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Organic Phosphate mineralization by bacteria isolated from polluted soil

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Abstract:
The phosphorus (P) content in average soils typically ranges around 0.05% (w/w), with only about 0.1% of this phosphorus readily accessible to plants. To become accessible to plants, organic phosphorus compounds must undergo hydrolysis facilitated by phosphatases, primarily driven by soil microorganisms and plant roots. Five highly effective phosphate-solubilizing bacteria were identified in polluted soil in Mumbai. Their ability to mineralize organic phosphate was evaluated using Pikovskaya's medium supplemented with organic phosphate sources such as sodium phytate and deoxyribonucleic acid (DNA). The isolated bacteria were identified as *Enterobacter mori strain LJP-k-10, Pseudomonas aeruginosa* JCM 5962(T), *Pseudomonas oleovorans subsp. oleovorans* DSM 1045(T), *Acinetobacter radioresistens DSM 6976(T),* and *Staphylococcus nepalensis* using 16s rRNA sequencing. The isolates exhibited varying abilities to mineralize sodium phytate and deoxyribonucleic acid (DNA). The successful utilization of this organic phosphate mineralization ability presents a promising opportunity for village farmers to improve soil health and increase agricultural productivity sustainably.

Keywords: Organic phosphate, Mineralization, 16s rRNA sequencing, sodium phytate, deoxyribonucleic acid (DNA).

Introduction:

Phosphorus (P) is a vital element necessary for sustaining life on Earth, as it plays a crucial role in facilitating various metabolic processes (TAO et al., 2008). It serves as a fundamental component in the structure of essential biomolecules such as DNA and RNA, while also acting as a key component in ATP, a primary energy carrier molecule within cells (Suleman et al., 2018).

Phosphorus is the second most vital nutrient after nitrogen, crucial for regulating plant growth and development. Its essential role in every cell makes it impossible to substitute with any other element(Arora et al., 2016). Many agricultural soils contain significant amounts of phosphorus compounds, much of which has accumulated due to the continuous and excessive application of chemical phosphate fertilizers(Alori et al., 2017; Henri et al., 2008; Timofeeva et al., 2022).

Organic phosphorus found in soil originates from sources such as animal and plant remnants, composts, and microorganisms (Suleman et al., 2018). Organic phosphorus is plentiful in soil and is a primary phosphorus source for plants (Turner et al., 2005). Moreover, organic phosphorus comprises approximately 30-80% of soil content and plays a crucial role in the soil phosphorus cycle. Within the total organic phosphorus content in the soil, roughly 60% is primarily found in the form of phytate and its derivatives (Singh et al., 2011). The most found inositol phosphate in the soil is Myoinositolhexakisphosphate commonly called phytate. It significantly contributes to the soil's organic phosphate pool (Gerke, 2015). In soil, deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are the two primary types of nucleic acids present, constituting significant portions of soil organic phosphorus(Abolfazli et al., 2012).

Phosphate-solubilizing bacteria offer potential benefits for agronomy; however, their presence in soil may not always be adequate to outcompete native bacterial populations. To establish sustainable crop systems, it is crucial to identify and characterize bacteria while prioritizing crop production and economic-environmental sustainability (Janati et al., 2022). Furthermore, waste disposal sites represent an underexplored ecological niche that could harbor microorganisms capable of solubilizing insoluble phosphate. This study aimed to isolate, identify, and characterize phosphate-solubilizing bacteria (PSB) from this unique habitat and demonstrate their potential to mineralize organic phosphate.

Materials and methods:

Phosphate solubilizing bacteria were isolated using commercially available dehydrated Pikovskaya's media obtained from Himedia, following the method outlined byKadiri et al., 2013. Ten grams of soil were mixed with 100 mL of sterile saline solution and agitated for 2 hours to

create a suspension. The suspension was then diluted, and 0.1 mL of appropriate dilutions were spread onto Pikovskaya's agar plates containing 5g/L of tricalcium phosphate as the sole phosphorus source for selectively screening phosphate solubilizing bacteria. The plates were then incubated at 30°C for three days. Following the incubation period, bacterial colonies exhibiting a clear zone of solubilization were selected and isolated onto fresh Pikovskaya's agar plates to confirm phosphate solubilization. The resulting colonies were subsequently transferred to PVK agar slants and stored at 4°C.

The solubilization index (SI) was determined by assessing both the colony diameter and the diameter of the halo zone, as outlined by (Mardad et al., 2013)

Solubilization index (SI) = Colony diameter + halo zone diameter Colony diameter

To determine the organic phosphate mineralization of the isolated bacteria, a modified Pikovskaya's broth was prepared by substituting tricalcium phosphate (TCP) with 0.5% concentrations of both deoxyribonucleic acid (DNA) and sodium phytate (Ramani, 2014). 100 mL of modified Pikovskaya's broth was aseptically inoculated with 1 mL of inoculum in each flask. The inoculated flasks were then placed in an incubator set at 28°C ± 0.2°C for 10 days to facilitate phosphate mineralization under static conditions. Parallel uninoculated flasks were also incubated to serve as controls. The experiment was conducted in triplicate. At regular intervals, 10 mL of medium was aseptically extracted from each flask and centrifuged at 10,000 revolutions per minute (rpm) for 20 minutes. The resulting supernatant was then analyzed for its water-soluble phosphate content using the molybdophosphoric acid blue method.

16S rDNA gene sequencing was done to identify the phosphate-solubilizing bacteria that were isolated. The sequencing procedure was done at the National Center for Cell Science-National Center for Microbial Resource (NCCS-NCMR) in Pune.

Results:

Five highly effective phosphate-solubilizing bacteria were identified from soil samples using a screening method that exclusively utilized tricalcium phosphate as the sole phosphorus source, leading to the formation of a halo zone on Pikovskaya's agar plates. Based on the zone of solubilization on pikovskaya's agar medium, the isolates were assessed for organic phosphate mineralization using the modified pikovskaya's agar medium by replacing tricalcium phosphate with sodium phytate and deoxyribonucleic acid (DNA). The isolates were identified using 16s RNA sequencing at the National Center for Cell Science-National Center for Microbial Resource (NCCS-NCMR). The isolated were identified as *Enterobacter mori strain LJP-k-10, Pseudomonas aeruginosa* JCM 5962(T), *Pseudomonas oleovorans subsp. oleovorans* DSM 1045(T), *Acinetobacter radioresistens DSM 6976(T),* and *Staphylococcus nepalensis.* The bacterial sequences were deposited in NCBI GenBank with accession numbers

a **b**

Figure 1: (a) Isolation of phosphate solubilizing bacteria (b) Control plate

The table below indicates the zone of solubilization on Pikovskaya's media and organic phosphate solubilization by the bacteria after 12 days in the presence of sodium phytate and deoxyribonucleic acid (DNA) as sources of organic phosphate.

Sr. No	Phosphate solubilizing bacteria	Phosphate solubilization index	Sodium phytate as an organic phosphate source (µg/ml)	Deoxyribonucleic acid (DNA) as an organic phosphate source (µg/ml)
1.	*Enterobacter mori strain LJP-k-10*	2.66	705.90±0.8	334.49±0.5
2.	*Pseudomonas aeruginosa* JCM 5962(T)	2.8	523.43±0.7	365.08±0.8
3.	*Pseudomonas oleovorans subsp. oleovorans* DSM 1045(T)	2.5	454.15±1.1	343.59±0.5
4.	*Acinetobacter radioresistens DSM 6976(T)*	3	753.41±0.5	506.85±0.7
5.	*Staphylococcus nepalensis*	3	542.64±0.5	438.23±0.4
6.	*S. aureus* ATCC (Positive control)	1.3	154.59±1.4	144.54±0.5

Table 1: Organic phosphate solubilization by the isolated phosphate solubilizing bacteria. (Values are mean ± SD of triplicates)

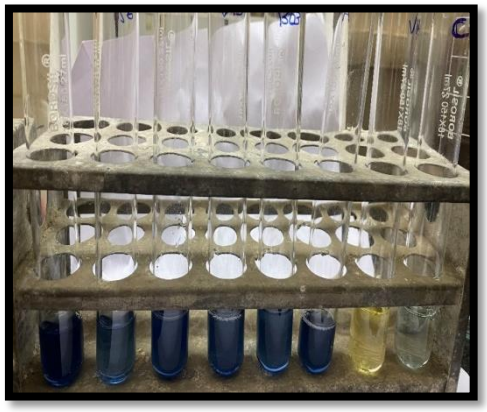

Figure 2: Quantitative estimation of phosphate mineralization in broth medium. The blue colour indicates positive phosphate solubilization.

Discussion:

Table 1 presents the amount of soluble phosphorus released from organic phosphorus compounds by phosphate-solubilizing bacteria. Each isolate demonstrated varying capacities to mineralize different organic phosphate compounds, resulting in variable amounts of release. The highest amount of phosphorus released was observed when sodium phytate was present for all organisms. The sequence of hydrolysis of various organic phosphates by the bacteria, leading to the subsequent release of soluble phosphorus, can be organized in descending order as follows:

Sodium phytate mineralization:

Acinetobacter radioresistens DSM 6976(T) >Enterobacter mori strain LJP-k-10 > Staphylococcus nepalensis > Pseudomonas aeruginosa JCM 5962(T) > *Pseudomonas oleovorans subsp. oleovorans* DSM 1045(T) > *S. aureus* ATCC (Positive control)

Deoxyribonucleic acid mineralization:

Acinetobacter radioresistens DSM 6976(T) > Staphylococcus nepalensis > Pseudomonas aeruginosa JCM 5962(T) > *Pseudomonas oleovorans subsp. oleovorans* DSM 1045(T) > *Enterobacter mori strain LJP-k-10 > S. aureus* ATCC (Positive control)

Acinetobacter radioresistens DSM 6976(T) demonstrates the highest proficiency in both sodium phytate and DNA mineralization. Moreover, it is also one of the highest forms of organic phosphate found in the soil. All the strains show phosphate mineralization under both the sources of organic phosphate tested.

The findings demonstrate that sodium phytate emerges as the most effective substrate for mineralization. A similar study reported by Ramani, 2014 has demonstrated calcium phytate as an efficient source of organic phosphorus.

Jorquera et al., 2008 isolated phosphate-mineralizing bacteria from rhizosphere soil. The study also demonstrated the mechanism of organic phosphate solubilization indicating the phosphoric hydroxylases in releasing phosphate from an organic phosphorus source. Out of a pool of 300 isolates, six phosphobacteria were chosen for their capability to utilize both Na-phytate and Ca-phosphate on agar media. These selected phosphobacteria were genetically identified as strains belonging to *Pseudomonas, Enterobacter, and Pantoea*. All the chosen strains exhibited the production of phosphatases, leading to a greater liberation of inorganic phosphate compared to uninoculated controls. The ability of the isolated phosphate-solubilizing bacteria used in our study was much higher than that reported in previous studies by (Wan et al., 2020).

Conclusion:

Given the growing apprehension regarding phosphorus pollution in several regions and the abundance of organic phosphorus in the form of phytate, phytases hold significant promise for both commercial and environmental utilization along with phosphate mineralizing bacteria. To our knowledge, we present the initial documentation of organic phosphate source solubilization by phosphorus-solubilizing bacteria isolated from waste dumping regions in Mumbai. In this study, we screened and isolated inorganic phosphate solubilizing bacteria to assess their abilities in inorganic P solubilization and organic P mineralization, assuming that these two groups were distinct from each other. Nevertheless, our findings, which resulted in the release of phosphate, revealed that all strains isolated from polluted soils in this study demonstrated both phosphate-solubilizing and phosphate-mineralizing capabilities. To harness the potential of these bacteria effectively, additional investigation into microbial-mineral interactions and genetic pathways is necessary. Novel research endeavours should focus on exploring how microbial biotechnology can be employed in agriculture to identify new phosphate-solubilizing bacteria aimed at cultivating sustainable crops across varying conditions.

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References:

1. Abolfazli, F., Forghani, A., & Norouzi, M. (2012). Effects of phosphorus and organic fertilizers on phosphorus fractions in submerged soil. Journal of Soil Science and Plant Nutrition, 12(2), 349–362. https://doi.org/10.4067/S0718-95162012000200014
2. Alori, E. T., Glick, B. R., & Babalola, O. O. (2017). Microbial phosphorus solubilization and its potential for use in sustainable agriculture. Frontiers in Microbiology, 8(JUN), 1–8. https://doi.org/10.3389/fmicb.2017.00971
3. Arora, N. K., Mehnaz, S., & Balestrini, R. (2016). Preface. Bioformulations: For Sustainable Agriculture, 5–6. https://doi.org/10.1007/978-81-322-2779-3
4. Gerke, J. (2015). Phytate (Inositol hexakisphosphate) in soil and phosphate acquisition from inositol phosphates by higher plants. A review. Plants, 4(2), 253–266. https://doi.org/10.3390/plants4020253
5. Henri, F., Laurette, N. G. O. N., Annette, D., & John, Q. (2008). Solubilization of inorganic phosphates and plant growth promotion by strains of Pseudomonas fluorescens isolated from acidic soils of Cameroon. African Journal of Microbiology Research, 2(7), 171–178.
6. Janati, W., Mikou, K., El Ghadraoui, L., & Errachidi, F. (2022). Isolation and characterization of phosphate solubilizing bacteria naturally colonizing legumes rhizosphere in Morocco. Frontiers in Microbiology, 13(September). https://doi.org/10.3389/fmicb.2022.958300
7. Jorquera, M. A., Hernández, M. T., Rengel, Z., Marschner, P., & De La Luz Mora, M. (2008). Isolation of culturable phosphobacteria with both phytate-mineralization and phosphate-solubilization activity from the rhizosphere of plants grown in a volcanic soil. Biology and Fertility of Soils, 44(8), 1025–1034. https://doi.org/10.1007/s00374-008-0288-0
8. Kadiri, D. D., Gorle, N., Varada, K., Peetala, R., & Peela, S. (2013). Isolation , Screening and Identification of Phosphate Solubilizing Bacteria From Different Regions of. 4(4), 518–526.
9. Mardad, I., Serrano, A., & Soukri, A. (2013). Solubilization of inorganic phosphate and production of organic acids by bacteria isolated from a Moroccan mineral phosphate deposit. African Journal of Microbiology Research, 7(8), 626–635. https://doi.org/10.5897/AJMR12.1431
10. Ramani, V. (2014). Organic phosphate mineralization by Bacillus sphaericus and Pseudomonas cepacia. Journal of Agricultural Technology, 10(4), 915–922. http://www.ijat-aatsea.com
11. Singh, S. M., Yadav, L. S., Singh, S. K., Singh, P., Singh, P. N., & Ravindra, R. (2011). Phosphate solubilizing ability of two arctic Aspergillus niger strains. Polar Research, 30(SUPPL.1). https://doi.org/10.3402/polar.v30i0.7283
12. Suleman, M., Yasmin, S., Rasul, M., Yahya, M., Atta, B. M., & Mirza, M. S. (2018).

Phosphate solubilizing bacteria with glucose dehydrogenase gene for phosphorus uptake and beneficial effects on wheat. PLoS ONE, 13(9), 1–28. https://doi.org/10.1371/journal.pone.0204408

13. TAO, G. C., TIAN, S. J., CAI, M. Y., & XIE, G. H. (2008). Phosphate-Solubilizing and -Mineralizing Abilities of Bacteria Isolated from Soils1 1 Project supported by the Scientific Research Foundation for the Returned Overseas Chinese Scholars, the Ministry of Education of the P.R. China. Pedosphere, 18(4), 515–523. https://doi.org/10.1016/S1002-0160(08)60042-9
14. Timofeeva, A., Galyamova, M., & Sedykh, S. (2022). Prospects for Using Phosphate-Solubilizing Microorganisms as Natural Fertilizers in Agriculture. Plants, 11(16), 1–23. https://doi.org/10.3390/plants11162119
15. Turner, B. L., Cade-Menun, B. J., Condron, L. M., & Newman, S. (2005). Extraction of soil organic phosphorus. Talanta, 66(2 SPEC. ISS.), 294–306. https://doi.org/10.1016/j.talanta.2004.11.012
16. Wan, W., Qin, Y., Wu, H., Zuo, W., He, H., Tan, J., Wang, Y., & He, D. (2020). Isolation and Characterization of Phosphorus Solubilizing Bacteria With Multiple Phosphorus Sources Utilizing Capability and Their Potential for Lead Immobilization in Soil. Frontiers in Microbiology, 11(April), 1–15. https://doi.org/10.3389/fmicb.2020.00752