EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 10
Issue: 12
184-190
Year: 2023

STUDY OF A PHOSPHATE SOLUBILISING STRAIN FROM MANGROVE SOIL ASA BIOINOCULANT FOR PLANTGROWTHPROMOTION

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Article Received on 01/10/2023 **Article Revised on 22/10/2023** **Article Accepted on 12/11/2023**

ABSTRACT

Background: The environment is negatively impacted with the use of chemical phosphate fertilizers. Unfortunately, most of the phosphorus precipitates in soil forming salt complexes, which are unavailable to plants. A practical and sustainable solution to this problem is the use of Phosphate Solubilizing Bacteria (PSB) which aids in increasing the availability of phosphorus to plants using natural processes. **Methods**: In this study, PSB were enriched from mangrove soil samples collected from Mumbai, India. A strain with maximum solubilization efficiency was screened using National Botanical Research Institute's Phosphate growth medium (NBRIP) and Pikovaskya's agar medium, and its activity was optimized using standard methods. The potential of selected strain as a bioinoculant was studied on seed germination (of black gram, cowpea, green gram and moth bean) and seedling characteristics (on day 3). **Results**: Among 12 isolates obtained in our study, *Azotobacter* spp. showed maximum solubilization efficiency (255% equivalent to 51µg/ml phosphate) in least time (3 days). Its growth was accompanied by pH reduction of 2.82 units by day 12, indicating production of organic acids. Optimum conditions for phosphate solubilization were identified as pH 5-7, room temperature (30°C) and presence of sucrose. As a bioinoculant, *Azotobacter* spp. showed upto 90% improvement in the root and shoot length of seedlings compared to control. **Conclusion**: Overall, our study indicates that mangrove soil serves as a natural reservoir for PSBs. Screening and application of potential PSB strains from mangrove samples as bioinoculants may be immensely helpful in sustainable approach to plant growth promotion in agricultural practices.

KEYWORDS: *Phosphate solubilizing bacteria, Pikovaskya's medium, Optimization, Azotobacter, Bioinoculant, Sustainable.*

INTRODUCTION

Phosphorus is a macronutrient required by living things for essential cellular functions like DNA replication, signal transduction, energy transfer and maintenance of cell membrane integrity.[1] Plants also utilize elemental phosphorus as the most important nutrient after nitrogen. For this reason, chemical fertilizers containing high concentration of phosphorus is applied to agricultural fields.[2] A 2001 report by Tiwari[3], stated that 98% districts in India are severely deficient in available forms of phosphorus and application of chemical fertilizers result in highly profitable yields. Hence, although over 90% of phosphorus in added chemical fertilizer is converted into insoluble salt complexes of calcium, iron and aluminium, they are continuously and increasingly used to meet the food demand.[2,3] As a result, phosphorus, along with other nutrients, has been washed off into the waterbodies from Indian soil, gradually decreasing its fertility and impeding nutrient cycling in nature.[4]

To reduce the impact of chemical phosphate fertilizers on our environment, sustainable strategies like use of organic fertilizers and inter-cropping systems has been suggested by researchers.[5] The application of Phosphate Solubilizing Bacteria (PSB) in agriculture is another potential, yet comparably less recognized, microbiological strategy that can replace chemical phosphate fertilizers, or at least reduce our dependence on them. PSBs recycle phosphorus from its complexes by solubilization due to production of organic acids or chelation.[6] In addition, most PSBs are reported to produce protease enzymes and Indole-3-Acetic Acid (IAA) that aid in plant growth promotion. The former compound aids the plants to overcome biotic and abiotic stress, and in seed germination, whereas the latter promotes RNA and protein synthesis.[7,8] In an ecosystem, PSBs are naturally enriched in phosphorus polluted but habitable areas which are rich in other nutrients. For instance, the abundance of cations in the interstitial waters surrounding mangroves causes precipitation of phosphates, making them unavailable to

plants. Muddy soil and clay particles in mangroves can also absorb and store insoluble phosphates carried by tides or agricultural activities of the nearby regions.[9] Both these factors contribute to phosphorus pollution in mangrove soil. At the same time, the microbial and plant diversity in mangroves promote the overall nutritional profile of the surrounding soil. Thus, PSBs can survive and recycle phosphorus from its insoluble forms leading to their natural enrichment. In turn, they promote phosphorus uptake by the plant roots in mangroves.

Statistically, the accumulated phosphorus in agricultural soil globally would have been sufficient to sustain 100 years of crop yield, if it were available.[4] Ignoring phosphorus pollution as an environmental issue still requires a challenging strategy to replace phosphate fertilizers and meet agricultural demands in near future. This is because, with the current mining rate of 7,100 million tones/annum of raw material, phosphorus reserves will deplete in around 500 years.[4] In this context, the present study was carried out with an objective to isolate a potential phosphate solubilizing bacteria from mangrove soil, optimize its growth conditions and study its application as a bioinoculant in plant growth promotion.

MATERIALS AND METHODS

Sample collection, enrichment and isolation of phosphate solubilizing bacteria

Mangrove soil samples (~30g) were collected, in pre-sterilized flasks, from Thane and Borivali creek regions situated in Mumbai, India. The collected samples were transferred to the laboratory on the same day and processed immediately. The phosphate solubilizing bacteria were enriched in 50mL National Botanical Research Institute's Phosphate growth (NBRIP) medium [composition in g/L: glucose (10), tricalcium phosphate (5), magnesium chloride (5), magnesium sulphate (0.25), potassium chloride (0.2), ammonium sulphate (0.1), pH 7] for 7 days before their isolation on NBRIP agar plates.

Screening of potential phosphate solubilizers

The isolates were spot inoculated on Pikovskaya'sagar medium [composition in g/L: glucose (10), tricalcium phosphate (5), potassium chloride (0.2), magnesium sulphate (0.1), sodium chloride (0.2), ammonium sulphate (0.5), manganese sulphate (0.002); ferrous sulphate (0.002), yeast extract (0.5), agar (15), pH 7] and incubated at Room Temperature (RT; ~30°C) for 3 days to determine their phosphate solubilization efficiency. After incubation, the comparative phosphorus solubilization index and efficiency were calculated using the formulas represented below.[10] Uninoculated Pikovskaya' sagar medium was used as control..

$$Solubilization\ Index\ (SI) = \frac{Colony\ diameter\ +\ Halo\ zone\ diameter}{\text{Colonydiameter}}$$

$$Solubilization\ Efficiency\ (SE) = \frac{Zone\ ratio}{\text{Colony}ratio}\ x\ 100$$

Quantitative estimation of solubilized phosphate was done colorimetrically (650nm) with standard phosphate assay.[11] For this purpose, the test isolates (0.1 O.D) were inoculated in Pikovskaya's liquid medium containing 0.5% tricalcium phosphate equivalent to 997μg/mL phosphorus. Incubation was carried out for 12 days and the inorganic phosphorus (Pi) released in the broth was estimated on day 3, 5, 7, 9 and 12, using 5mL aliquots, from standard curve of potassium orthophosphate. Test isolates inoculated in saline was used as control. The pH of the medium was also noted.

Identification of potential isolate

The potential isolate was identified based on cultural, morphological and biochemical tests and comparison of observations with Bergey's Manual of Determinative Bacteriology.[12]

Optimization of growth conditions for potential phosphate solubilizing bacteria

The potential isolate (0.1 O.D) was inoculated in 50mL NBRIP medium (pH 7) and incubated under standard conditions of Room Temperature (RT; 30°C) and shaker for 4 days. The phosphate solubilization activity was optimized by varying one growth parameter at a time while keeping the others constant. The growth parameters included in our study were temperature (30°C and 37°C), pH (5, 6, 7), and carbon sources (1% glucose, fructose, maltose and sucrose).[13, 14]

Determination of plant growth promoting ability of potential isolate

The production of Indole Acetic Acid (IAA) and proteolytic enzymes by potential isolate were detected to confirm its ability to promote plant growth. To check IAA production, the isolate (0.1 O.D) was inoculated in 5mL nutrient broth with and without tryptophan and incubated at 25°C for 7 days in a shaker incubator. Bacterial cells were separated from the culture broth by centrifugation and the supernatant was mixed with Salkowski's reagent (in 1:2 ratio). The mixture was incubated in dark for 60min at RT and the absorbance of the medium was measured colorimetrically at 530nm. The concentration of IAA in the culture medium was

determined using the standard curve prepared using pure IAA. The proteolytic activity of the isolate was determined by observing a clear zone surrounding a spot inoculated on casein yeast extract agar plate containing 7% skim milk powder, after 72 h incubation at RT.

Study of application of potential isolate as bioinoculant

The potential isolate (0.1 O.D) was inoculated in 50mL NBRIP medium and incubated under optimized conditions of growth. The cells were harvested by centrifugation and used as a bioinoculant for plant growth promotion. In the present study, the efficacy of bioinoculant was studied on germination of seeds of blackgram, cowpea, green gram and moth bean. The seeds (n=4) were soaked in distilled water for 10h and surface sterilized with 2% sodium hypochlorite for 5-7 mins. At the time of sowing seeds in the pot filled with soil, it was treated with 10^7 CFU/mL of test isolate. The pots were watered everyday as required and checked for seed germination.[15,16] After 3 days of growth, the root length, shoot length and number of leaves were noted. Along with potential isolate obtained in this study, a laboratory isolate of *Azotobacter* spp. was also used as a bioinoculant for comparative studies. Untreated seeds were used as control. Overall, 4 pots were set up, including

1. Control (with untreated seeds)
2. Pot with seeds treated with potential isolate
3. Pot with seeds treated with laboratory isolate of *Azotobacter* spp.
4. Pot with seeds treated with both strains

RESULTS AND DISCUSSION

Screening and isolation of potential phosphate solubilizers

Twelve morphologically distinct colonies were obtained on NBRIP agar plates. Among these, 8 isolates showed halo zones on Pikovskaya's agar medium indicating their potential phosphate solubilization efficiency. The solubilization index and efficiency of these isolates are represented in Table 1. Previous studies have indicated that it is possible for phosphate solubilizing isolates to not show a halo zone on agar plates due to varying diffusion rates of different organic acids secreted by them.[9,10] Hence, all 12 isolates were screened quantitatively for their phosphate solubilizing potential in this study. Quantitative assay indicated potential phosphate solubilization by 6 isolates, which was accompanied by significant reduction in initial pH (7.0) of the medium (Table 2). The 4 isolates observed on Pikovskaya'sagar medium without halo zones showed comparatively low phosphate solubilization efficiency. Isolate PSB10 showed maximum efficiency (255%) by solubilizing 51µg/ml phosphate in least time (3 days), and pH reduction (2.82 units by day 12). Hence, it was selected as the most potential candidate in our study.

Table 1: Phosphate solubilization index and efficiency of isolates.

Isolates	Solubilization index	P solubilizatione fficiency
PS2	2.7	170%
PS3	3.0	200%
PS5	2.58	158%
PS7	3.33	233%
PS9	3.22	222%
PS10	3.5	255%
PS11	3.36	236%
PS12	3.06	206%

Table 2: Quantitative phosphate solubilization and pH reduction of the growth medium.

Isolates	Solubilized phosphates in µg/ml					pH on day 3 and day 12
	Day3	Day5	Day7	Day9	Day12	
PS2	48.5	47	45	41.5	38	5.49 – 4.20
PS5	42.5	46	49.5	41.5	37.5	5.45 – 4.25
PS7	39	46	41.5	36.5	35.5	5.66 – 5.01
PS10	51	45	40	38	37.5	5.41 – 4.18
PS11	35	43	42.5	36.5	35.5	5.46 – 5.30
PS12	43	44	41.5	36.5	35	5.35 – 4.25

The halo zones formed on screening media can be due to production of organic acids, polysaccharides or phosphatase enzymes by PSB strains. Since the phosphate solubilization was accompanied by reduction in pH of the media in our study, it may be due to production of organic acids. Other studies have reported detection of organic acids like gluconic acid, formic acid, malic acid, lactic acid, acetic acid (5.0 mg/l) and citric acid in the medium along with phosphate solubilization by PSB strains.[9, 10]

Similar to our study, *Serratia* sp., isolated from mangrove soil of the Mahanadi river delta, Odisha, India showed solubilization efficiency of 44.84µg/ml, which was accompanied by 3.85 unit pH drop in growth medium.[9] A much higher phosphate solubilization

efficiency of 219.64±0.330 µg/mL (SI= 2.85) by *Pseudomonas aeruginosa* KUPSB12 was reported by Paul and Sinha.[17] The strain was isolated from river Ganga, West Bengal, India. In addition to phosphate solubilization, it showed broad spectrum antibacterial activity against pathogenic strains. Thus, it may provide additional advantage in agricultural activities.A PSB strain showing 99% similarity to *Burkholderia cepacia*, isolated from an agriculture field of Uttarakhand, India, also showed a high SI of 4.88 ± 0.69 and solubilized 305.49 ± 10.7281 µg/ml phosphates in 8 days.[10] Overall, all these studies highlighted the efficacy of simple screening techniques in isolation of potential PSB strains that can be used for agricultural as well as non-agricultural applications.

Identification of potential isolate

Isolate PSB10 was identified as Azotobacter spp. based on cultural, morphological and biochemical tests. The characteristics for identification tests are represented in Table 3.

Table 3: Characteristics of Azotobacter spp.

Microscopic characteristics	Gram negative motile rod shaped bacteria
Sugar fermentation	Positive test for Glucose, Fructose, Mannose, Sucrose, Arabinose and Mannitol
	Negative test for Lactose and Maltose
Other biochemical tests	Positive test for Voges Prausker's, Citrate utilization and Nitrate reduction
	Negative test for Indole, Methylred and Urease

Azotobacter spp. is widely recognized for their nitrogen fixing ability. However, there are several reports of their potential to solubilize phosphates and produce phytohormones.[18, 19, 20] Also, *Azotobacter* spp. adapt quickly to the soil conditions.[18] Hence, introducing a low phosphate solubilizing strain to polluted soil may result in improved efficacy of the introduced strain over time. Hence their application as biofertilizers or bioinoculants can be specifically beneficial in chemical fertilizer affected agricultural soil.

Optimization of growth conditions for potential phosphate solubilizing bacteria

Optimizing the solubilization parameters for the bioinoculant strain can significantly boost the application output. In this study we optimized only the basic parameters for phosphate solubilization, since it is difficult to control environmental conditions outside the laboratory. Also, strains that show optimum activity under stringent environmental conditions are not suitable to be used as a bioinoculant. Instead, a more versatile and adaptive strain is ideal for field studies. Phosphate solubilization by *Azotobacter* spp. was found to be optimum at pH 5-7 (on day 3), room temperature (30°C) and presence of sucrose (Table 4). Most soil has pH between 6-7 and an average temperature of 30°C during summers in India.[21] Also sucrose being the end product of photosynthesis is abundant in planted green fields and agricultural areas. Hence, the above optimum conditions suggest adaptive nature of *Azotobacter* spp. isolated in our study. It also shows that application of this strain in different soil may most probably not reduce its phosphate solubilizing efficiency (since all three parameters are common in Indian soil).

Table 4: Phosphate solubilized under different parameters of optimization.

Optimization conditions	**Solubilized phosphates in µg/ml**		
	Day2	**Day3**	**Day4**
pH			
pH5	33.5	48.5	32.5
pH6	34	50.5	32
pH7	36.5	51.5	34
Carbon source			
Glucose	28.5	32	34
Fructose	38	45	32
Sucrose	40	43.5	45
Maltose	39	41.5	31
Temperature			
RT	37.5	44	46
37°C	33.5	46	32

A common trend was observed at different pH and temperature on strain activity in this study. The solubilization potential increased gradually from day 2 to day 4 at different temperatures and presence of carbon sources. However, maximum activity was observed at pH 7 on day 3, and reduced significantly on day 4. Similarly, at pH 5 and 6, maximum activity was observed on day 3, which reduced on day 4. Thus our results indicate that pH and incubation time are critical optimization criterion for phosphate solubilization by *Azotobacter* spp., as compared to other parameters. Similar to our findings, *Azotobacter vinelandii* strains

O2, O4 and O6 also showed phosphate solubilization at wide pH range (5 – 10) with optimum activity at pH 7 on day 3. These strains were also tolerant to 45°C temperature and up to 5% NaCl concentration.[18] Optimum solubilization by *Serratia* sp., was observed at 45°C on 48h incubation at 100 rpm in growth medium (pH 5.0) containing glucose and ammonium sulphate.[9]

Study of application of potential isolate as bioinoculant

The isolated phosphate solubilizing Azotobacter spp. (PSB) as well as laboratory strain of Azotobacter spp. (LSA) showed considerable improvement in seed germination and growth characteristics of seedlings compared to control. Interestingly, seeds treated with both PSB and LSA showed significantly enhanced growth. The observations of pot studies on day 3 are indicated in Table 5. Overall our results showed improved germination and growth characteristics in the order of treatment with (PSB + LSA) > PSB > LSA > control. In this study, PSB strain was capable of producing IAA (217 µg/ml) as well as protease enzyme. In contrast, the laboratory strain lacked these characteristics as well as phosphate solubilization potential. In spite of their difference in abilities, PSB and LSA treatment results were comparable. Combined treatment (PSB + LSA), however, showed an astounding 65 - 145% increase in root length, and 120 – 264% increase in shoot length.

PSB strains are isolated from various soils indicating their abundance in nature.[9,10,17,18,21] However, the microbial load of these potential strains are too low to produce competitive outputs in any given soil region. Hence, use of PSB as bioinoculant is a natural intervention for sustainable agricultural practices. An endophytic bacteria of *Erythrina brucei* (*Acinetobacter soli*) reportedly increased shoot length and weight of wheat plants by 14.6% and 53.8% respectively. Similarly, its use as bioinoculant increased root length and weight by 35.9% and 57.1% respectively.[22] In another study, *Pantoea* sp. S32 significantly improved root and shoot growth of rice seedling in experimental and reclaimed soil, in China.[23] Bioinoculation of rhizospheric and endophytic bacterial isolates (consortium) of wheat resulted in improved root and shoot length as well as grain yield of its cultivars.[24] Overall, there have been reports of plant growth promotion by PSB isolates alone and in consortium. However, to the best of our knowledge, a synergy between two strains with different characteristics, resulting in improved seed germination and seedling characteristics is not reported in literature.

Table 5: Seed germination and seedling characteristics observed on Day 3.

Seeds	Observations on Day 3	Control	Laboratory strain of Azotobacter spp. (LSA)	Phosphate solubilizing Azotobacter spp. (PSB)	PSB + LSA
Black gram	No. of seeds germinated (n=4)	2	4	4	4
	No. of leaves	-	2 leaves each on 2 seeds	-	2 leaves each on 4 seeds
	Ave. Root length (mm)	26	29 [11.53]	33 [26.92]	43 [65.38]
	Ave. Shoot length (mm)	5	12 [140]	8 [60]	18.2 [264]
Cowpea	No. of seeds germinated (n=4)	1	2	2	4
	No. of leaves	-	-	-	-
	Ave. Root length (mm)	20	26 [30]	28 [40]	38 [90]
	Ave. Shoot length (mm)	6	14 [133.33]	10 [66.66]	14 [133.33]
Green gram	No. of seeds germinated (n=4)	4	4	4	4
	No. of leaves		2 leaves on 1 seeds	2 leaves each on 2 seeds	2 leaves each on 2 seeds
	Ave. Root length (mm)	26	35 [34.61]	39 [50]	63 [142.30]
	Ave. Shoot length (mm)	18	28 [55.55]	30 [66.66]	44 [144.44]
Moth bean	No. of seeds germinated (n=4)	2	3	3	4
	No. of leaves	-	-	2 leaves on 1 seeds	2 leaves each on 2 seeds
	Ave. Root length (mm)	20	26 [30]	33 [65]	49 [145]
	Ave. Shoot length (mm)	12	19 [58.33]	23 [91.66]	26.5 [120.83]

Values in square brackets indicate % increase compared to control

CONCLUSION

Considerable literary and experimental evidence suggest that PSBs stimulate plant growth and fruit/grain yield. In contrast, many researchers believe that co-production of phytohormones and other metabolites by PSBs are largely responsible for the observed effect. In this study, PSB as well as laboratory strain (without solubilization potential) of Azotobacter showed comparable efficacy in seed germination and development of roots and shoots. Co-inoculating both strains resulted in significantly

higher efficacy compared to control as well as that observed with single isolates. These observations indicate that though soluble phosphorus, IAA and enzymes promote plant growth, there exist unidentified factors that may work alone or in synergy during the growth.

REFERENCES

1. Kumar S, Kumar S, Mohapatra T. Interaction between macro- and micro-nutrients in plants. Front Plant Sci, 2021; 12: 665583.
2. Solangi F, Zhu X, Khan S, Rais N, Majeed A, Sabir MA, Iqbal R, Ali S, Hafeez A, Ali B, Ercisli S, Kayabasi ET. The global dilemma of soil legacy phosphorus and its improvement strategies under recent changes in agro-ecosystem sustainability. ACS Omega, 2023; 8(26): 23271-23282.
3. Tiwari KN. Phosphorus needs of indian soils and crops. Better Crops Int, 2001; 15.
4. Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springerplus, 2013; 2: 587.
5. Wu L, Gao JE, Ma XY, Li D. Application of modified export coefficient method on the load estimation of non-point source nitrogen and phosphorus pollution of soil and water loss in semiarid regions. Environ SciPollut Res Int, 2015; 22: 10647–10660.
6. Tian J, Ge F, Zhang D, Deng S, Liu X. Roles of phosphate solubilizing microorganisms from managing soil phosphorus deficiency to mediating biogeochemical P cycle. Biol, 2021; 10(2): 158.
7. Ratnaningsih HR, Noviana Z, Dewi TK, Loekito S, Wiyono S, Gafur A, Antonius S. IAA and ACC deaminase producing-bacteria isolated from the rhizosphere of pineapple plants grown under different abiotic and biotic stresses. Heliyon, 2023; 9(6): e16306.
8. Sritongon N, Boonlue S, Mongkolthanaruk W, Jogloy S, Riddech N. The combination of multiple plant growth promotion and hydrolytic enzyme producing rhizobacteria and their effect on Jerusalem artichoke growth improvement. Sci Rep, 2023; 13(1): 5917.
9. Behera BC, Yadav H, Singh SK, Mishra RR, Sethi BK, Dutta SK, Thatoi HN. Phosphate solubilization and acid phosphatase activity of *Serratia* sp. isolated from mangrove soil of Mahanadi river delta, Odisha, India. J Genet EngBiotechnol, 2017; 15(1): 169-178.
10. Pande A, Pandey P, Mehra S, Singh M, Kaushik S. Phenotypic and genotypic characterization of phosphate solubilizing bacteria and their efficiency on the growth of maize. J Genet EngBiotechnol, 2017; 15(2): 379-391.
11. Wang Z, Zhang H, Liu L, Li S, Xie J, Xue X, Jiang Y. Screening of phosphate-solubilizing bacteria and their abilities of phosphorus solubilization and wheat growth promotion. BMC Microbiol, 2022; 22(1): 296.
12. Bergey's Manual of Determinative Bacteriology (7th ed.). Am J Public Health Nations Health, 1964; 54(3): 544.
13. Zhang J, Wang PC, Fang L, Zhang QA, Yan CS, Chen JY. Isolation and characterization of phosphate-solubilizing bacteria from mushroom residues and their effect on tomato plant growth promotion. Pol J Microbiol, 2017; 66(1): 57-65.
14. Poonguzhali S, Madhaiyan M, Sa T. Isolation and identification of phosphate solubilizing bacteria from Chinese cabbage and their effect on growth and phosphorus utilization of plants. J MicrobiolBiotechnol, 2008; 18(4): 773-777.
15. Oteino N, Lally RD, Kiwanuka S, Lloyd A, Ryan D, Germaine KJ, Dowling DN. Plant growth promotion induced by phosphate solubilizing endophytic Pseudomonas isolates. Front Microbiol, 2015; 6: 745.
16. Khan MR, Khan SM. Effects of root-dip treatment with certain phosphate solubilizing microorganisms on the fusarial wilt of tomato. BioresourTechnol, 2002; 85(2): 213-215.
17. Paul D, Sinha SN. Isolation and characterization of phosphate solubilizing bacterium *Pseudomonas aeruginosa* KUPSB12 with antibacterial potential from river Ganga, India. Annals Agrarian Sc, 2017; 15(1): 130-136.
18. Nosrati R, Owlia P, Saderi H, Rasooli I, Ali Malboobi M. Phosphate solubilization characteristics of efficient nitrogen fixing soil *Azotobacter* strains. Iran J Microbiol, 2014; 6(4): 285-295.
19. Tejera N, Lluch C, Martinez-Toledo MV, Gonzalez-Lopez J. Isolation and characterization of *Azotobacter* and *Azospirillum* strains from the sugarcane rhizosphere. Plant Soil, 2005; 270: 223–232.
20. Farajzadeh D, Yakhchali B, Aliasgharzad N, Sokhandan-Bashir N, Farajzadeh M. Plant growth promoting characterization of indigenous *Azotobacteria* isolated from soils in Iran. CurrMicrobiol, 2012; 64: 397–403.
21. Das BS, Wani SP, Benbi DK, Muddu S, Bhattacharyya T, Mandal B, Santra P, Chakraborty D, Bhattacharyya R, Basak N, Reddy NN. Soil health and its relationship with food security and human health to meet the sustainable development goals in India, Soil Security, 2022; 8: 100071.
22. Berza B, Sekar J, Vaiyapuri P, Pagano MC, Assefa F. Evaluation of inorganic phosphate solubilizing efficiency and multiple plant growth promoting properties of endophytic bacteria isolated from root nodules Erythrinabrucei. BMC Microbiol, 2022; 22: 276.
23. Chen Q, Liu S. Identification and characterization of the phosphate-solubilizing bacterium *Pantoea* sp. S32 in reclamation soil in Shanxi, China. Front Microbiol, 2019; 10: 2171.
24. Emami S, Alikhani HA, Pourbabaee AA, Etesami H, Motasharezadeh B, Sarmadian F. Consortium of

endophyte and rhizosphere phosphate solubilizing bacteria improves phosphorous use efficiency in wheat cultivars in phosphorus deficient soils. Rhizosphere, 2020; 14: 100196.