Ann. For. Res. 65(1): 11062-11070, 2022
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
https://www.e-afr.org/

AGRICULTURAL POTENTIAL OF PHOSPHATE SOLUBILIZING MICROORGANISMS OF MANGROVE ECOSYSTEM

Joshi Chinmay[1] and Soni Manish[2]

1. Department of Microbiology and Biotechnology, C.U.Shah University, Wadhwan City, Surendranagar, Gujarat, India.
2. School of Engineering and Technology, Department of Biotechnology, Jaipur National University, Jaipur, Rajasthan, India

Abstract

This investigation aimed to isolate potent phosphate solubilizing endophytic (PPSE) bacteria from mangrove. Different biochemical tests were performed to tested for Indole acetic acid (IAA), siderophore and Hydrogen Cyanide (HCN) producing ability. A highly potent PPSE bacterium was selected. Then effect of carbon, nitrogen, pH, and incubation time on phosphate solubilizing ability of the isolated S23 was studied. Further, two different phosphate requiring plants cluster bean and sorghum were tested with the isolated S23 to check their plant growth after the bacterial treatment. Data suggested the and phylogenetic analysis revealed it to be *Acinetobactor rudis*-S23 excellent growth in treated cluster bean and sorghum plants compared to the control.

Keywords: Hydrogen Cyanide, Indole acetic acid, phosphate solubilizing endophytic bacteria, *Acinetobactor rudis*

Introduction

Phosphorus (P) is known to be one of the crucial elements for the growth and development of plants such as, photosynthesis, respiration and energy storage (Fernández et al., 2014). It is very fascinating to know that soil is a major reservoir of P however, due to insolubility of P (04-1.2 g/kg) in soil plants cannot utilize it themselves (IMF and Unctad 2011; Joe et al., 2018). For agricultural crops, it is very necessary to provide P supplements in adequate quantity. Therefore, many farmers use chemical P fertilizers however, consequences include soil toxicity. Earlier reports suggested that P fertilizers (about 80%) interfere with plant sorption ability in the soil by causing precipitation reaction if reacts with Ca^{2+} in calcareous soils or Al^{3+} and Fe^{3+} in acidic soils which result reduction in plant sorption capacity and reduced plant growth (Gyaneshwar et al., 2002). This hampers the growth of most agricultural crops. Reports suggested that, there are a group of microorganisms named as PPSE a reside in the soil capable of solubilizing P by means phosphatase and organic acid production (Illmer and Schinner 1995). *Burkholderia, Pseudomonas, Pantoea, Achromobacter, Agrobacterium, Flavobacterium, Micrococcus, Serratia, Acinetobacter, and Aerobacter* are major PPSE microbes and also known as biofertilizers which have been become a great research topic for agricultural researchers (Castagno et al., et al., 2011; Chen et al., 2006; Peix et al., 2009; Hu et al., 2 010; Chauhan et al., 2015). These PPSE are eco-friendly in behaviour and are having high plant growth stimulating ability (Adhikari et al., 2019; Kumar et al., 2012).

In this context, the objectives of the present study are to isolate and characterize potent PPSE bacterial strains from mangrove ecosystem for the plant growth promotion of useful agricultural crops such as cluster beans, sorghum etc.

Materials and methods

Isolation of PPSE bacteria

Soil sample from mangrove soil was collected and PPSE bacteria were isolated using NBRIP solid medium (g/l) [D-glucose (10), $MgSO_4$ $7H_2O$ (0.25), $MgCl_2$ $6H_2O$ (5), 0.1 g/l $(NH_4)_2SO_4$ (0.1), KCl (0.2)] supplemented with Tricalcium Phosphate (5g/l) as a sole P source and incubated for 7 days at 30°C. The bacterial isolates showing halo zone around their colonies were selected, purified and stored at 4°C on NBRIP medium (Nautiyal 1990).

Preparation of inoculum

The purified PPSE bacterial isolates were grown in the NBRIP medium and incubated at 30°C for 24 h to prepare culture inoculum.

Studies on qualitative estimation of PPSE bacterial isolates

Each isolated PPSE bacterial strains with an inoculum (10 µl) was added on NBRIP agar medium supplemented TCP (5g/l) and incubated for 14 days at 30°C. All experiments were performed in triplicates and the sterile NBRIP agar medium was served as a control (Premono et al., 1996).

Studies on siderophore producing ability of PPSE bacteria

All selected PPSE bacteria isolates were grown on Chrome-Azurol S (CAS) medium (Schwyn and Neilands, 1987). Fresh inoculum of PPSE bacterial strains were spotted on CAS agar plates and incubated at 28°C for 4 days. Development of orange halo zone around bacterial colonies were observed in the siderphore producing strains. The experiments were performed in duplicates.

Indole-3-Acetic Acid Production

All selected PPSE bacterial stains were analyzed for IAA production in order to check their P solubilization ability. LB broth with 0.1% L-tryptophan (30 ml) was prepared, sterilized and inoculated with PPSE bacterial strains (200 µl) at 28°C for 72 h in dark under shaking conditions of 140 rpm/min. After 72 h, culture broth was harvested, centrifuged at 10,000 rpm for 10 min. Cell pellet was discarded, cell free broth (2 ml) was mixed with Salkowski reagent and kept in dark for 30 min. Change in the color of the Salkowski reagent treated cell free broth was measured using UV spectrophotometer at 530 nm (Gordon and Weber 1951).

HCN Production

All selected PPSE bacterial strains were inoculated on nutrient agar supplemented with 0.44% glycine to check HCN production. After inoculation, 2% Sodium carbonate socked filter paper and 0.5 ml picric acid solution was added on the top of nutrient agar plate lid and incubated for. for 4 days at 28°C The change in color of the medium from yellow to brown was recorded to screen HCN production by selected PPSE bacterial strains (Bakker and Schippers 1987).

Identification of Isolated Strains and Phylogenetic tree analysis

PPSE bacterial strains were isolated from mangrove ecosystem and after screening crude DNA was extracted to analysis their phylogeny. DNA was extracted using was performed using the

PureLink® Genomic DNA. Mini Kit (Invitrogen, K1820-01) as per the manufacturer instructions. The degenerative primers 27F (farword) (5'AGAGTTTGAT CCTGGCTCAG-3') and 1492R (reverse) (5'-ACGGTTAC CTTGTTACGACTT-3') were used for the 16S rRNA sequencing and PCR studies. The obtained electropherograms were checked to prepare phylogenetic tree using

Strains	IAA (µg/mL)	Siderophore	Hydrogen Cyanide
Control	0.12	-	-
S1	3.9	+	+
S2	2.53	+	-
S3	**7.69**	**++**	**-**
S4	2.66	+	-
S5	**7.47**	**++**	**-**
S6	2.05	++	-
S7	1.37	+++	-
S8	**8.73**	**++**	**-**
S9	2.53	+++	-
S10	0	-	-
S11	1.64	++	-
S12	**8.49**	**+**	**-**
S13	4.77	+++	+
S14	3.12	++	-
S15	4.06	+	-

MEGA7 software (Kumar et al., 2016).

Optimization of carbon, nitrogen and pH

Selected PPSE bacterial isolated was tested for the optimization of carbon, nitrogen sources, pH and incubation time in NBRIP medium to get maximum phosphate solubilization potential (Pallavi and Gupta 2013).

Studies of plant growth promotion on Cluster bean and Sorghum

Plant growth promoting potential of selected *A. rudis*-S23 was studied on Cluster bean and Sorghum plants (Manzoor et al., 2017).

Results

Isolation of potent PPSE bacteria

87 isolates were selected at 8^{th} dilution in NBRIP solid medium and the PPSE bacterial strains were selected based on their biochemical and morphological differences. Out of which S16-S30 bacterial strains were selected and further screening tests these isolated strains were done using HCN test, IAA and Siderophore production in order to estimate their phosphate solubilization ability.

Table 1

Data suggested that, S3, S5, S8, S12, S20. S23 and S27 were having potent HCN, IAA and Siderophore producing ability and their phylogenetic tree was prepared.

Isolates	Name of an organism	NCBI Accession Number
S3	*Acinetobacter sp.*	MW581934
S5	*Acinetobacter calcoaceticus*	MW581950
S8	*Acinetobacter sp.*	MW581951
S12	*Acinetobacter rhizosphaerae*	MW581952
S20	*Acinetobacter sp.*	MW582294
S23	*Acinetobacter rudis*	MW582308
S27	*Rhizobium sp.*	MW582305

Table 2

Phylogenetic analysis revealed that S3, S5, S8, S12, S20 and S23 belongs to *Acinetobacter* sp. while S27 is a *Rhizobium*

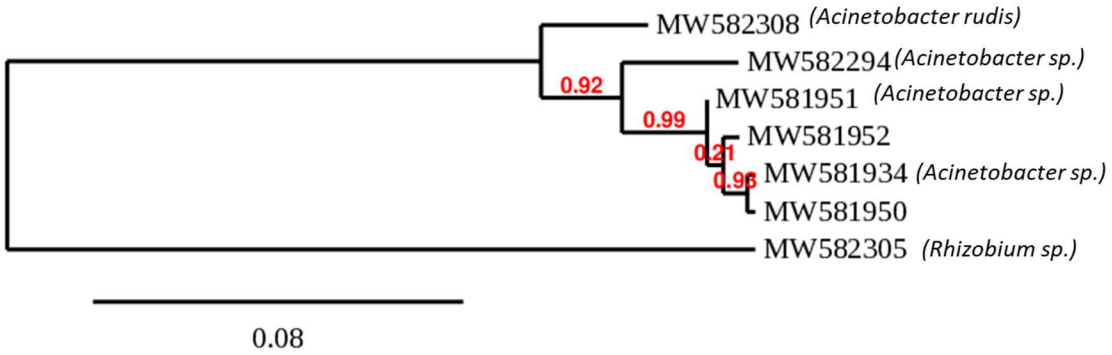


Fig 1

Among all these strains, S23 i.e. *A. rudis*-S23 was having highest ability for HCN, IAA and Siderophore production which was selected and stored for further studies.

Quantitative estimation of P solubilization

The P solubilization in NBRIP liquid medium, by the isolated bacterial strains was studied.

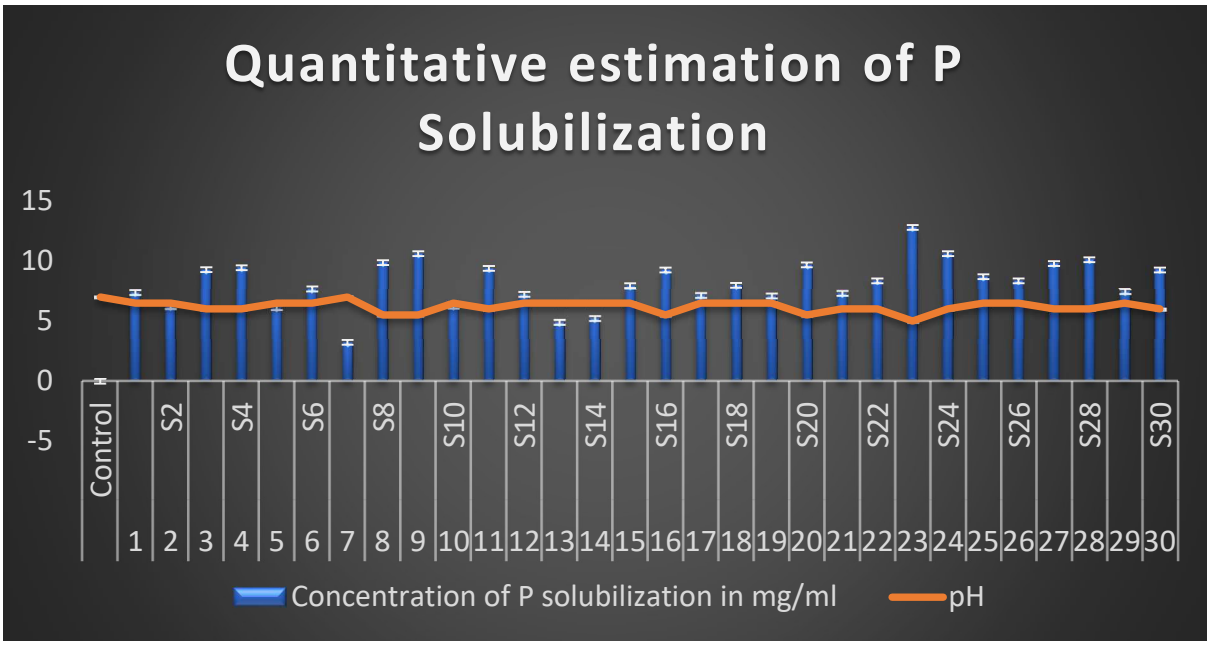


Fig 2

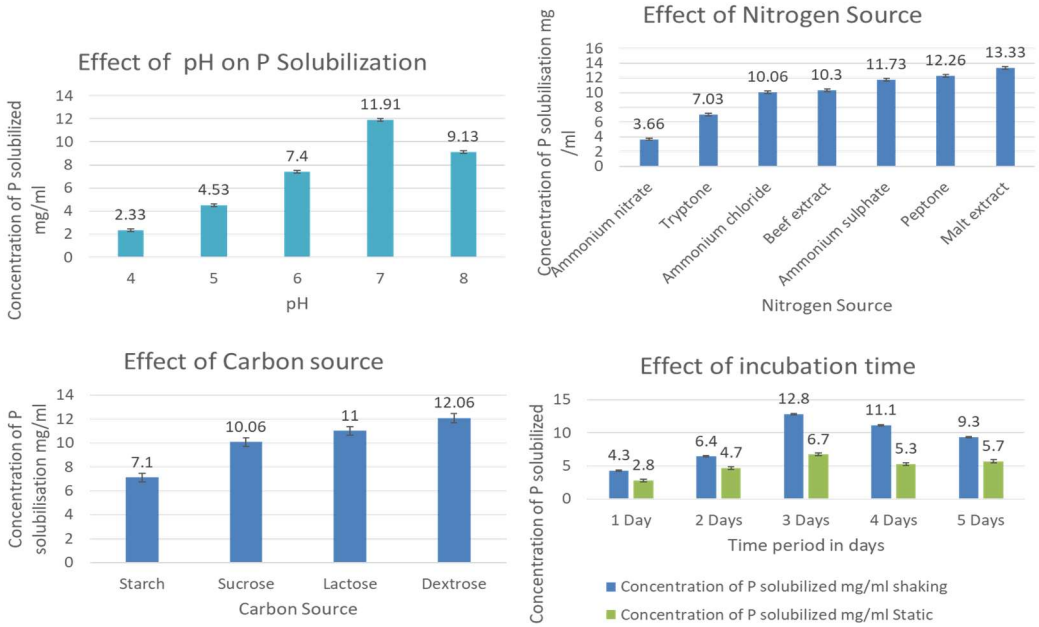
Data revealed that *A. rudis*-S23 possess maximum P solubilization ability (12.8 mg/ml) in NBRIP liquid medium as compared to other isolates including S3 (9.27 mg/ml), S5 (6.61mg/ml), S8 (9.87 mg/ml), S12 (7.25 mg/ml), S20 (9.28 mg/ml) and S27 (9.78 mg/ml). These results are in agreement with earlier studies as reported by Premono et al., 1996, Aliyat et al., 2020.

Optimization of pH, carbon sources, nitrogen sources and incubation time

On the basis of secondary screening *A. rudis*-S23 was selected for further studies. Optimization of the selected isolate *A. rudis*-S23 was done with respect to pH (4,5, 6,7,8), carbon (starch, sucrose, lactose, dextrose) and nitrogen sources (Ammonium nitrate, tryptone, ammonium chloride, beef extract, ammonium sulphate, peptone, malt extract). data suggested that when *A. rudis*-S23 was used the highest phosphate solubilization was observed at pH -7 (11.91 mg/ml), with malt extract (13.33 mg/ml), with dextrose (12.06 mg/ml) and after 3 days of incubation time at 30 °C (12.8 mg/ml) under shaking conditions (Fig.2). Similarly, Son et al. 2006 identified *Pantoea agglomerans* to be the efficient phosphate solubilizer which solubilises 900mg/l Phosphate at pH 7.5. This observation is in agreement with Maheswar and Sathiyavani (2012) who reported that for *Bacillus subtilis*, isolated from groundnut rhizosphere soil, pH 7 was most suitable for phosphate solubilisation. On the other hand, among all carbon sources, dextrose was found to most suitable carbon source yielding high P solubilization (12.06 mg/ml) compared to the other used carbon source (Fig. 2). This result is in agreement with the findings as reported by Kongbrailatpam and Putatunda 2018 on *Bacillus subtilis* PSBN B4 obtained from Pineapple. In case of nitrogen sources, our findings showed malt extract to best nitrogen source showing highest P solubilization (13.33 mg/ml) as compared to the other nitrogen sources used.

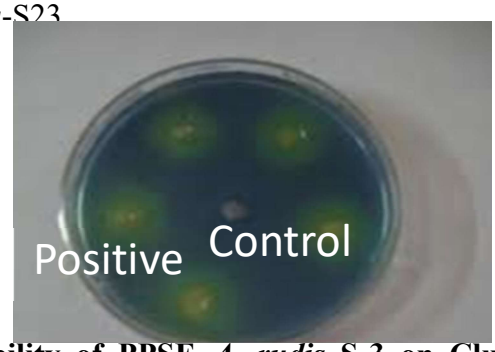
Pallavi and Gupta (2013) observed best nitrogen sources in ammonium sulphate. However, Seshadhari et al. (2010) observed ammonium nitrate was very effective for phosphate solubilisation by *Aspergillus*. Optimization of the incubation period was done in which the effect

of shaking and non-shaking conditions was also assessed which showed 3 days incubation time under shaking conditions are optimum to get higher P solubilisation. which is in agreement with the earlier studies (Banergee et al., 2010, Pramod and Dhevendaran **1987, Saini et al., 2015)**



Siderophore, HCN and IAA production by PPSE bacteria

Orange halo zone formation on CAS blue agar medium confirmed the siderophore production by PPSE bacterial isolates (Table 1). All selected bacterial isolates (S16-S30) demonstrated a significant amount IAA and HCN production (Table 1). Highest siderophore, HCN and IAA production was reported in case of *A. rudis*-S23



Studies on *plant growth promoting* ability of PPSE *A. rudis* S-3 on Cluster bean and Sorghum

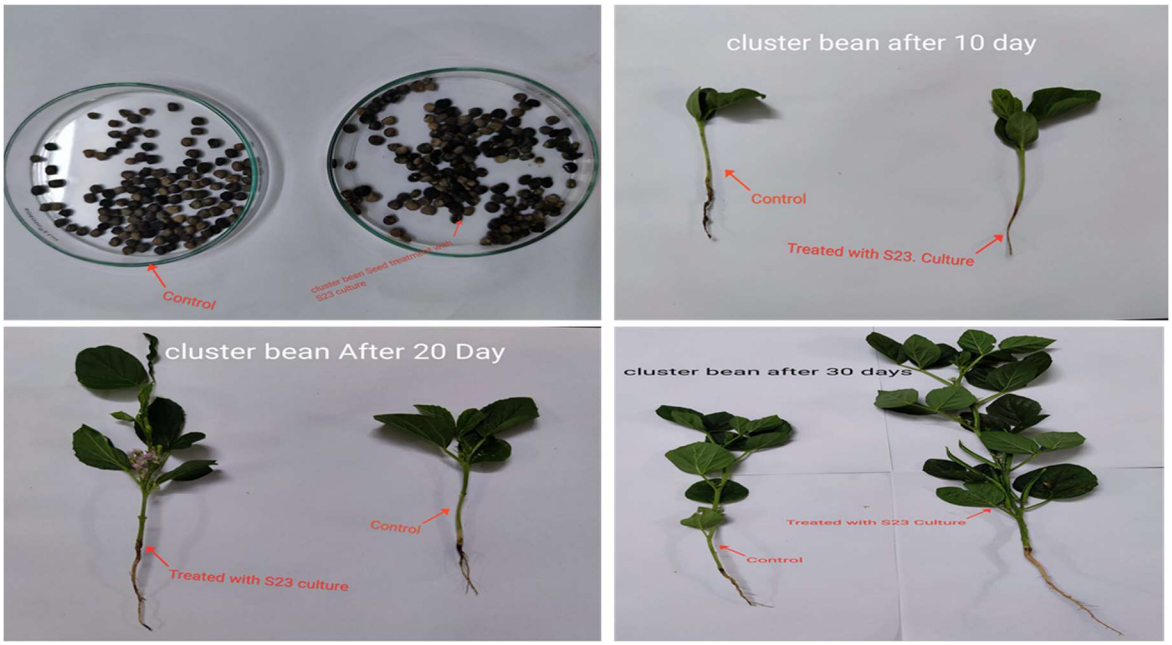
Data revealed that when Cluster bean and Sorghum plants were treated with PPSE *A. rudis* S-23, increased plant growth of these plants were observed after 10, 20 and 30 days as compared to control

.

Days	Control Plant height (Cm.)	Treated with S23 plant height (Cm.)	Flowering control Plant	Flowering treated Plant	Fruiting control plant	Fruiting treated plant
10	10.80 cm.	12.30 cm.	No	No	No	No
20	13.40 cm.	18.60 cm.	No	Yes	No	No
30	37.5 cm.	55.5 cm.	YES	YES	NO	YES

Table 3

Table 3 clearly showed the significant difference in flowers and fruits of *A. rudis* S-23 treated Cluster bean plants after 10, 20 and 30 days as compared to control. All these results are in agreement with the earlier findings of PPSE bacteria on plants growth promotion (Manzoor et al., 2017).



Discussion

Microorganism based Bio-Fertilizer are replacement of toxic chemical based fertilizers. Consistent use of chemical fertilizers causes, soil toxicity, imbalance of soil beneficial microbial consortia and nutrient depletion in the soil especially different minerals like p, $Ca^{2+,}$ Mg^{2+}, carbon and nitrogen. In order to improve soil nutrient quality and to minimize soil toxicity we require alternative agent such as Phosphate solubilizing bacteria which have strong ability to soluble unavailable phosphate in to available form to plants and Microorganisms based bio fertilizers are 100% organic and eco-friendly to nature. These phosphate solubilizing bacteria also called as bio-fertilizers which can easily enhance the soluble inorganic phosphorus concentration in the soil and thus help farmers to get higher crop productivity. In the present investigation, out of the fourteen isolates *A. rudis*-S23 mangrove ecosystem was found to be the most efficient phosphate solubilizing strain. Our findings showed that *A. rudis*-S23 displayed appreciable amount of phosphate solubilizing activity and increased the growth of Cluster bean and Sorghum which make

suitable candidate to be used as bio fertilizer. *A. rudis*-S23 can be used as a good bio fertilizer for agriculture land of saline region. *A. rudis*-S23 can be used for preparation of phosphate rich organic manure (PROM) as an efficient bio culture. This all are the future prospective scope for our research outcome.

Conclusion

In the present investigation, out of the fourteen isolates *A. rudis*-S23 mangrove ecosystem was found to be the most efficient phosphate solubilizing strain during lab as well as pot experiments.

References

1. 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.

2. Seshadri, S., Ignacimuthu, S. and Lakshminarasimhan, C., 2010, "Effect of Nitrogen and Carbon sources on the inorganic Phosphate solubilisation by different **Aspergillus niger** strains," Chem. Engg. Comm., 191, 1043-1052.

3. Banerjee, S., Palit, R., Sengupta, C., and Standing, D., 2010, "Stress induced phosphate solubilization by **Arthrobacter** sp. and **Bacillus** sp. isolated from tomato rhizosphere," Aus. J. Crop Sci., 4, 378-383.

4. Promod, K., and Dhevendaran, K., 1987, "Studies on phosphobacteria in Cochin backwater," J. Mar. Biol. Ass. India, 29, 297-305.
Saini, K., Vyas, A., and Putatunda, C., 2015, "**In vitro** phosphate solubilization by maize rhizosphere bacteria," Res. J. Pharma. Bio. Chem. Sci., 6, 897-902.

5. Saini, K., Vyas, A., and Putatunda, C., 2015, "**In vitro** phosphate solubilization by maize rhizosphere bacteria," Res. J. Pharma. Bio. Chem. Sci., 6, 897-902.

6. Fatima Z., Aliyat, Mohamed Maldani, Mohammed E Guilli, Laila Nassiri and Jamal Ibijbijen 2020. Isolation and Characterization of Phosphate Solubilizing Bacteria from Phosphate Solid Sludge of the Moroccan Phosphate Mines, 14, 16-24.

7. Awasthi R, Tewari R, Nayyar H. Synergy between plants and psolubilizing microbe in soils: effects on growth and physiology of crops. Int Res J Microbiol 2011; 2: 484-503.

8. Fernández VP, Guzmán CA, Peirce TM, McBeath M, Khayet L, McLaughli MJ. Effect of wheat phosphorus status on leaf surface properties and permeability to foliar-applied phosphorus. Plant Soil 2014; 384:20

9. Joe MM, Deivaraj S, Benson A, Henrya AJ, Narendrakumar G. Soil extract calcium phosphate media for screening of phosphatesolubilizing bacteria. Agric Nat Resour (Bangk) 2018; 52(3): 305-8.

10. IMF O, UNCTAD W.. Price Volatility in Food and Agricultural Markets: Policy Responses. Rome: FAO 2011.

11. Gyaneshwar P, Kumar GN, Parekh LJ, Poole PS. Role of soil microorganisms in improving P nutrition of plants. Plant Soil 2002; 245: 83-93.

12. Illmer P, Schinner F. Solubilization of inorganic calcium phosphates solubilization mechanisms. Soil Biol Biochem 1995; 27: 257-63.
13. Adhikari P, Pandey A. Phosphate solubilization potential of endophytic fungi isolated from Taxus wallichiana Zucc. Roots. Rhizosphere 2019; 9: 2-9.
14. Kumar A, Kumar A, Devi S, Patil S, Payal C, Negi S. Isolation, screening and characterization of bacteria from rhizospheric soils for different plant growth promotion (PGP) activities: An in vitro study Recent Res Sci Technol 2012; 4(1): 01-5.
15. Castagno LN, Estrella MJ, Sannazzaro AI, Grassano AE, Ruiz OA. Phosphate-solubilization mechanism and in vitro plant growth promotion activity mediated by Pantoea eucalypti isolated from Lotus tenuis rhizosphere in the Salado River Basin (Argentina) J Appl Microbiol 2011; 110(5): 1151-65.
16. Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl Soil Ecol 2006; 34:33-41.
17. Peix A, Ramírez-Bahena MH, Velázquez E. Historical evolution and current status of the taxonomy of genus Pseudomonas. Infect Genet Evol 2009; 9(6): 1132-47.
18. Hu XJ, Li ZJ, Cao YC, Zhang J, Gong YX, Yang YF. Isolation and identification of a phosphate-solubilizing bacterium Pantoea stewartia subsp. stewartii g6, and effects of temperature, salinity, and pH on its growth under indoor culture conditions. Aquacult Int 2010; 18(6):1079-91.
19. Chauhan H, Bagyaraj DJ, Selvakumar G, Sundaram SP. Novel plant growth promoting rhizobacteria—Prospects and potential. Appl Soil Eco 2015; 95: 38-53.
20. Schwyn B, Neilands JB. Universal chemical assay for the detection and determination of siderophores. Anal Biochem 1987; 160(1): 47-56.
21. Bakker AW, Schippers B. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp-mediated plant growth-stimulation. Soil Biol Biochem 1987; 19: 451-7.
22. Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 2016; 33(7): 1870-4.
23. Gordon SA, Weber RP. Colorimetric estimation of indoleacetic acid. Plant Physiol 1951; 26(1): 192-5.
24. Manzoor, M., M. Kaleem Abbasi, and Tariq Sultan. "Isolation of phosphate solubilizing bacteria from maize rhizosphere and their potential for rock phosphate solubilization–mineralization and plant growth promotion." Geomicrobiology Journal 34.1 (2017): 81-95.