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Exploration and Characterization of High-Efficiency Phosphate-Solubilizing Bacteria Isolates from Chickpea Rhizospheric Soil

Vikash Kumar[1], Hirendra Kumar Chourasia[2], Kumari Rajani[3] and Ravi Ranjan Kumar[4]

[1]Dept. of Biotechnology, [2]Dept. of Botany, T. M. Bhagalpur University, Bhagalpur, Bihar (812 007), India
[3]Dept. of Seed science and Technology, [4]Dept. of Molecular Biology and Genetic Engineering, Bihar Agricultural University, Sabour, Bhagalpur, Bihar (813 210), India

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Corresponding rajani.dsstiari@gmail.com

0000-0002-6300-4282

ABSTRACT

The study was conducted during April, 2021 to June, 2022 to study the isolation and identification of high effective strains of phosphate solubilizing bacteria and evaluation the ability to solubilize tricalcium phosphate *in vitro*. Microorganisms that live in soil are actively involved in the carbon, nitrogen, sulphur, and phosphorus cycles in nature, enabling them to maintain ecological balance. Phosphate-solubilizing microbes are used as agricultural biofertilizers and play an essential role in plant nutrition by enhancing phosphate uptake. Phosphate is one of the most important macronutrients necessary for the growth and development of plants. Numerous microorganisms present in the rhizosphere solubilize insoluble phosphorus, making it readily available to plants. Fourteen phosphate-solubilizing bacterial colonies were identified on Pikovskaya's agar medium, containing insoluble tricalcium phosphate (TCP) from rhizospheric soil. Among them, eight colonies showing clear halo zones around the microbial growth were considered as phosphate-solubilizing. All isolates showing the highest phosphate solubilization index (PSI), ranging from 2.8 to 4.03, were selected for further qualitative and quantitative studies. Out of these eight potent isolates, two strains showed the maximum PSI: V_7 (4.03) and V_8 (3.85) in agar plates, along with a high production of soluble phosphate, measuring 448.03 and 441.43 mg l^{-1}, and a greater reduction in pH in the broth culture. The morphological, biochemical, and molecular identification of V_7 and V_8 strains revealed them to belong the *Acinetobacter* and *Paenibacillus* genera, respectively, and were phylogenetically close to *Acinetobacter baumannii* and *Paenibacillus lautus* species.

KEYWORDS: *Acinetobacter*, isolates, *Paenibacillus*, phosphate, rhizosphere, solubilization

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1. INTRODUCTION

Phosphorus (P) is the second-most important macronutrient for the nourishment and development of all living forms on earth, including plants, animals, and microbes. It is a crucial part of phospholipids, ATP, and ADP along with nucleic acids. (Khan et al., 2010), It is also essential for the composition of cellular membranes, photosynthesis, macromolecular biosynthesis, respiration and play a significant part in the fixation of nitrogen in leguminous crops (Hutchins et al., 2019), along with a number of other metabolic activities like energy transfer and signal transduction (Zaidi et al., 2009 and Elser, 2012). So, the sufficient amount of P into plant system provides batter growth and development. But this highly vital element is one of the least accessible (only 0.1% of total soil phosphorus reserve) to the plants (Takahashi and Anwar, 2007, Zhu et al., 2018). In order to supply nutrients to encourage plant growth and hence increase crop yield. Chemical-based fertilizers have been applied extensively in agriculture everywhere the world. Modern cropping systems have undoubtedly benefited from chemical fertilizers, but their frequent application has adversely affected the health of agricultural soils, which leads to lower output yields and less effective nutrient use. (Zhang et al., 2021) Both organic and inorganic forms of phosphorus are found in soil; the average amount of phosphorus in soil is 0.05% (Ahmed et al., 2019). The low availability of P in soil is primarily due to its highly reactive nature as it readily forms insoluble complexes with other soil components like insoluble phosphates of iron, aluminium, and calcium (Khan et al., 2007 and Izhar Shafi et al., 2020). High P application rates can pose a serious risk to the environment through leaching, runoff and erosion, while excessive P application is likely to adversely affect soil efficiency due to various abiotic and biotic biochemical processes, including sorption, fixation, and immobilization (Bindraban et al., 2020; Bargaz et al., 2021). Therefore, the development of chemical fertilizer substitutes is currently fascinating to scientists.

The application of biological fertilisers that including microorganisms, has consequently attracted more scrutiny. Application of these beneficial microbes, individually or in combination with fertilizers, as nutrient-providers can assist in replenish and maintain long-term soil fertility through promoting healthy soil biological activity, suppressing pathogenic soil organisms, boosting microbial activity in the rhizosphere (Rossmann et al., 2017), and improving plant health (Biswas and Narayanasamy, 2006 and Billah et al., 2020). In addition to reducing the high cost of producing phosphatic fertilizers, the utilisation of phosphate solubilizing microorganisms in agriculture would also mobilize insoluble phosphorus in the fertilizers in the soils to which they are applied (Rodriguez and Fraga, 1999). It has been demonstrated that inoculating soil with phosphate-solubilizing microorganisms releases soluble phosphorus, fosters plant growth, and preserves plants towards pathogen infection (Verma et al., 2010). Several strains of phosphate solubilizing bacteria are produced IAA. Majorly, it improves the state of plants in all stages of growth, because of increasing the root exudates proportion in the rhizosphere (Glick, 2012). The efficient phosphate solubilizing bacteria majorly belongs to *Bacillus, Pseudomonas* and actinomycetes (de Sousa et al., 2021 and Bouizgarne, 2022).

The goal of the current study is to isolation and identification of high effective strains of phosphate solubilizing bacteria and evaluation the ability to solubilize tricalcium phosphate in vitro. In additionally establish the phylogenetic diversity of selected potent isolates with other bacteria that can potentially dissolve phosphate.

2. MATERIALS AND METHODS

The study has been carried out during April, 2021 to June, 2022 to study the selection of efficient strains of phosphate solubilizing bacteria form rhizospheric soil and evaluation their ability to solubilize tricalcium phosphate in vitro.

2.1. Collection of soil samples

Soil samples were collected from rhizosphere of chickpea plant at the agricultural farm of BAU Sabour Bhagalpur. Chickpea plants were uprooted carefully with their rhizosphere soil intact and collected in sterile plastic bags. Plants were shaken vigorously to remove soil from the roots which was then collected in a sample bag and stored at 4°C until further examination.

2.2. Serial dilution and agar plating

One gram of soil sample was mixed in 9 ml of 0.8% sterile NaCl solution. Turbid solution of soil was serially diluted. Serial dilution was carried out in 5 sterile test tubes each containing 9 ml of 0.8% sterile NaCl solution. 1ml of the turbid solution was added into the first test tube containing 9 ml NaCl solution resulting in a dilution of 10^{-1}. Then 1ml from this test tube was transferred to the second test tube giving a dilution of 10^{-2}. This was followed till a dilution of 10^{-4} obtained. Further, 0.1 ml aliquots of the serially diluted samples were placed on the middle of a sterile Pikovskaya's agar medium (Sonam et al., 2011) plates and then they were spread over the surface with the help of a sterile L- shaped glass rod. Incubated the plate at 30°C for a period of 48-72 h (2–3 days) in shaker cum incubator (moxcare) and observed the colonies.

2.3. Selection of phosphate solubilizing bacterial colonies

At the end of the incubation period, all plates were examined

to select colonies. The phosphate-solubilizing colonies were chosen based on the formation of halo zones on the medium (Bouizgarne et al., 2023) and sub-cultured on Pikovskaya's agar medium for further characterization.

2.4. Solubilization efficiency on the solid media

The phosphate-solubilizing activity of each isolate was assessed by inoculating a pinpoint of PSB strains on PKA medium supplemented with tricalcium phosphate (TCP) plates under aseptic conditions. The plates were then incubated at 28±2° C for 4–6 days with continuous observation of colony diameter and halo zone formation. Phosphate solubilization efficiency was calculated on the 6th day of incubation using the formula mentioned below (Ramkumar and Kannapiran, 2011).

SE%=(Z-C)/C×100

Were,

Z: Solubilization zone (in mm)

C: Colony diameter (in mm)

SE: solubilization efficiency

2.4.1. Solubilization index

Solubilization index of PSB on solid Pikovskaya's agar media was calculated according to the equation (Premono et al., 1996)

Solubilization index (SI)=(Total diameter(Colony+Halo zone))/Colony diameter

2.5. In vitro solubilization of tricalcium phosphate

The phosphorus solubilizing potential of PSB isolates was tested *in vitro* by estimating available phosphorus in Pikovskaya's broth, amended with 0.5% tricalcium phosphate as a substrate. Cultures of the phosphate-solubilizing bacteria were inoculated into 100 ml of Pikovskaya's broth in 250 ml flasks in triplicate replications, along with an equal number of uninoculated controls. The cultures were incubated for six days at 30°C on a rotary shaker (Moskcare). On the 6th day of incubation, the amount of Pi (phosphate) released in the broth was estimated. The broth cultures of bacteria were centrifuged at 9000 rpm for 20 minutes using an Eppendorf centrifuge to separate the supernatant from the cell growth and insoluble phosphate. The ascorbic acid colorimetric method, as described by Murphy and Riley (1962), was used to determine the concentration of assimilable P. For the colorimetric assay, 1 ml of supernatant was mixed with 160 µl of the reaction mixture and incubated for 5 minutes at room temperature. The optical density (OD) was measured at a wavelength of 880 nm, and the soluble P content was estimated using a standard KH_2PO_4 solution (concentrations ranging from 0 to 1 mg l^{-1}). Additionally, changes in the pH of the broth were measured using a digital pH meter (LabMan).

2.6. Molecular identification and phylogenetic analysis

The *16S rRNA* gene sequence of selected isolated bacterial strain was retrieved to assess the phylogenetic association using the *16S rRNA* gene specific universal primers: 27F and 1492R (Table 1).

Table 1: Details of primer used for molecular identification.

Primer	Sequences	References
27 F	5'-AGAGTTTGATCCTGGCT-CAG-3'	Chen and Liu (2019)
1492R	5'-TACGGTTACCTTGTTAC-GACTT-3'	

The reaction was performed with a final volume of 25 µl, containing 2X green PCR master mix (Thermo scientific), 10 pmol of each primer, and 2 µl of DNA template. The thermocycling was performed with in a thermocycler (Eppendorf Mastercycler nexus) and started with an initial denaturation for 4 min at 94°C, followed by 35 cycles (94°C for 30s, 55°C for 60 s and 72°C for 90s) and a final extension at 72°C for 10 min. PCR products were checked by electrophoresis in 1% agarose gel to evaluate the size of the amplicons.

The positive amplicons were purified with the Gene JET Gel Extraction Kit (Thermo scientific, Lithuania) according to the manual instructions. Purified DNA fragments were sequenced in both directions using the same primers as for PCR. The 16S rDNA sequence isolated from selected strain's was sequenced and sequence data were aligned and compared with available published sequences of bacterial lineage at NCBI GenBank (http://www.ncbi.nlm.nih.gov) using the BLAST (N) programme (Zhang at al., 2000). The completed sequence was entered into GenBank (Thompson et.al., 1997) to get accession number. In addition, previously reported phosphate solubilizing bacterial sequences were downloaded from GenBank and aligned all the sequences in alignment explorer tool of the MEGA (Molecular Evolutionary Genetic Analysis software) by using Clustal-W. (Tamura et al., 2021) The phylogenetic tree was prepared using the Neighbour-Joining method.

2.6. Statical analysis

The results were presented as mean values ± standard error of the three replicates. Data were statistically analysed using OPSTAT software. The significant difference between means was calculated by one way ANOVA ($p \leq 0.05$).

3. RESULTS AND DISCUSSION

3.1. Soil sample and isolation PSB

In the present study, rhizospheric soil samples from chickpea cultivation areas were used to isolate phosphate-

solubilizing bacteria (PSB). The selection of strains was based on the formation of distinct halo zones in serially diluted spread plates on Pikovskaya's agar medium. (Panda et al., 2013) The first qualitative indicator of PSB activity is the development of halos around colonies. Additionally, colonies without halos were isolated, and their solubilization ability was tested. To ensure their effectiveness and stability, we initially selected 14 isolates and co-inoculated them onto Pikovskaya's agar medium. Based on their ability to solubilize tricalcium phosphate (TCP), eight isolates were classified as PSB.

3.2. Qualitative estimation of PSB

The eight isolates were individually tested on Pikovskaya's agar and in broth media. The qualitative assessment was confirmed through the zone of solubilization (Figure 1) on Pikovskaya's agar media.

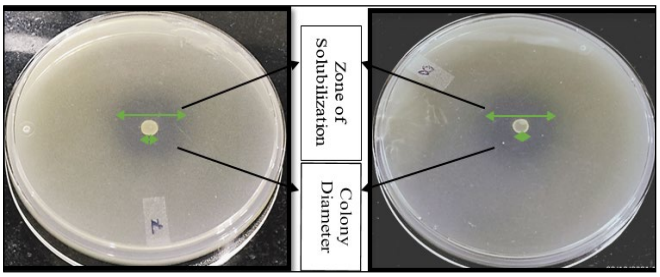

Figure 1: Qualitative estimation of phosphate solubilization through zone of solubilization

The formation of a halo zone around all isolates was observed after the 4th day of incubation. The results showed that colony diameters ranged from 6.9 mm to 9.6 mm, with a corresponding range in the zone of solubilization from 14.5 mm to 27.1 mm. Similar experimental results were reported by Kumar et al., (2010), Walpola and Yoon (2013), and Tariq et al. (2022). The solubilization index results ranged from 2.8 to 4.03 on average (Table 2).

The maximum solubilization index (SI) were calculated by V_7 isolates 4.03 with solubilization efficiency (SE) 303 followed by V_8 isolates SI 3.85 and SE 285. Similarly at the same time, the lowest calculated SI 2.80 and SE 180. The SI and SE of each bacterial strain shows its potential ability to solubilize phosphate. Greater value of SI and SE showed the stronger activity of the phosphatase enzyme so releasing P from organic compounds, and the clear halo zone will be obtained by PSB through the insoluble phosphate was processed into the soluble form. The formation of clear zone around the bacterial colony could be the production of polysaccharide or either production of organic acid (Halder & Chakrabarty, 1993) or either may be due to production of bacterial enzyme phosphatase (Paul and Sinha, 2013) or phytase (Patel et al., 2010).

3.3. Quantitative estimation of PSB strain and pH lowering of broth medium

The quantitative estimation of PSB strain is determined by growing a pure culture of bacterial isolates on the liquid Pikovskaya media. All the eight isolates (V_1–V_8) efficiently solubilized inorganic phosphate in the medium containing 0.5% tri-calcium phosphate. (Table 2) The result shows that amount of soluble phosphate concentration varies with different isolated PSB strain. The highest value 448.70 mg l^{-1} and 441.10 mg l^{-1} obtained by V_7 and V_8 whereas the minimum solubilization 281.17 mg l^{-1} were obtained by V_2 with respect to uninoculated broth. Similarly, (Perez et

Table 2: Qualitative and quantitative measurement of phosphate solubilization of selected isolates

Isolate	Colony diameter (mm)	Zone diameter (mm)	SE %	SI	pH change	TCP (mg l^{-1})
V_1	8.8±0.145	17.9±0.737	204±5.132	3.04±0.051	5.03±0.088	289.93±2.839
V_2	6.9±0.371	14.7±0.581	213±8.083	3.13±0.081	5.31±0.017	281.17±4.574
V_3	7.2±0.491	14.5±0.361	212±7.000	3.04±0.144	4.77±0.032	354.43±4.013
V_4	9.2±0.441	16.5±0.500	180±5.548	2.80±0.055	4.87±0.026	323.87±2.161
V_5	9.6±0.348	19.8±0.536	207±2.186	3.07±0.022	4.63±0.025	354.87±3.402
V_6	8.3±0.353	17.0±0.543	205±8.413	3.04±0.084	4.53±0.015	364.07±3.768
V_7	8.9±0.067	27.1±0.521	303±4.041	4.03±0.040	3.99±0.047	448.03±7.125
V_8	9.2±0.296	26.2±0.906	285±0.880	3.85±0.009	4.11±0.018	441.43±4.157
CD	1.032	1.868	17.374	0.220	0.123	12.810
SEm±	0.341	0.618	5.746	0.073	0.041	4.236

All PSB isolate characterized for colony diameter (mm), Zone of solubilization (zone diameter) Percentage solubilization efficiency (SE%), Solubilization index (SI), pH changes and Tri calcium phosphate (TCP) quantitively solubilized. The results were presented as mean values±standard error of the three replicates)

al., 2007) found about 580 µg ml^{-1}. P solubilization from the liquid cultures containing the dissolution of tricalcium phosphate by phosphate solubilizing strains.

All PSB isolates were studied for their characteristic of lowering the pH of broth medium. Generally, there is negative correlation between the degree of phosphorus solubilization and the reduction of the medium pH. (Mohamad et al., 2019). Present result showed that the maximum phosphate solubilization has greater reduction in pH and lower solubilization less reduction in pH. Figure 2.

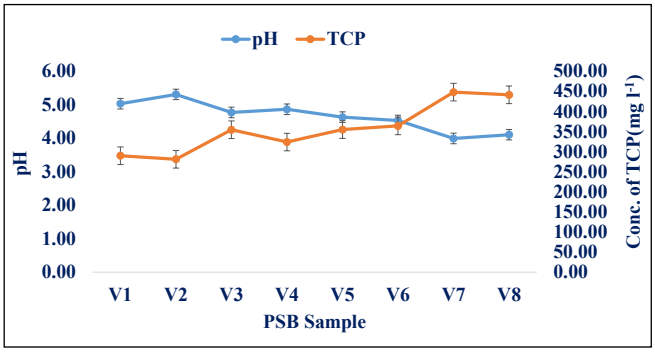

Figure 2: Relationship between phosphate solubilization and pH of the broth

Broth pH of each PSB isolates showed a tremendous decrease in pH from 7.0 to 3.9. The maximum decrease in pH 3.9 were recorded in V_7 followed by V_8 isolate 4.1. The minimum decreasing in pH 5.3 were observed in V_2 isolates. Similar result was found by Park et al. (2009) in *Pseudomonas fluorescens* RAF15 isolate, maximum pH drops 4.0 was recorded. In another experiment of Paul and Sinha (2017) were also recorded same declination in pH of broth containing *Pseudomonas aeruginosa* KUPSB12 isolates.

3.4. Physiochemical characterization of selected isolates

Based on highest solubilization of phosphate, grater lowering of pH, maximum solubilization index and efficiency, two isolates V_7 and V_8 were characterised. The physiochemical characterization included nature of isolates, biochemical activity and the carbohydrate utilization test documented in table 3. Both isolates showed Gram's negative, coccobacilli in shape and nonmotile. The biochemical properties, indole, methyl red, Voges-Proskauer, citrate and catalase found positive test whereas urease, oxidase and gelatinase negative in V_7 isolate. The other isolate V_8 showed indole, methyl red, Voges-Proskauer, catalase, oxidase positive and citrate, urease, gelatinase test found negative. The result of carbohydrate utilization test mentioned in table 3. The V_7 isolate shows positive for glucose fructose maltose sucrose and negative inositol mannitol as carbon source. Similarly, the V_8 isolate showed glucose fructose and inositol positive whereas sucrose, maltose and mannitol negative carbohydrate teats.

Table 3: Physical and biochemical characterization of selected PSB

Sl. No.	Physical and biochemical test	PSB Isolates	
		V_7	V_8
Physical characters			
1.	Gram reaction	Gram-negative	Gram-positive
2.	Cell shape	Coccobacilli	Coccobacilli
3.	Motility	Non-motile	Non-motile
Biochemical test			
4.	Indole test	Positive	Positive
5.	Methyl red test	Positive	Positive
6.	Voges-proskauer test	Positive	Positive
7.	Citrate utilization test	Positive	Negative
8.	Urease test	Negative	Negative
9.	Oxidase test	Negative	Positive
10.	Catalase	Positive	Positive
11.	Gelatine liquification test	Negative	Negative
Carbohydrate utilization test			
12.	Glucose	Positive	Positive
13.	Fructose	Positive	Positive
14.	Sucrose	Positive	Negative
15.	Maltose	Positive	Negative
16.	Inositol	Negative	Positive
17.	Manitol	Negative	Negative

3.5. Molecular identification and phylogenetic analysis

The molecular identification of selected isolates using 16S rRNA sequencing. The partial sequence of targeted part of 16S rRNA was submitted to the Gene bank with accession number OK510279 and OK337612 for strain V_7 and V_8 respectively. The initial comparison of 16S rRNA sequences with reference strains acquired using BLAST at the NCBI-Gene Bank database which revealed a sequence identity of 94% and 90% with *Acinetobacter* sp. and *Penibacillus* sp. (Table 4).

The FASTA sequence was deposited in GenBank under the accession number OK510279 and OK337612 (Figure 3)

Molecular identification of selected isolates and potentially efficient PSB strains based on the sequencing of 16S rRNA and phylogenetic affiliation confirmed that the V_7 isolates and belonged to the genus *Acinetobacter* and V_8 isolate *Penibacillus* respectively (Figure 4).

The phylogenetic tree revealed that the isolate V_8 (VK1)

Table 4: Molecular identification of selected PSB isolates to genomic level

Sl. No.	PSB strain	Types	Accession no.	Nucleotides length (bp)	Percentage identity
1.	V_7 (VK2)	*Acinetobacter* sp.	OK510279	1040	94
2.	V_8 (VK1)	*Paenibacillus* sp.	OK337612	1083	90

>VK2 (V7)

TCTGAGGGGAGGATTACCATGCAAGTCGAAGCGGGGGAAGGAAGCTTGCTACTGGACCTAGCGGCGGACGGGTGAGTAAT
GCTTAGGAATCTGCCTATTAGTGGGGGACAACATCTCGAAAGGGATGCTAATACCGCATACGTCCTACGGGAGAAAGCAG
GGGATCTTCGGACCTTGCGCTAATAGATGAGCCTAAGTCGGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACG
ATCTGTAGCGGGTCTGAGAGGATGATCCGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGG
GAATATTGGACAATGGGGGGAACCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGCCTTATGGTTGTAAAGCACTTTAA
GCGAGGAGGAGGCTACTCTAGTTAATACCTAGGGATAGTGGACGTTACTCGCAGAATAAGCACCGGCTAACTCTGGGCCA
GCAGCCGCGGTAATACAGAGGGTGCGAGCGTTAATCGGATTTACTGGGCGTAAAGCGTGCGTAGGCGGCTTATTAAGTCG
GATGTGAAATCCCCGAGCTTAACTTGGGAATTGCATTCCATACTGGTGAGCTAGAGTATGGGAGAGGATGGTAAAATTCC
AGGTGTAGCGGTGAAAATGCGTAAAAAATCTGGAGGAATACCGATGGCGAAGGCAGCCATCTGGCCTAATACTGACGCTG
AAGGTACGAAAGCCTGGGGGAACAAACCGGGATTAAAATACCCTGGGTAGTCCATGCCCGTAAACGAATGTCTACTAACC
CGTTGGGGGCCTTTTGAGGGCTTTTAGGGGCGGCAGCCTAACGCCAAAAAGTAGACCGCCCGGGGAAGTACGGGTCCCAA
GAATAAAAACTCAAATGAAATTGACGGGGGGCCCCCCCCAACCGGTGGAACAATGGGGTTTTAATTCCATGCCACCCCCA
AAAAACCTTTTCCTGGGCCTTGGACAAAACAAAAAAAATTTTCCCCAAAAAGGGATTGGGGGCCCTTCCGGGAAAACTAA

VK1 (V8)

TCCTAAACATGCAAGTCGAAGCGGACTTGATGGAGTGCTTGCACTCCTGATGGTTAGCGCGCGTGACGGGTGAAGTAGCA
CGTACGCAACCTGCCCTCTAGACTGGGATAACTACCGGAAACGGTAGCTAATACCGGATAATTTATTTTGCAGCATTGTG
GAATAATGAAAGGCGGAGCAATCTGTCACTTGAGGATGGGCCTGCGGCGCATTAGCTAGTTGGCGGGGTACCGGCCCACC
AAGGCGACAATGCGTAGCCGACCTGAGAGGGTGAACGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGC
AGCAGTAGGGAATCTTCCCCAATGGGCGAAAGCCTGACGGAGCAACGCCGCCTGAGTGATGAAGGTTTTCGGATCGTAAA
GCTCTGTTGCCAAGGAAGAACGTCTTCTAGAGTAACTGCTAGGAGAGTGACGGTACTTGAGAAGAAAGCCCCGGCTAACT
ACGTGCCACCAGCCGCGGTAATACTTAGGGGGCAAGCGTTGTCCGGAATTATTGGGGCGTAAAGCGCCCGCAGGGGGGTT
CTTTAAGTCTGGTGTTTAAACCCGGAGGCTCAACTTCGGGTCGCACTGGAAAACTGGGGAAACTTGAAGTGCAAAAGAAG
AAAAGGGAAATTCCACGTGTAGCGGGGAAATGCCGAAAATATGTGGAGGAACACCAATGGCGAAAGGGGAATCTCTGGGG
TGGTAACTGACGCTGAGGCCCCAAAAGCGTGGGGGAGCCAACCGGATTAAAATCCCCGGGTAGGCCCACCCCGTAAACCG
AAGAATGCTAAGGGTTAAGGGGTTCCAAACCCCTTGGGGGCCGAAGTTAACCCTTAAAGCATTCCCGCCTGGGGGAAACA
GGTCGCCAAAAATGAAACCCAAAGGAATTGGTGGGGGACCCCGCCCCCACCACTGGGAGTATGTGGTGTTATTTCCAAAC
CCCCCGGAAAAAACCTTTACAATATTTTGAAATTCCCTCTGAACTCCTCAGAAAAAAGAGCGGAGCCTCTCGGAGCAAAG
GGTGACACGGGAGGCAGGGTGTGTGTACGCCCCTACTCGCGAG

Figure 3: FASTA sequence of the selected PSB isolate (V_7 and V_8)

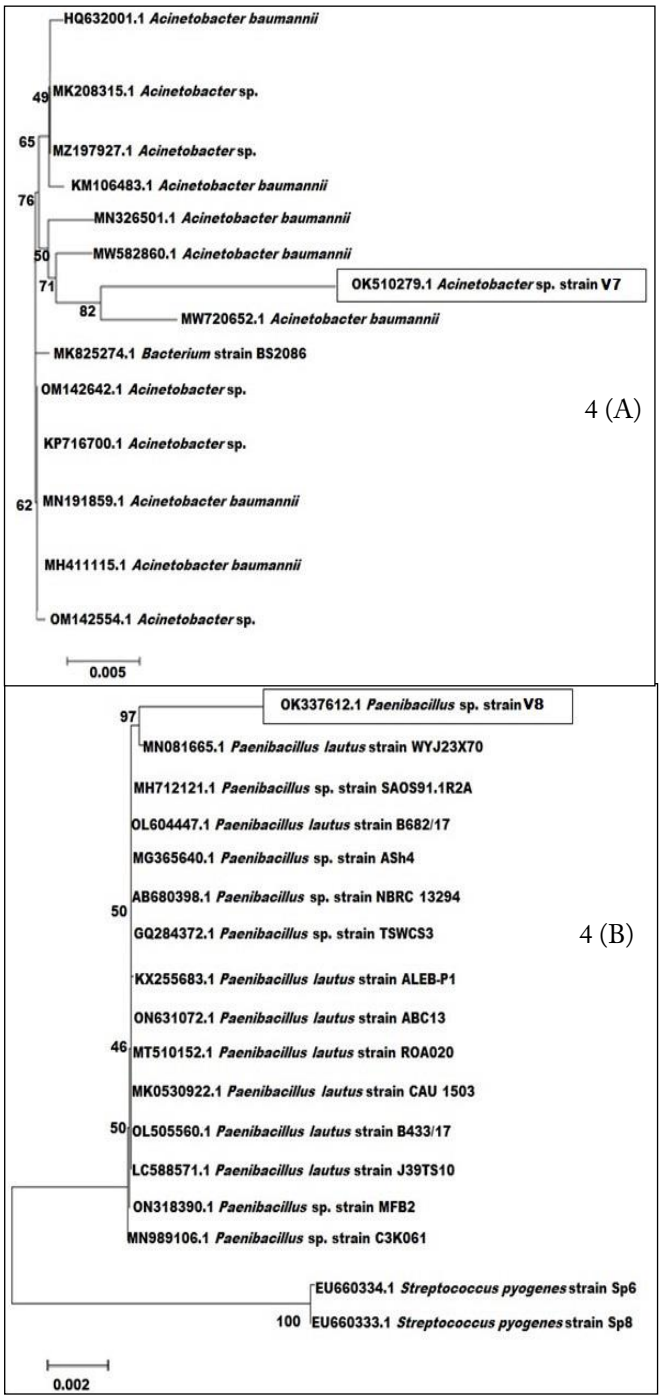

Figure 4(A) and 4(B): Phylogenetic trees showing the evolutionary position and relationship of Acinetobacter (V_7) and *Paenibacillus* sp. (V_8) with other bacterial isolates. The tree was constructed neighbour joining method with 1000 bootstrap replicates

closely related to the *Penibacillus lautus* strain with Accession No. MN081665. Whereas isolate V7(VK2) has closely related to the *Acinetobacter baumanni* strain with Accession No. MW720652 with its closest neighbours.

Isolation of bacterial strains of these genera have already been obtained from various soils and found to have inorganic phosphate (IP) solubilization such as, *Acinetobacter calcoaceticus Goosen* et al. (1989) and *Acinetobacter baumannii* (Nugroho et al., 2020). In some *Paenibacillus* species such as *Paenibacillus mucilaginosus* (Hu et al., 2006), *Paenibacillus elgii* (Narayan et al., 2010), *Paenibacillus kribbensis* (Marra et al., 2012), *Paenibacillus polymyxa, Paenibacillus macerans* (Wang et al., 2012), and *Paenibacillus xylanilyticus* (Pandya et al., 2015), the Phosphate Solubilization ability has been confirmed.

4. CONCLUSION

The isolated potent phosphate-solubilizing bacteria, with Acinetobacter (V_7) and Paenibacillus (V_8) exhibiting superior phosphate solubilization, pH reduction, and efficiency in solubilizing tricalcium phosphate. Molecular identification confirmed their evolutionary proximity to established strains, highlighting their potential as efficient biological fertilizers for sustainable agriculture. These findings emphasize the significance of phosphate-solubilizing bacteria in optimizing plant growth and soil fertility, paving the way for future studies to harness the agricultural benefits of these microbial isolates.

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