of Plant Pathogenic Bacteria in Ornamentals by the PCR Technique. Diagnosis and Identification of Plant Pathogens. 11: 189-192.

Manulis, S., Shafrir, H., Epstein, E., Lichter, A. and Barash, I. (1994). Biosynthesis of indole-3-acetic acid via the indole-3-acetamide pathway in *Streptomyces* spp. Microbiology. 140: 1045-1050.

Parth Vinodrai Bhatt and Bharatkumar Rajiv Manuel Vyas, (2014). Screening and characterization of plant growth and health promoting rhizobacteria. Int. J. Curr. Microbiol. App. Sci. 6: 139-155.

Pathom-Aree, W., Stach, J.E., Ward, A.C., Horikoshi, K., Bull, A.T. and Goodfellow, M. (2006). Diversity of actinomycetes isolated from Challenger Deep sediment from the Mariana Trench. Extremophiles. 10: 181-189.

Pedregosa, A.M., Pinto, F., Monistrol, I.F. and Laborda. F, (1991). Regulation of acid and alkaline phosphatases of Cladosporium cucumerinum by inorganic phosphate. Mycological Research. 95: 720-724.

Pikovskaya, R.I, (1948). Mobilization of phosphorus in soil in connection with vital activities by some microbial species. Microbiologia. 17: 362-370.

Pisano, M.A., Sommer, M.J. and Lopez, M.M. (1986). Application of pretreatments for the isolation of bioactive actinomycetes from marine sediments. Applied Microbiology and Biotechnology. 25: 285-288.

Puente, M. E., Bashan, Y., Li, C.Y. and Lebsky, V.K., (2004). Microbial populations and activities in the rhizosphere of rock-weathering desert plants. Root colonisation and weathering of igneous rocks. Plant Biology. 6: 629-642.

Saif, Saima, *et al*., (2014). Role of phosphate-solubilizing Actinomycetes in plant growth promotion: current perspective. *Phosphate Solubilizing Microorganisms*. Springer, Cham. 137-156.

Salem MA, AL-ZayadnehW, Abdul-Jaleel C. (2010). Effects of compost interactions on the alterations in mineral biochemistry, growth, tuber quality and production of Solanum tuberosum. Frontiers of Agriculture in China. 4: 170-174.

Shirting, E.B. and Gottlieb, D, (1966). Methods for characterization o Streptomyces species. International Journal of Syst Bacteriol. 16: 313-340.

Sudhansupal, (1999). Selection of acid tolerant strains of phosphate solubilizing bacteria in soil of Uttar Pradesh, Himalayas. Indian Journal of Agricultural Science. 69: 787-82.

Vurukonda, S.S.K.P., Giovanardi, D. and Stefani, E. (2018). Plant growth promoting and biocontrol activity of Streptomyces spp. as endophytes. International Journal of Molecular Sciences. 19(4): 952.

Yang, Y.J., Yu, F.L., Chen, L., Zeng, J. M., Yang, S.J., Li, S.F. and Wang, H.S. (2003). Elite germplasm evaluation and genetic stability of tea plants. J. Tea. Sci. 23: 1-8.

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 Complementary and Alternative Medicine. 2011.