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Research Article

Isolation and Compatibility investigation of Phosphate-Solubilizing Rhizosheric Bacteria from *Triticum aestivum* with Preferred Agrochemicals for Sustainable Agriculture

Chauhan S, Neha and Negi DS*

Department of Microbiology, Himachal Pradesh University, Summerhill, Shimla, India

***Corresponding Author:** Negi DS, Associate Professor, Department of Microbiology, Himachal Pradesh University, Summerhill, Shimla, Himachal Pradesh, India

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Abstract

The study explores the symbiotic relationship between Triticum aestivum and phosphate- solubilizing bacteria (PSB) in the rhizosphere. This research investigated the isolation and characterization of phosphate-solubilizing bacteria (PSB) from the rhizospheric soil of Triticum aestivum (Wheat) plants. Out of 57 isolates, 24 strains exhibited notable P-solubilization, with isolate PD-1 demonstrating the highest solubilization potential at 555.5 ug/ml. The study delves into the plant growth-promoting activities of these isolates, revealing diverse capabilities.

Quantitative analyses highlighted the multifaceted potential of isolate PD-8, which not only demonstrated substantial P-solubilization (505.0 ug/ml) but also exhibited remarkable indole acetic acid (IAA) production, reaching 11 ug/ml in the presence of L-tryptophan. Four isolates, including PD-8, demonstrated hydrogen cyanide (HCN) production, with PD-8 further displaying antifungal activity.

Compatibility testing with common pesticides showcased the resilience of PD-8, tolerating Carbendazim and Mancozeb up to 100 ppm. However, incompatibility was observed with Metalaxyl-M. These findings position PD-8 as a versatile bioinoculant, offering a comprehensive suite of plant growth-promoting attributes coupled with adaptability to specific pesticides.

The study underscores the potential of PD-8 as a promising candidate for integrated agricultural practices, promoting sustainable crop productivity and effective disease management. This research contributes valuable insights into harnessing specific microbial strains for enhanced agricultural sustainability.

Keywords: Plant Growth Promoting Rhizobacteria (PGPR); Hydrogen Cyanic Acid (HCN); Indole Acetic Acid (IAA); *Triticum aestivum*; Agricultural Sustainability

Introduction

The rhizosphere, a dynamic interface influenced by plant roots, is vital for plant health due to a myriad of microbial interactions [1]. It also a pivotal arena for dynamic interactions between plants and a diverse microbial community, influencing nutrient dynamics [2]. Historical practices involving the mixing of legume and non-legume soils laid the foundation for scientific exploration, with the introduction of "Nitragin" in the late 19th century for leguminous crops [3].

Root exudates create a selective environment, favoring beneficial bacteria like Plant Growth Promoting Rhizobacteria (PGPR), constituting 2-5% of rhizobacteria and playing a pivotal role in enhancing plant growth [4,5].

Modern agricultural practices, characterized by high-yielding varieties and chemical fertilizers, have increased crop production but at the expense of soil damage, fertility loss, and environmental challenges [6,7]. To address these issues, there is a growing interest in sustainable alternatives, such as biofertilizers, particularly PGPR, to optimize soil productivity while preserving its health [8,9].

Biofertilizers, emerging as promising alternatives to chemical fertilizers, demonstrate positive effects on plant growth and health, whether symbiotic or free-living [10]. PGPR, constituting 2-5% of rhizobacteria, employ diverse mechanisms to enhance plant growth at various developmental stages [5,11].

Integrated Nutrient Management (INM) emphasizes the crucial role of PGPR, such as Azotobacterand Rhizobium, in enhancing nutrient cycling and promoting plant growth [12,13]. Challenges associated with phosphorus mobility in moist soils are addressed through the influence of soil conditions and plant species on the bioavailability of inorganic phosphorus [14,15].

Commercial biofertilizers, incorporating strains like Azotobacter, Rhizobium, Azospirillum, and Burkholderia, have proven efficacy in enhancing crop yields [16]. The integration of phosphate and potassium solubilizing bacteria with rock phosphates and potassium rocks provides an eco- friendly alternative to conventional fertilizers [17].

The cycling of phosphorus, a critical macronutrient, between organic and inorganic forms in soil is dynamic, with phosphate-solubilizing bacteria (PSB) playing a pivotal role in sustaining plant nutrition by solubilizing various phosphate compounds [18,19].

Concentrated in the rhizosphere, PGPR influences plant physiology through enzymatic activities, organic acid production, antifungal properties, and the release of various metabolites [20,21]. Certain PGPR, exemplified by *Pseudomonas putida*, demonstrate compatibility with agrochemicals, showcasing resilience in the presence of fungicides and pesticides [22]. However, caution is warranted, as excessive agrochemical use may adversely impact soil microbialcommunities and fertility [23].

The intricate interactions within the rhizosphere, coupled with the strategic application of PGPR and sustainable nutrient management practices, present promising avenues for optimizing agricultural productivity.

In the agro-climatic conditions of Himachal Pradesh, this study aims to isolate and screen phosphate-solubilizing bacteria from rhizospheric soils. Additionally, it seeks to evaluate the PGPR activities of these bacteria and assess their compatibility with commonly used pesticides. Addressing these objectives contributes to the development of sustainable agricultural practices tailored to local conditions.

Materials and Methods

Study area

The current study was conducted at the microbiology laboratory of Himachal Pradesh University (H.P.),state of India. Study areas lies within the geographical coordinates of N 31° 10’ 0.012” and E 77° 34’ 59.988 and altitudinal gradients of 2197 m.a.s.l.(meters above sea level). Shimla presents mainly mountainous and hilly landscape. The average temperature during summer is between 19 and 28 °C (66 and 82 °F), and between −1 and 10 °C (30 and 50 °F) in winter.

Isolation and preliminary screening

The research, conducted at the microbiology laboratory of Himachal Pradesh University (H.P.), focused on studying phosphate-solubilizing bacteria (PSB) isolated from the rhizospheric soil of *Triticum aestivum* (wheat) and their competence with commonly used pesticides. Soil samples from wheat rhizospheres in Himachal Pradesh were collected and stored at 4°C [24].

Assessing the plant growth enhancement characteristics of the chosenisolates.

Phosphate solubilization

For qualitative assessment of phosphate solubilization, a Pikovskaya medium containing tricalcium phosphate [25] was utilized. Bacterial cultures were spotted onto the medium and then incubated at 30°C for 48 hours. The presence of a distinct halo zone surrounding the culture spot served as an indicator of the isolate’s ability to solubilize phosphate and this was determined by the calculation of phosphate solubilization index (PSI) through the following formula [26]:

$$PSI = \frac{\text{total diameter(colony + halo zone)}}{\text{Colony diameter}}$$

Quantitative estimation of phosphate solubilization in broth was carried out Vanadomolybdo phosphoric acid method done by Fiske and Subba Rao [27].

Indole acetic acid

To evaluate the production of indole acetic acid (IAA) in the bacterial isolates, a bacterial suspension (10% v/v) was introduced into Luria Bertani (LB) broth supplemented with 50 µg ml−1 L-tryptophan. After an incubation period of 48 hours at 28 ± 2°C, the cultures underwent centrifugation at 10,000 g for 10 minutes. The concentration of IAA in the resulting culture supernatant was determined using the Salkowski reagent method [28].

HCN production

Hydrogen cyanide (HCN) production was assessed using the qualitative method described by Kremer and Souissi [29]. Bacterial isolates were streaked onto King's B agar medium supplemented with 4.4 g glycine L−1. Circular Whatman no.1 filter paper, saturated with a 0.05% picric acid solution in 2% sodium carbonate, was placed in the lid of each Petri plate. The plates were sealed air-tight with Parafilm and then incubated at 30°C for 48 hours. A change in color of the filter paper disc from yellow to reddish-brown was interpreted as indicative of HCN production.

Ammonia production

For the detection of ammonia production, a method outlined by Dye (30) was employed. Specifically, 1 ml of Nessler's reagent was added to a 72-hour-old culture grown in peptone broth, and the presence of a yellowish-brown coloration was observed.

Morphological and biochemical characterization of isolates

Morphological analyses, encompassing assessments of shape, color, edge properties, motility, andthe presence of endospores, in addition to Gram staining, were carried out in accordance with the procedures outlined in references [31,32]. Furthermore, biochemical examinations, including the indole test, urease test, catalase test, Voges–Proskauer (VP) test, methyl red (MR) test, and citrate test, were conducted following the methodologies delineated in the aforementioned references. Biochemical identification was performed using "Bergey's Manual of Systemic Bacteriology" [31].

Compatibility of the isolate with some commonly used Pesticides

The best isolate underwent exposure to varying concentrations of 3 agrochemicals, including three fungicides (Carbendazim, Mancozeb, and Metalaxyl-M) undergoing assessment and analysis to check bacterial isolate's tolerant capacity against these pesticides was determined [33,34]. This comprehensive methodology provides insights into PSB dynamics and their potential application in integrated disease management.

Results

Phosphate solubilization and IAA production by PGPR isolates

Phosphate solubilization capability of the bacterial isolates was assessed using both qualitative and quantitative methods. Out of 57 isolates, 24 strains exhibited notable P- solubilization, showing zone of solubilization of more than 8mm diameter The phosphate-solubilization capacity of the isolate was evaluated by examining the presence of extensive clear zones surrounding the bacterial colonies on PVK agar plates. The solubilization index (PSI) was subsequently calculated based on these observations. (Figure 1 and 2) with isolate PD-1 demonstrating the highest solubilization potential at 555.5 ug/ml (Figure 3).

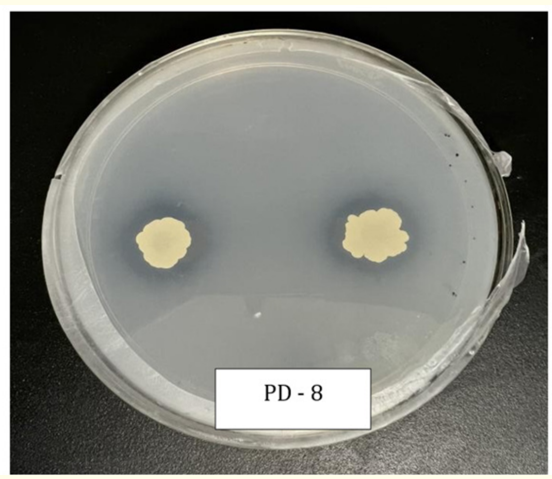

Figure 1: Phosphate solubilization zone of PD-8.

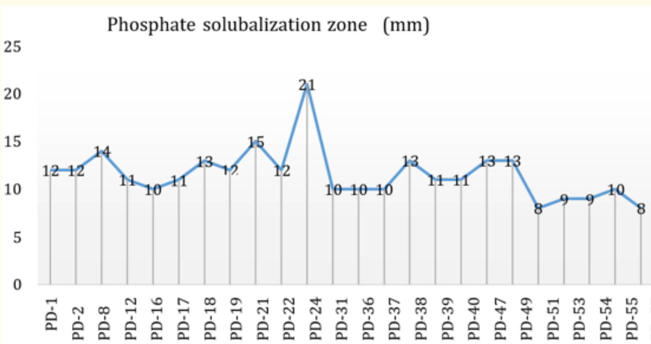

Figure 2: Data of phosphate Solubalization zone by different isolates.

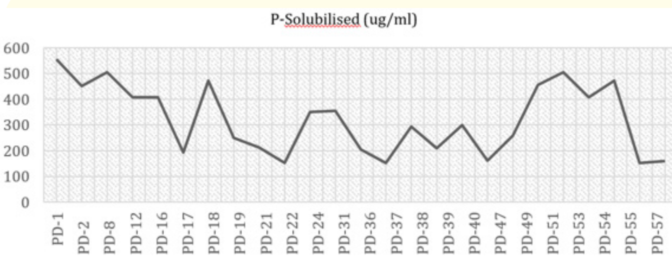

Figure 3: Concentration of Phosphate solubilization of the isolates.

Bacterial isolates PD-8 and PD-1 exhibited poor results in citrate and indole tests. Morphological and biochemical characteristics of PD-8 align with typical phenotypic traits of the genus *Bacillus sp*. (35) whereas PD-1 resemble *Pseudomonas sp.* [36].

Qualitative evaluation of P- solubilization was performed by IAA production where the recorded range of IAA production in isolates lie between 6ug/ml -14ug/ml (Figure 4). PD-1 and PD-8 both had shown the IAA production of 11 ug/ml after 3 days. PD-12 showed the maximum amount of IAA production of 14 ug/ml.

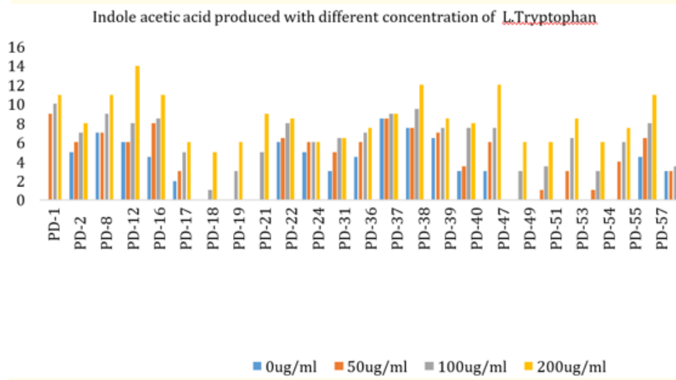

Figure 4: IAA concentration produce by isolates in ug/ml.

Characterization of plant growth promoting Activities of PD-8

Following various incubation period on their respective media, an analysis was conducted to evaluate the plant growth-promoting capabilities of isolate PD-8. The findings revealed positive results for the production of hydrogen cyanide (HCN), antifungal activity, and ammonia.

Agrochemicals tolerance among bacterial isolates

Out of fifty-seven isolates, PD-8 was singled out due to its promising plant growth-promoting (PGP) attributes. Subsequently, PD-8 was subjected to different concentrations of three fungicides (ranging from 0 to 100 ppm) (Table 1). The results revealed that the isolate exhibited tolerance to carbendazim and mancozeb at concentrations up to 100 ppm. This resilience suggests its potential suitability for integration into disease management programs.

Discussion

In this extensive study, the isolation of phosphate-solubilizing microorganisms from the rhizospheric soil of *Triticum aestivum* (Wheat) was meticulously conducted through both qualitative

S. No.	Pesticide	Concentration in ppm										
1	Carbendazim	0	10	20	30	40	50	60	70	80	90	100
		+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
2	Mancozeb	+++	+++	+++	+++	+++	+++	++	++	++	++	++
3	Metalaxyl-M	+++	+++	++	++	++	++	++	----	----	----	----

Table 1: Compatibility of PD-8 with the pesticides.

and quantitative screening methods. A total of 57 isolates were obtained, with PD-24 exhibiting the highest zone of solubilization (21 mm), closely followed by PD-21 (21 mm), PD-8 (14 mm), and PD-47 (13 mm). Further quantitative assessments focused on 18 isolates with solubilization zones exceeding 10 mm. During the incubation period, the pH of the culture broth ranged from 7.0 to 3.6. Notably, PD-1 demonstrated superior phosphate solubilization, reaching 555.5 μg/ml on the 9th day, followed by PD-8 (505.0 μg/ml) and PD-18 (472.5 μg/ml). PD-22 andPD-37 exhibited the least solubilization at 152.0 μg/ml and 152.5 μg/ml, respectively. The findings of this investigation surpass those documented in [37], where *Aneurinibacillus aneurinilyticus* CKMV1 was isolated from the rhizosphere of Valeriana jatamansi, demonstrating phosphate solubilization of 260 μg/ml and indole-3-acetic acid (IAA) production of 8.1 μg/ml. However, they fall short compared to other studies reporting higher IAA production ranging from 19.2 to 22 μg/ml [38] and phosphate solubilization of 334 ± 0.8 μg/ml [39].

Beyond phosphate solubilization, the study explored plant growth-promoting activities. Indole acetic acid (IAA) production after 72 hrs of incubation at 30°C revealed that PD-37 produced the highest IAA (8.5 μg/ml), while PD-1, PD-18, PD-19, and PD-21 showed no IAA production. PD-1 excelled in IAA production under varied L-tryptophan concentrations, with the highest at 200 μg/ml.

In the context of plant growth-promoting rhizobacteria (PGPR) activities, PD-8 showcased noteworthy hydrogen cyanide (HCN) production, and all isolates were tested for antifungal activity, with PD-8 displaying such activity. Ammonia production screening indicated that all strains, except PD-1 and PD-36, exhibited ammonia production, demonstrating the potential for fermentative ammonia production.

Exploring the compatibility of PD-8 with commonly used pesticides, the study revealed its resilience and excellent growth across all concentrations of carbendazim tested. This robust compatibility aligns with the findings of Leha and Venkataraman (2001), indicating favorable interactions between PD-8 and carbendazim.

This multifaceted analysis emphasizes the unique attributes of PD-8 as a phosphate-solubilizing and plant growth-promoting bacterium, showcasing promising potential for sustainable agricultural applications.

Conclusion

In general, numerous beneficial plant growth-promoting rhizobacteria (PGPR) strains have been identified to enhance plant growth and yields. Among the isolated bacteria, PD-8 demonstrates notably high phosphate solubilization efficiency in both agar and broth media, alongside significant indole-3-acetic acid (IAA) production.Furthermore,PD-8 also showed compatibility with two of the tested pesticide. The tolerance to pesticides exhibited by bacterial isolates are likely to enhance their ability to survive in polluted soil environments. The selected rhizobacterial isolate in this study demonstrated inherent pesticide tolerance and the production of various plant growth-promoting substances. These advantageous characteristics render rhizobacteria an appealing and sustainable alternative for crop production. The findings suggest that the formulation of PGPR strains with potential activity can enhance nutrient availability in the rhizosphere by sequestering nutrients, thereby mitigating leaching. While the current study underscores the potential of PGPR to positively influence wheat growth parameters, further research into the molecular characteristics of these strains and optimal application methods for PGPR inoculation may facilitate the exploitation of these microorganisms. It is evident that microorganisms continually evolve in response to their surroundings. However, the conclusions drawn from this study are based on laboratory experiments, and further research is warranted to validate these findings in real-world field conditions. Additionally, elucidating the molecular mechanisms underlying the developmen pesticide tolerance among rhizobacteria requires further investigation.

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Conflict of Interest

The authors possess no conflict of interest.

Bibliography

1. Kennedy AC. “Bacterial diversity in agroecosystems”. Invertebrate biodiversity as bioindicators of sustainable landscapes. Elsevier, (1999): 65-76.

2. Singh., *et al*. “Climate Change and Nutrients Dynamics of Soil”. *Climate Change and the Microbiome: Sustenance of the Ecosphere* (2021): 521-549.

3. Liebman A. "Legumes and soil organic matter transformations in Upper Midwest agroecosystems". Diss. University of Minnesota, (2018).
4. Kumar V., *et al*. "Rhizosphere: A functional perspective". *Critical Reviews in Microbiology* 3 (2014): 221-230.
5. Antoun Hani. "Plant-growth-promoting rhizobacteria". (2013): 369-385.
6. Singh V., *et al*. "Does a rhizospheric microorganism enhance K+ availability in agricultural soils?". *Microbiological Research* 169.5-6 (2014): 337-347.
7. Aloo BN., *et al*. "Impacts of soil erosion on crop productivity". In Soil Erosion Research for the 21st Century (2017): 137-147.
8. Roopnarain A., *et al*. "Soil fertility challenges and Biofertiliser as a viable alternative for increasing smallholder farmer crop productivity in sub-Saharan Africa". *Cogent Food and Agriculture* 3.1 (2017): 1400933.
9. Bauddh K., *et al*. "Sustainable agricultural approaches for enhanced crop productivity, better soil health, and improved ecosystem services". *Ecological and Practical Applications for Sustainable Agriculture* (2020): 1-23.
10. Sarıhan O., *et al*. "Plant-bacterial symbiosis: an ecologically sustainable agriculture production alternative to chemical fertilizers". *Revisiting Plant Biostimulants. IntechOpen*, (2022).
11. Maheshwari KD., *et al*. "Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: Action mechanisms and future prospects". *Ecotoxicology and Environmental Safety* 156 (2018): 225-246.
12. Egamberdieva D., *et al*. "Beneficial effects of plant growth-promoting rhizobacteria on improved crop production: prospects for developing economies". Bacteria in agrobiology: Crop productivity. Berlin, Heidelberg: Springer Berlin Heidelberg, (2013): 45-63.
13. Kumar R., *et al*. "Sustaining productivity through integrated use of microbes in agriculture". *Role of Microbial Communities for Sustainability* (2021): 109-145.
14. Toledo L., *et al*. "Phosphorus dynamics in the soil–plant–environment relationship in cropping systems: A review". *Applied Sciences* 11.23 (2021): 11133.
15. Muhammad I., *et al*. "Phosphorus mobilization in plant–soil environments and inspired strategies for managing phosphorus: A review". *Agronomy* 12.10 (2022): 2539.
16. Lee K.D., *et al*. "Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber". *Plant soil and Environment* 52.3 (2006): 130.
17. Verma JP., *et al*. "Potassium solubilizing microorganisms for sustainable agriculture" (2016): 331.
18. Afzal A., *et al*. "Rhizobium and phosphate solubilizing bacteria improve the yield and phosphorus uptake in wheat (*Triticum aestivum*)". (2008): 85-88.
19. Tian J., *et al*. "Roles of phosphate solubilizing microorganisms from managing soil phosphorus deficiency to mediating biogeochemical P cycle". *Biology* 10.2 (2021): 158.
20. Bhandari BS., *et al*. "Rhizosphere associated PGPR functioning". *Journal of Pharmacognosy and Phytochemistry* 8.5 (2019): 1181-1191.
21. Swapnil P., *et al*. "PGPR-mediated induction of systemic resistance and physiochemical alterations in plants against the pathogens: Current perspectives". *Journal of Basic Microbiology* 60.10 (2020): 828-861.
22. Singh R., *et al*. "Plant growth-promoting microbes: diverse roles in agriculture and environmental sustainability". *Probiotics and Plant Health* (2017): 71-111.
23. Olalekan A., *et al*. "Plant disease management: leveraging on the plant-microbe-soil interface in the biorational use of organic amendments". *Frontiers in Plant Science* 12 (2021): 700507.
24. Rao YS., *et al*. "Phosphorus". In H. G. Glick (Ed.), Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties (pp. 891-921). Soil Science Society of America. (1993): 891-921.

25. Pikovskaya RI. "Mobilization of phosphorus in soil in connection with vital activity of some microbial species". *Mikrobiologiya* 17 (1948): 362-370.
26. Pande A., *et al*. "Phenotypic and genotypic characterization of phosphate solubilizing bacteria and their efficiency on the growth of maize". *Journal of Genetic Engineering and Biotechnology* 15.2 (2017): 379-391.
27. Gordon SA., *et al*. "Colorimetric estimation of indoleacetic acid". *Plant Physiology* 26.1 (1951): 192.
28. Souissi T., *et al*. "Cyanide production by rhizobacteria and potential for suppression of weed seedling growth". *Current Microbiology* 43 (2001): 182-186.
29. Dye DW. "The inadequacy of the usual determinative tests for the identification of Xanthomonas spp". *New Zealand Journal of Science* 5.4 (1962).
30. Leila R., *et al*. "Use of plant growth promoting bacteria as an efficient biotechnological tool to enhance the biomass and secondary metabolites production of the industrial crop Pelargonium graveolens L'Hér. under semi-controlled conditions". *Industrial Crops and Products* 154 (2020): 112721.
31. Bergey and Hendricks D. "Bergey's manual of determinative bacteriology". Lippincott Williams & Wilkins, (1994).
32. Helaly AA., *et al*. "Effects of growth-promoting bacteria on growth, yield and nutritional value of collard plants". *Annals of Agricultural Sciences* 65.1 (2020): 77-82.
33. Mohammad S., *et al*. "In vitro investigation to explore the toxicity of different groups of pesticides for an agronomically important rhizosphere isolate Azotobacter vinelandii". *Pesticide Biochemistry and Physiology* 157 (2019): 33-44.
34. Mohammad S., *et al*. "Mesorhizobium ciceri as biological tool for improving physiological, biochemical and antioxidant state of Cicer aritienum (L.) under fungicide stress". *Scientific Reports* 11.1 (2021): 9655.
35. yaneshwar P., *et al*. "Isolation, biochemical characterization and production of biofertilizer from Bacillus megaterium". *International Journal of Life-Sciences Scientific Research* .2.6 (2016): 749-752.
36. Dipak P and Sankar S.N. "Isolation and characterization of phosphate solubilizing bacterium Pseudomonas aeruginosa KUPSB12 with antibacterial potential from river Ganga, India". *Annals of Agrarian Science* 15.1 (2017): 130-136.
37. Chauhan A., *et al*. "Tricalcium phosphate solubilization and nitrogen fixation by newly isolated Aneurinibacillus aneurinilyticus CKMV1 from rhizosphere of Valeriana jatamansi and its growth promotional effect". *Brazilian Journal of Microbiology* 48 (2017): 294-304.
38. Somayeh E., *et al*. "Consortium of endophyte and rhizosphere phosphate solubilizing bacteria improves phosphorous use efficiency in wheat cultivars in phosphorus deficient soils". *Rhizosphere* 14 (2020): 100196.
39. Lumyong S., *et al*. "Screening and optimization of indole-3-acetic acid production and phosphate solubilization from rhizobacteria aimed at improving plant growth". *Current Microbiology* 62 (2011): 173-181.