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Bacillus stercoris and *Enterobacter quasihormaechei* as phosphate-solubilizing bacteria: Isolation, characterization, and abiotic stress response

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ABSTRACT

Phosphobacteria are key suppliers of phosphate, a crucial nutritional component for plant growth. After being isolated from the rhizosphere of vegetable plants, a total of 20 phosphate-solubilizing bacteria (PSB) were tested for their ability to dissolve phosphate in soil and promote plant growth by producing phytohormones. Only two of the twenty isolates tested positive by converting to dark pink, and these bacteria were capable of producing phytohormones (auxin). Hence, these bacteria were selected for further investigation after testing positive for auxin production. Auxin is regarded as a vital regulator, particularly for plant growth. It regulates seed germination and elongation of the plant's roots and shoots. After screening for phosphate-solubilization activity, it was revealed that the phosphate-solubilizing index of these isolates was recorded as 3.16 and 3.33. Under *in vitro* conditions, both plant growth-promoting bacteria were further studied for growth up to 72 h in terms of salinity, pH, and temperature. In this study, both P7 and P9 isolates showed maximum growth at pH 7.0 and 9.0, temperatures of 30°C and 47°C, and salinity of 4% and 10%, respectively. In addition, biochemical tests and *16S rRNA* gene sequence analysis were done, identifying isolates P7 and P9 as *Bacillus stercoris* and *Enterobacter quasihormaechei*, respectively. This study emphasizes that *B. stercoris* and *E. quasihormaechei* can be advantageous in sustainable agriculture by optimizing plant growth and increasing crop output.

1. INTRODUCTION

Phosphorus is an important macronutrient. It is required for several biological and physiological processes in plants, such as photosynthesis, signal transduction, and energy transfer. Although soil contains a significant amount of phosphorus, much of it exists in forms that plants are unable to absorb. Chemical fertilizers can meet the phosphorus needs of plants during crucial growth stages; however, they consistently harm the environment and cause soil damage, pollute water, and deplete phosphorus mineral reserves [1]. Africa, South America, and Eastern Europe had the greatest rates of soil P depletion. The lack of P in agricultural soil will also have an effect on food security around the world in the upcoming years [2]. Phosphorus makes up 0.05% of the normal soil, but only 10% of this mineral is available to plants since it does not dissolve completely and remains embedded in the soil [3]. About 98% of India's soil doesn't have enough accessible phosphorus [4]. A survey of Indian soil found that only 2.2% of the 363 districts had high phosphorus levels, 51.5% had medium levels, and 46.3% had low levels [5]. Phosphorus is generally taken up by plants as the primary orthophosphate ion, H_2PO_4. The deficiency of phosphorus reduces the size of the plant and develops a deep green color. Phosphorus is an essential plant nutrient that is added to the soil in the form of soluble inorganic phosphate [6,7]. The precipitated inorganic phosphate is solubilized through the action of mineral and organic acids produced by bacteria and fungi [8,9]. Plant root-associated PSBs are considered a viable alternative to inorganic phosphate fertilizers for enhancing plant growth and yield [10,11]. Rhizospheric bacteria play an important role in the biogeochemical cycling of both macro and micronutrients, for example, nitrogen, phosphorus, iron, zinc, manganese, and copper [12]. Several studies revealed that Phosphate-solubilizing *Pseudomonas* and *Arthrobacter* spp. could improve plant health by boosting phosphate absorption [13,14]. Potential microbial communities promote plant development and disease resistance as well. They are regarded as environmentally beneficial farming methods that mitigate the negative effects of traditional fertilizers on the environment [15]. Phosphate-solubilizing bacteria (PSB) have the ability to solubilize phosphorus and make it available to the plant for its absorption [16]. Plant growth-promoting bacteria (PGPB) can enhance the yield of various crops, for example, paddy, soybeans, legumes, maize, canola, palm, potato, tomato, wheat, and peppers. Microbes increase crop output, by improving plant development and stress resilience [17]. PSB have

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significant potential for immobilizing metals in the soil, which helps to create a non-toxic environment for plants. PSB is the source of lead (Pb) immobilization; therefore, it could be exploited to phytostabilize Pb-contaminated soils by improving soil structure and plant growth. This method not only reduces the bioavailability of lead but also facilitates the restoration of ecosystems that have been impacted by heavy metal pollution [18]. The aim of this study is to isolate and identify PSB from the rhizosphere of different plant species and evaluate their plant growth-promoting (PGP) traits, analyze the effect of various parameters on their phosphate-solubilizing activity. The study offers a sustainable approach in agriculture.

2. MATERIALS AND METHODS

2.1. Sample Collection

Soil samples were collected from the rhizospheric region of 7 vegetable plant species, that is, mustard, potato, peppermint, spinach, lentil, tomato, and chili, from Agriculture University Gwalior, India. Residues and stones were removed, and fresh soil samples were stored in sealed bags at 4°C [19].

2.2. Isolation and Screening of PSB

Serially diluted soil samples were prepared up to 10^6 dilutions. About 100 µL of each dilution were placed on Pikovskaya agar medium containing tricalcium phosphate as the sole source of phosphate [21]. Each sample was plated in triplicate with suitable soil concentrations. After incubation at 28°C for 72 h under aseptic conditions, all the isolates containing a clear halo zone around the colony were isolated as PSB. Halo zones and colony diameter were measured to calculate the value of the phosphate-solubilizing index (PSI) and solubilizing efficiency (SE).

$$PSI = \frac{Colony\ diameter\ +\ Halo\ diameter}{Colony\ diameter}$$

$$SE\ = \frac{Halo\ diameter}{Colony\ diameter} \times\ 100$$

2.3. Morphological and Phenotypic Characterization

The bacterial isolates were cultured using Pikovskaya's agar media. Gram staining, endospore staining, and capsule staining were used to study the morphology of isolates according to standard procedures. The stained cells were observed using a compound microscope. The staining reaction and cell morphology of potential PSB strains were noted. The motility of both bacterial strains was assessed. Bacterial isolates that showed growth turbidity around the stab line were considered motile. Isolates were also tested for carbohydrate fermentation, Methyl Red (MR), Voges-Proskauer (VP), urease, gelatinase, indole, citrate, oxidase, and catalase tests. The selected bacterial isolates were characterized according to Bergey's manual of determinative bacteriology [20].

2.4. Study of Phytohormone (Indole Acetic Acid [IAA]) Production

Gordon and Weber's method was used to determine the IAA production in bacterial culture, it is commonly known as auxin. The test tubes containing 0.05 g/L^{-1} (50 µg/mL) of L-tryptophan and 10 mL of nutrient broth, each were used to inoculate the bacterial cultures. The tubes were centrifuged for 10 min at 10,000 rpm following a 48-h incubation period. As a result, 1 mL of supernatant was combined with 2 mL of Salkowski reagent and allowed to sit at room temperature for 25–30 min. The presence of IAA was indicated by the appearance of a dark pink color against the control, which showed a light amber or light yellow color. All of the samples that came out negative looked like the control [22].

2.5. Optimization of PSB Isolates under *In Vitro* Conditions

Growth of the isolates was monitored by measuring the optical density turbidometrically on days 0, 24, 48, and 72 h using a spectrophotometer at 600 nm (Model- Shimadzu UV-1800). The experiment was arranged in three replicates.

(1) pH: The growth of the bacteria was tested in a pH ranging from acidic to basic (3, 5, 7, 9, and 11), and neutral (7.0) pH was considered to be the control
(2) Temperature: The bacterial isolates were characterized for their optimum temperature, and the effect of the temperature ranges from low to high (20°C, 30°C, 37°C, and 47°C) was studied, and room temperature 37°C was considered as a control
(3) Salinity: Four different concentrations (0%, 1%, 4%, 7%, and 10%) of salts were studied against 0% salt (control) for their effect on the growth of PSB.

2.6. Molecular Identification of the PSB Strains

PSB isolates were identified through analysis of 16S rRNA sequences. Genomic DNA was isolated using the HiPurA Bacterial DNA Purification Spin Column Kit (MB505-250PR, HiMedia, India) and analyzed on a 1% agarose gel electrophoresis. The bacterial-specific *16SrRNA* gene (1500 bp) was amplified using primers F27 (5'AGAGTTTGATCMTGGCTCAG 3') and 1492R (5' GGTTACCTTGTT ACGACTT 3') [23]. Polymerase chain reaction (PCR) amplification was carried out using an Applied Biosystems Veriti Thermal Cycler as follows: Denaturation at 94°C for 5 min, followed by 34 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 1.30 min, and a final cycle at 72°C for 7 min. PCR results were sequenced by NCIM CSIR-NCL in Pune. The DNA sequence was submitted to GenBank for homology analysis through the BLASTN tool [24]. The DNA sequence was uploaded to NCBI through GenBank, and multiple sequence alignments were performed with Clustal W [25]. The phylogenetic tree was generated using the neighbor-joining method in MEGA 11 [26], with a bootstrap of 1000 iterations [27], and evolutionary relationships were calculated.

2.7. Statistical Analysis

The Prism version 3 statistical software was used for statistical analysis. All comparisons of means were carried out using the one-way analysis of variance test. Multiple comparisons were carried out using Tukey's multiple range test. Statistical significance was defined as $P < 0.05$. The probability value of $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$ for selected isolates was compared.

3. RESULTS AND DISCUSSION

3.1. Isolation and PSI Evaluation of PSB

In the present study, PSB were isolated from 7 vegetable rhizospheric soils from the agriculture university, Gwalior, India. A total of 20 bacterial isolates were obtained from the collected soil sample. These isolates were qualitatively evaluated for phosphate-solubilization. A total of 16 isolates out of 20 showed a clear halo zone around their colonies, and their PSI was calculated [Figure 3]. The phosphate-solubilization potential of PSB was determined by measuring the PSI. The results indicated a varied ability among the isolates to solubilize

phosphate. The formation of a halo zone around the bacterial colonies shows their ability to solubilize phosphates by releasing organic acids [28]. In this study, 16 selected bacterial isolates exhibited a PSI range of 1.75 and 3.33. The highest PSI value was detected in the P9 strain, followed by P7, which is 3.16, as listed in Table 1 and Figure 2. Similar results were achieved by Pande *et al*. and Nacoon *et al*., who found that the PSI in different bacterial isolates ranged between 2.56 and 4.50 [29,30].

3.2. Qualitative Assessment of IAA Production

IAA production is another PGP trait in bacteria, reported by several researchers [31,32]. Phytohormone (IAA) synthesis was further qualitatively assessed in the isolated bacteria. Over 72 h of incubation in nutrient broth containing L-tryptophan, it was found that only two isolates, P7 and P9, out of the twenty showed positive results by changing from light amber to dark pink [Figure 4]. Hereby, the samples that did not show any color change exhibited negative results and resembled the control. The results were revealed that these two isolates were capable of producing a significant amount of IAA. Phosphate solubilizers that produce auxin have a noteworthy impact on improving plant development based on seed germination [33]. As auxin was found to promote cell elongation, particularly in stems and young leaves, it resulted in an increase in shoot length. Auxin also has a role in the development of adventitious and lateral roots, among other aspects of root growth [34,35]. Furthermore, auxin induces seed dormancy and regulates seed germination by stimulating abscisic acid (ABA) signaling pathways, resulting in ABA deposition that subsequently suppresses growth [36]. Moreover, auxin also impacts seed developmental parameters, such as seed weight and seed size, resulting in enhanced crop production [37]. In a relevant study by Thakur and Parikh, auxin-producing rhizobacterial species *Burkholderia kururiensis, Burkholderia cenocepacia, Enterobacter cloacae*, and *Bacillus subtilis* demonstrated a significant enhancement in shoot height and plant dry weight, thereby affirming that auxin-producing bacteria serve effectively as biofertilizer inoculants to promote plant growth [38]. Similarly, *Pseudomonas* and *Acinetobacter* spp. were also identified as auxin-producing, PGP bacterial strains [39].

3.3. Growth Optimization of Selected Strains under Abiotic Stress

In a further study of abiotic stress, PSB could tolerate temperatures as high as 45°C, large concentrations of NaCl (up to 5%), and a broad starting pH range of 5.0–10 [40]. Plant species may withstand all types of stress with the help of rhizobacterial IAA [41]. PSB isolates (P9 and P7) were chosen and tested for their best growth [Table 2] on the basis of their auxin-producing ability under different conditions, including salt levels (0%, 1%, 4%, 7%, and 10%), pH levels (3, 5, 7, 9, and 11), and temperatures (20°C, 30°C, 37°C, and 47°C). The *in vitro* conditions can have a significant impact on the growth of plant growth-promoting bacteria (PGPB). It controls factors, such as nutrient availability, pH, temperature, and the presence of other microbes; hence, it influences their ability to promote plant growth [42]. The study examined the growth of selected bacterial strains under abiotic stress. The strains were grown at different pH, temperature, and salt levels for 72 h to assess their long-term effects on growth and survival. The P9 strain grew mostly at neutral pH 7.0, while P7 was at alkaline pH 9.0 [Figure 5]. The optimal temperature for growth was 30°C; however, the P7 exhibited the highest growth at 47°C [Figure 6]. For the majority of species, a temperature of 47°C is considered stressful. Under such conditions, heat-shock proteins, membrane composition, and enzyme activity are adaptations that facilitate bacterial survival [43,44]. High salinity negatively impacts plant growth; the P7 strain showed the highest growth at 10% NaCl concentration, while P9 was at 1% salt and showed tolerance up to 4% NaCl [Figure 7]. Salinity tolerance is beneficial for plant growth in salt-affected soils [45]. A study by Gupta *et al*. found comparable outcomes for various bacterial strains

Table 1: Phosphate-solubilization index (PSI), solubilization efficiency in % (SE), and phytohormone (indole acetic acid) production ability of the selected isolates with their harvesting plant.

S. No.	Crop plant	Isolate	PSI index	SE (%)	Phytohormone
1	Lentil	P1	2.3	133	Negative
		P2	2.5	150	Negative
		P3	2.6	166	Negative
2	Tomato	P4	2.71	171	Negative
		P5	2.75	175	Negative
3	Chili	P6	1.75	133	Negative
		P7	3.16	216	Positive
		P8	2.2	120	Negative
		P9	3.33	233	Positive
4	Mustard	D1	Negative	Negative	Negative
5	Potato	D2	Negative	Negative	Negative
		D3	2.5	150	Negative
6	Peppermint	D4	2.4	140	Negative
		D5a	Negative	Negative	Negative
		D5b	2.2	128	Negative
		D5c	2.8	180	Negative
7	Spinach	D6a	2.4	140	Negative
		D6b	3	200	Negative
		D6c	Negative	Negative	Negative
		D6d	2.5	150	Negative

Table 2: Effect of pH, temperature, and salt on the growth of selected bacterial isolates.

pH	P7	P9
3	0.053±0	1.093±0.006
5	0.08±0.005	1.061±0.005
7	0.185±0.094	1.105±0.001
9	0.395±0.002	1.098±0.012
11	0.294±0.016	0.617±0.015
Temperature	**P7**	**P9**
20°C	0.234±0.024	1.093±0.006
30°C	0.187±0.038	1.061±0.005
37°C	0.164±0.018	1.105±0.001
47°C	0.356±0.12	1.098±0.012
Salt Conc.	**P7**	**P9**
0%	0.082±0.01	1.138±0.066
1%	0.119±0.024	1.259±0.067
4%	0.15±0.019	1.194±0.024
7%	0.17±0.003	0.53±0.007
10%	0.837±0.064	0.136±0.001

Data are presented in Mean±SE, n=3.

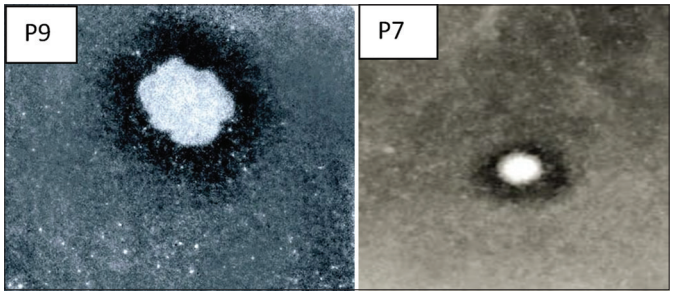

Figure 1: Phosphate-solubilizing bacteria showing a clear halo zone on Pikovskaya agar medium containing tricalcium phosphate ($Ca_3(po_4)_2$).

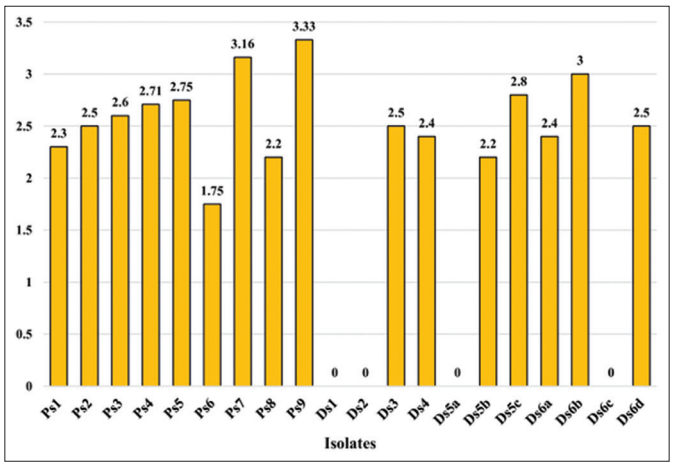

Figure 2: Graph represents phosphate-solubilizing index of all phosphate-solubilizing bacteria isolates.

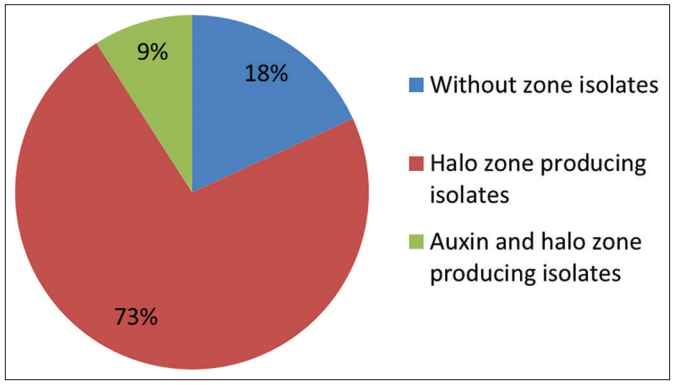

Figure 3: Graphical representation of total rhizospheric phosphate-solubilizing bacteria isolates that include indole acetic acid production, halo zone formation, and no halo zone.

grown under nearly identical environmental conditions: Optimal bacterial growth occurs at a pH range of 6.8–8.8, a temperature range of 28°C–37°C, and salt concentrations between 1% and 2% [46]. Our results were also supported by Shruti *et al*. and Arindam *et al*., who similarly found that diverse bacterial strains exhibited optimal growth at alkaline pH [47,48].

3.4. Characterization of Selected Strains

Both selected strains were then identified using biochemical tests for probable identification up to the genus level. Several tests were performed for biochemical analysis of the selected isolates, namely,

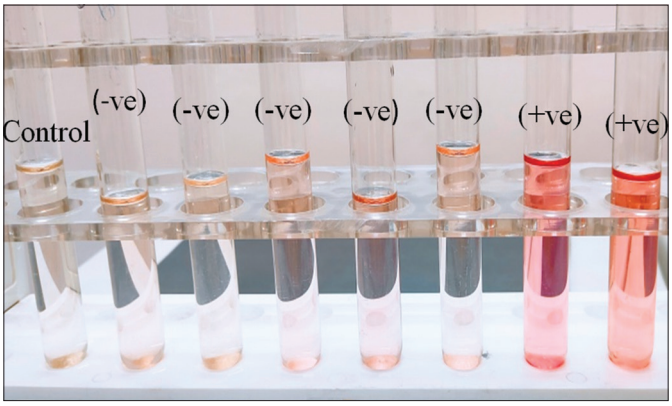

Figure 4: Qualitative estimation of indole acetic acid production (pink color developed) in comparison to control, (+VE) is a positive test, and (-VE) is a negative test.

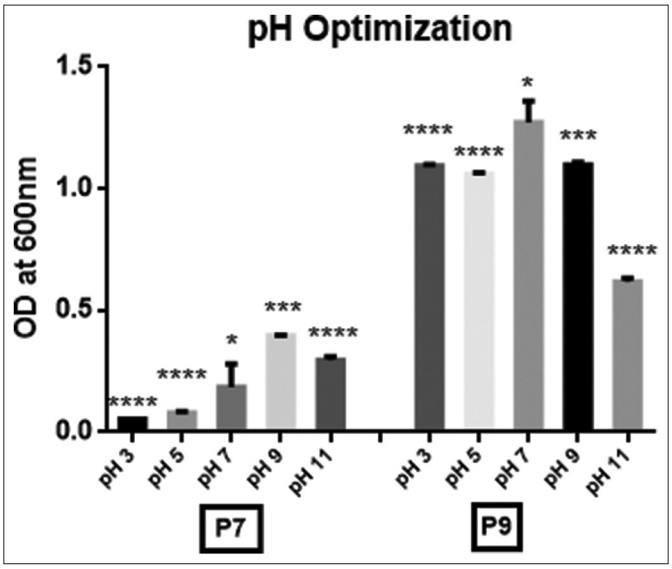

Figure 5: Growth of selected phosphate-solubilizing bacteria isolates at 72 h and different pH levels (3, 5, 7, 9, and 11). Significant difference between the growth of both bacterial isolates was evaluated (at $P < 0.05$).

amylase, indole, oxidase, nitrate, citrate, urease, bile esculine, catalase, MR, and VP tests. Results for some of the common tests are listed in Table 3. As shown in the table, isolate P9 is gram-negative, motile, and isolate P7 is gram-positive, short rod-shaped, and non-motile. Isolate P9, is negative for amylase, indole, and oxidase tests, and positive for the nitrate, citrate, urease, bile esculine, catalase, and MR VP tests. However, isolate P7 shows negative for the indole, oxidase, citrate, and MR tests and positive for other tests [Table 4]. In the carbohydrate fermentation test, both isolates, P7 and P9, produce negative results for lactose only but positive results for dextrose, sucrose, maltose, xylulose, and rhamnose sugars. In rhamnose fermentation, the P9 isolate produces gas, whereas the P7 isolate does not show any change [Table 4]. Reiner revealed that an organism fermenting a specific carbohydrate produces organic acids and gas [49]. However, an organism that is unable to ferment the provided glucose shows negative results.

3.5. Molecular Sequencing of Selected Bacterial Strains

The *16S rRNA* gene sequence of both strains showed 99% similarity with *Bacillus stercoris* and *Enterobacter quasihormaechei* from the

Table 3: Morphological and molecular identification of selected phosphate-solubilizing bacteria isolates.

Selected isolates	Gram staining	Endospore staining	Capsule staining	Motility	Colony morphology	Genus and Species	Accession No.
P7	G+ve, Purple, short rod	Negative	Negative	Non-motile	Lobate, large, white, mucoid	*Bacillus stercoris*	PQ394968
P9	G -ve, Pink, short rod	Negative	Negative	Motile	Entire, large, white, smooth	*Enterobacter quasihormaechei*	PQ394969

Where, G+ve=Gram-positive, G-ve=Gram-negative.

Table 4: Characterization of selected bacterial isolates.

Biochemical Tests	P7	P9
Amylase	+	−
Catalase	+	+
Citrate	−	+
Esculin hydrolysis	+	+
Gelatinase	+	+
Nitrate reductase	+	+
Oxidase	−	−
Growth at 6.5% NaCl	+	+
Indole	−	−
Methyl red	−	+
Urease	+	+
Voges-Proskauer	+	+
Sugar fermentation test		
Dextrose	+	+
Sucrose	+	+
Lactose	−	−
Maltose	+	+
Xylulose	+	+
Rhamnose	+	+

Where (+) indicates positive test and (−) indicates negative test.

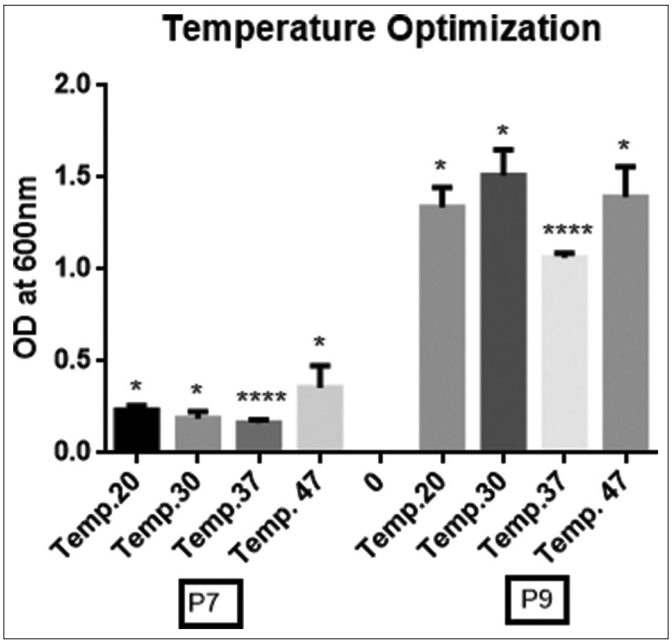

Figure 6: Growth of selected phosphate-solubilizing bacteria isolates at 72 h and different temperatures (20°C, 30°C, 37°C, and 47°C). Significant difference between the growth of both bacterial isolates was evaluated (at $P < 0.05$).

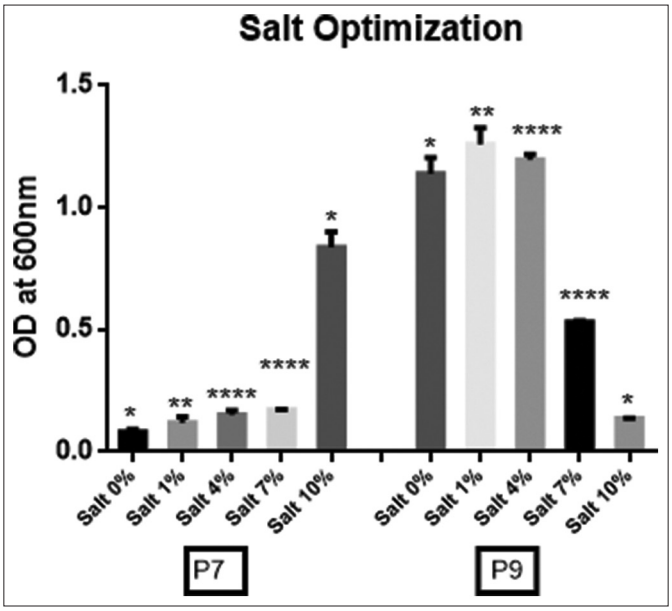

Figure 7: Growth of selected phosphate-solubilizing bacteria isolates at 72 h and different salt concentrations (0, 1, 4, 7, and 10). Significant difference between the growth of both bacterial isolates was evaluated (at $P < 0.05$).

GenBank database. The 16S rRNA gene sequence is a component of the bacterial ribosome that comprises highly conserved variable regions utilized for bacterial characterization, identification, and phylogenetic analysis at both the genus and species levels [50]. The result revealed that isolate P7 (accession no. PQ394968) was closely related to *B. stercoris* and P9 (accession no. PQ394969) to *E. quasihormaechei* [Figures 8 and 9]. Both isolates, *B. stercoris* P7 and *E. quasihormaechei* P9, have the potential to produce IAA and phosphate-solubilization. Pengproh and Khianngam reported that the same species have also shown phosphate-solubilization and plant growth-promoting activity [51,52]. However, findings reveal that both species perform well as effective biofertilizers by regulating seed germination and enhancing the growth of roots and shoots.

The study was concluded that *E. quasihormaechei* P9 and *B. stercoris* P7 strains showed maximum PSI of 3.33 and 3.16, respectively [Figure 1]. Among all isolated strains, only P9 and P7 exhibit auxin production property. The isolated strain *B. stercoris* P7 performs well at extreme conditions, showing maximum growth at pH 9.0, a 10% NaCl concentration, and high temperature at 47°C. On the other hand, *E. quasihormaechei* P9 showed best growth at pH 7.0, 30°C temperature, and can also tolerate up to 4% NaCl concentration. Moreover, Chen *et al*., identified PSB *Enterobacter hormaechei, Pseudomonas grimontii*, *Pantoea roadsii*, and allowed them to increase plant height

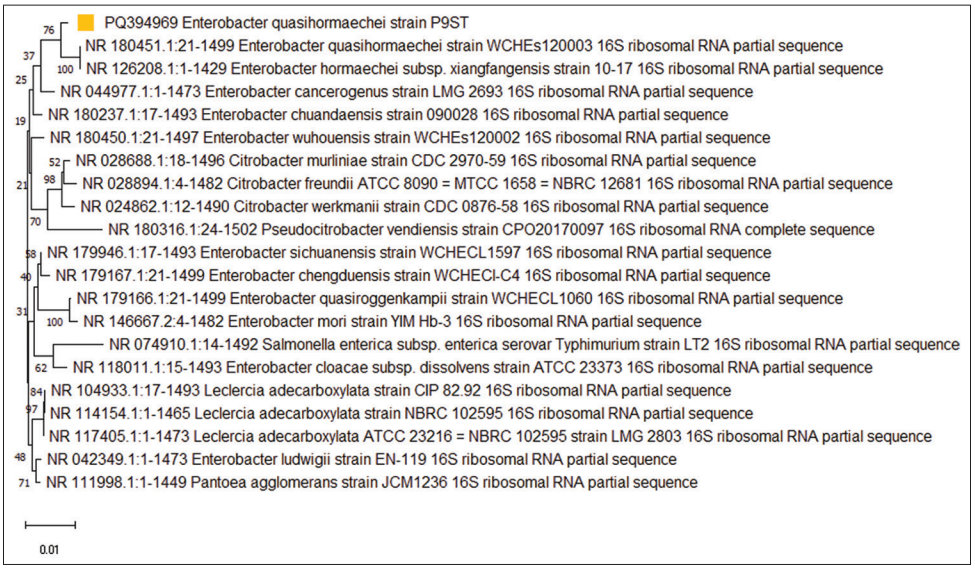

Figure 8: Phylogenetic analysis of the *16S rRNA* gene sequence of strain PQ394969 *Enterobacter quasihormaechei* P9ST constructed by the neighbor-joining method with 1000 bootstraps.

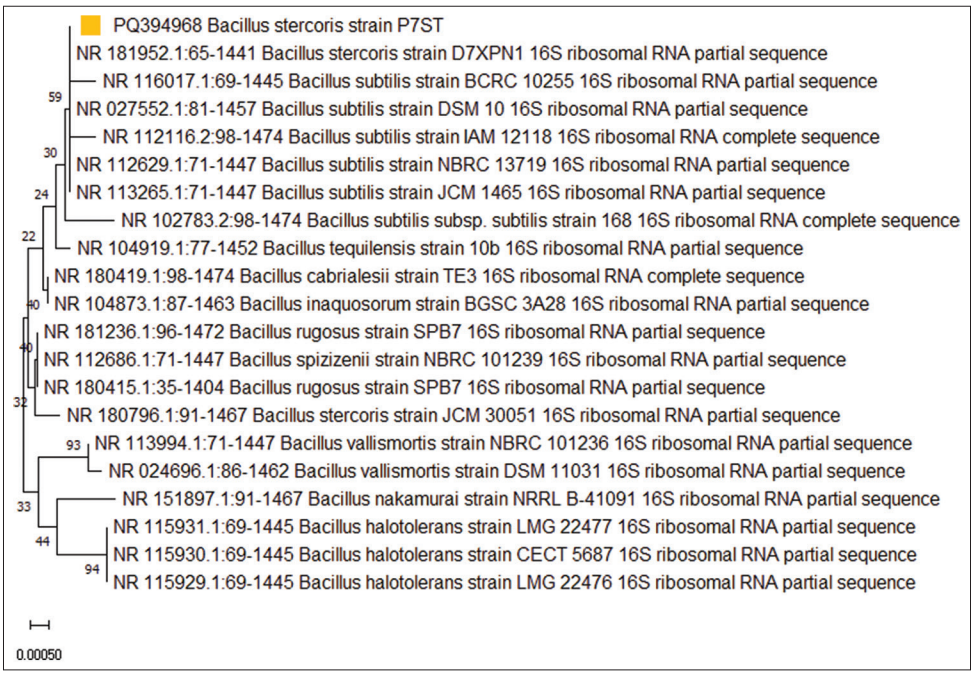

Figure 9: Phylogenetic analysis of the *16S rRNA* gene sequence of strain PQ394968 *Bacillus stercoris* P7ST constructed by the neighbor-joining method with 1000 bootstraps.

and biomass production [53]. In another study, Xess *et al*., identified *B. subtilis, Bacillus circulans, Pantoea dispersa*, and *Pseudomonas syringae* as potential PSBs that increase total phosphate concentration in plant tissues [54]. As a result, this study additionally improves our understanding of the potential for phosphate-solubilization and auxin production in isolates that may be beneficial in the formation of roots and seeds, as well as shoot elongation. Furthermore, it might be used in sustainable agriculture as an eco-friendly fertilizer. However, more information on several PGP characteristics, such as nitrogen fixation, HCN gas production, siderophore synthesis, metal remediation, and antibacterial activity, is required to utilize these PSBs as significant and efficient elements in sustainable agriculture. Further investigation is the purpose of future research that would ensure an integrated approach to the development of biofertilizer.

4. CONCLUSION

In the present investigation, PSB were isolated from the rhizospheric region of vegetable plants. Two isolates out of 20 were found as potential PSB based on clear zones formed by the bacterial colonies. These isolates were found positive for the auxin test and have shown satisfactory results in the different parameters of abiotic stress. PSB can promote plant growth by increasing root growth, phosphate availability, auxin production, and nutrient uptake. As a result, both

bacteria species could be employed as biofertilizers to increase plant height and aid in root and seed development. Both isolates were further selected for biochemical investigation and 16S rRNA molecular sequencing, identified as *B. stercoris* and *E. quasihormaechei.* Both of the strains are known as PSB and are considered efficient biofertilizers for better quality and quantity of crops. Moreover, this is a cost-effective, non-toxic, and eco-friendly alternative to chemical fertilizer, which produces detrimental effects on human health and the ecosystem. These PSBs are therefore believed to be promising in sustainable farming and have the potential to improve soil health and nutrient availability in addition to increasing crop yield.

5. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the present journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

6. FUNDING

There is no funding to report.

7. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

8. ETHICAL APPROVALS

This study does not involve experimentson animal or human subjects.

9. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

10. PUBLISHER'S NOTE

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The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

REFERENCES

1. Cheng Y, Narayanan M, Shi X, Chen X, Li Z, Ma Y. Phosphate-solubilizing bacteria: Their agroecological function and optimistic application for enhancing agro-productivity. Sci Total Environ. 2023:25;901:166468.
2. Samantaray A, Chattaraj S, Mitra D, Ganguly A, Kumar R, Gaur A, *et al*. Advances in microbial based bio-inoculum for amelioration of soil health and sustainable crop production. Cur Res Microb Sci. 2024;7:100251.
3. Illmer P, Barbato A, Schinner F. Solubilization of hardly-soluble AlPO4 with P-solubilizing microorganisms. Soil Biol Biochem. 1995;27(3):265-70.
4. Ghosh AB, Hasan R. Phosphorus fertility status of soils of India. Bull Indian Soc Soil Sci. 1979;12:1-8.
5. Kanwar JS, Goswami NN, Kamath MB. Phosphorus management of Indian soils-problems and prospects. Fertil News. 1982;27(2):43-52.
6. Singh S, Kapoor KK. Solubilization of insoluble phosphate cultured from different sources. Environ Ecol. 1994;12:51-5.
7. Illmer P, Schinner F. Solubilization of inorganic calcium phosphates solubilization mechanisms. Soil Biol Biochem. 1995;27(3):257-63.
8. Kucey R. Phosphate-solubilizing bacteria and fungi in various cultivated and virgin Alberta soils. Can J Soil Sci. 1983;63(4):671-8.
9. Mattey M. The production of organic acids. Crit Rev Biotechnol. 1992;12(1-2):87-132.
10. De Freitas JR, Banerjee MR, Germida JJ. Phosphate-solubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus* L.). Biol Fertil Soils. 1997;24:358-64.
11. Richardson AE. Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Funct Plant Biol. 2001;28(9):897-906.
12. Dotaniya ML, Meena VD. Rhizosphere effect on nutrient availability in soil and its uptake by plants: A review. Proc Natl Acad Sci India Section B Biol Sci. 2015;85:1-2.
13. Lifshitz R, Kloepper JW, Kozlowski M, Simonson C, Carlson J, Tipping EM, *et al*. Growth promotion of canola (rapeseed) seedlings by a strain of *Pseudomonas putida* under gnotobiotic conditions. Can J Microbiol. 1987;33(5):390-5.
14. Rodríguez H, Fraga R. Phosphate-solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv. 1999;17(4-5):319-39.
15. Alewell C, Ringeval B, Ballabio C, Robinson DA, Panagos P, Borrelli P. Global phosphorus shortage will be aggravated by soil erosion. Nat Commun. 2020;11(1):4546.
16. Zhu Y, Xing Y, Li Y, Jia J, Ying Y, Shi W. The role of phosphate-solubilizing microbial interactions in phosphorus activation and utilization in plant soil systems: A review. Plants. 2024;13(19):2686.
17. Reed L, Glick BR. The recent use of plant-growth-promoting bacteria to promote the growth of agricultural food crops. Agriculture. 2023;13(5):1089.
18. Park JH, Bolan N, Megharaj M, Naidu R. Isolation of phosphate-solubilizing bacteria and their potential for lead immobilization in soil. J Hazard Mater. 2011;185(2-3):829-36.
19. Barillot CD, Sarde CO, Bert V, Tarnaud E, Cochet N. A standardized method for the sampling of rhizosphere and rhizoplan soil bacteria associated to a herbaceous root system. Ann Microbiol. 2013;63:471-6.
20. Holt JG, Krieg NR, Sneath PH, Staley JT, Williams ST. Bergey's Manual of Determinate Bacteriology. Baltimore: Williams & Wilkins. 1994. p. 786-8.
21. Pikovskaya RI. Mobilization of phosphorus in soil connection with the vital activity of some microbial species. Microbiology. 1948;17:362-70.
22. Gordon SA, Weber RP. Colorimetric estimation of indoleacetic acid. Plant Physiol. 1951;26(1):192.
23. Clarridge JE 3rd. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. Clin Microbiol Rev. 2004;17(4):840-62.
24. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990;215(3):403-10.
25. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 1994;22(22):4673-80.

26. Tamura K, Stecher G, Kumar S. MEGA11: Molecular evolutionary genetics analysis version 11. Mol Biol Evol. 2021;38(7):3022-7.
27. Felsenstein J. Confidence limits on phylogenies: An approach using the bootstrap. Evolution. 1985;39(4):783-91.
28. Kesaulya H, Baharuddin B, Zakaria B, Syaiful SA. The ability phosphate-solubilization of bacteria rhizosphere of potato Var. Hartapel from Buru Island. Int J Curr Microb Appl Sci. 2015;4(1):404-9.
29. 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 Eng Biotechnol. 2017;15(2):379-91.
30. Nacoon S, Jogloy S, Riddech N, Mongkolthanaruk W, Kuyper TW, Boonlue S. Interaction between Phosphate-solubilizing bacteria and arbuscular mycorrhizal fungi on growth promotion and tuber inulin content of *Helianthus tuberosus* L. Sci Rep. 2020;18; 10(1):4916.
31. Kudoyarova GR, Vysotskaya LB, Arkhipova TN, Kuzmina LY, Galimsyanova NF, Sidorova LV, *et al*. Effect of auxin-producing and Phosphate-solubilizing bacteria on mobility of soil phosphorus, growth rate, and P acquisition by wheat plants. Acta Physiol Plant. 2017;39:1-8.
32. Phringpaen W, Aiedhet W, Thitithanakul S, Kanjanasopa D. Ability of phosphate-solubilizing bacteria to enhance the growth of rice in phosphorus-deficient soils. Trends Sci. 2023;20(12):7032.
33. Lebrazi S, Niehaus K, Bednarz H, Fadil M, Chraibi M, Fikri-Benbrahim K. Screening and optimization of indole-3-acetic acid production and phosphate-solubilization by rhizobacterial strains isolated from *Acacia cyanophylla* root nodules and their effects on its plant growth. J Genet Eng Biotechnol. 2020;18:1-2.
34. Rayle DL, Cleland RE. The acid growth theory of auxin-induced cell elongation is alive and well. Plant Physiol. 1992;99(4):1271-4.
35. Sosnowski J, Truba M, Vasileva V. The impact of auxin and cytokinin on the growth and development of selected crops. Agriculture. 2023;13(3):724.
36. Liu X, Zhang H, Zhao Y, Feng Z, Li Q, Yang HQ, *et al*. Auxin controls seed dormancy through stimulation of abscisic acid signaling by inducing ARF-mediated ABI3 activation in *Arabidopsis*. Proc Natl Acad Sci. 2013;110(38):15485-90.
37. Cao J, Li G, Qu D, Li X, Wang Y. Into the seed: Auxin controls seed development and grain yield. Int J Mol Sci. 2020;21(5):1662.
38. Thakur A, Parikh SC. Auxin hormone production and plant growth promotion by Phosphate-solubilizing bacteria of groundnut rhizosphere. Int J Innov Res Sci Eng Technol. 2015;4(9): 8539-48.
39. Leinhos V, Vacek O. Biosynthesis of auxins by phosphate-solubilizing rhizobacteria from wheat (*Triticum aestivum* and rye (*Secale cereale*). Microbiol Res. 1994;149(1):31-5.
40. Nosrati R, Owlia P, Saderi H, Rasooli I, Malboobi MA. Phosphate-solubilization characteristics of efficient nitrogen fixing soil *Azotobacter* strains. Iran J Microbiol. 2014;6(4):285.
41. Lebrazi S, Fadil M, Chraibi M, Fikri-Benbrahim K. Screening and optimization of indole-3-acetic acid production by *Rhizobium* sp. strain using response surface methodology. J Genet Eng Biotechnol. 2020;18(1):21.
42. Puri A, Padda KP, Chanway CP. *In vitro* and in vivo analyses of plant-growth-promoting potential of bacteria naturally associated with spruce trees growing on nutrient-poor soils. Appl Soil Ecol. 2020;149:103538.
43. Richter K, Haslbeck M, Buchner J. The heat shock response: Life on the verge of death. Mol Cell. 2010;40(2):253-66.
44. Niu Y, Xiang Y. An overview of biomembrane functions in plant responses to high-temperature stress. Front Plant Sci. 2018;9:915.
45. Gao Y, Zou H, Wang B, Yuan F. Progress and applications of plant growth-promoting bacteria in salt tolerance of crops. Int J Mol Sci. 2022;23(13):7036.
46. Gupta R, Kumari A, Sharma S, Alzahrani OM, Noureldeen A, Darwish H. Identification, characterization and optimization of Phosphate-solubilizing rhizobacteria (PSRB) from rice rhizosphere. Saudi J Biol Sci. 2022;29(1):35-42.
47. Shruti K, Arun K, Yuvneet R. Potential plant growth-promoting activity of rhizobacteria *Pseudomonas* sp in *Oryza sativa*. J Nat Prod Plant Resour. 2013;3(4):38-50.
48. Arindam Chakraborty AC, Mala RH, Roshni Rajgopal RR, Mohini Jain MJ, Rashmi Yadav RY, Siddalingeshwara KG, *et al*. Isolation and characterization of potential plant growth promoting rhizobacteria from non-rhizospheric soil. Int J Curr Microbiol Appl Sci. 2014;3(4):432-8.
49. Reiner K. Carbohydrate fermentation protocol. Energy. 2012;11:12.
50. Johnson JS, Spakowicz DJ, Hong BY, Petersen LM, Demkowicz P, Chen L, *et al*. Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. Nat commun. 2019;10(1):5029.
51. Pengproh R, Thanyasiriwat T, Sangdee K, Saengprajak J, Kawicha P, Sangdee A. Evaluation and genome mining of *Bacillus* stercoris isolate B. PNR1 as potential agent for fusarium wilt control and growth promotion of tomato. Plant Pathol J. 2023;39(5):430.
52. Khianngam S, Meetum P, Chiangmai PN, Tanasupawat S. Identification and optimisation of indole-3-acetic acid production of endophytic bacteria and their effects on plant growth. Trop Life Sci Res. 2023;34(1):219.
53. Chen J, Zhao G, Wei Y, Dong Y, Hou L, Jiao R. Isolation and screening of multifunctional Phosphate-solubilizing bacteria and its growth-promoting effect on Chinese fir seedlings. Sci Rep. 2021;11(1):9081.
54. Xess N, Sao S. Effect of carbon and nitrogen source on phosphate-solubilization and impact of phosphate-solubilizing bacteria, rock phosphate and organic waste treatments on maize plants. Appl Ecol Environ Sci. 2020;8(6):499-504.

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