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Isolation and Preparation of Efficient Local Phosphate Solubilizing Bacteria Biofertilizer to Improve Productivity of *Manilkara Zapota (L.) P. Royen.*

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A B S T R A C T

Phosphate Solubilizing Bacteria (PSB) play a crucial role in improving soil fertility and plant growth by converting insoluble forms of phosphorus into forms that plants can readily absorb. Continuous use of chemical phosphate fertilizers negatively impacts soil health and microbial diversity, leading to nutrient imbalances and reduced sustainability in agricultureConsidering the variation in climatic and edaphic factors across different regions, the efficiency and viability of natural bio-agents like PSB differ from place to place. PSB solubilize phosphate through mechanisms such as acid production, chelation, enzymatic activity, and exchange reactions. They enhance phosphorus availability in the soil, promoting better root development, higher nutrient uptake, and improved crop productivity.

With the objective of formulating an efficient biofertilizer for the local farming community, the present research was undertaken to isolate an efficient strain of PSB for sapota fruit crop of farmers. The PSB strains were isolated from the rhizosphere soil of the sapota crop at using plate assay methods. The isolates were tested for phosphate solubilization efficiency in vitro on both solid and liquid media. The most effective PSB strain was formulated into a biofertilizer using a lignite carrier, ensuring high viability and long-term effectiveness.The biofertilizer efficacy and PSB viability were assessed, showing a significant solubilization zone in plate assays and a viable count of 9×10^8 CFU/g. The applied PSB biofertilizer significantly enhanced sapota crop yield and quality by increasing phosphorus availability, reducing dependency on chemical fertilizers, and improving soil health. This research highlights that region-specific PSB biofertilizers can contribute to sustainable agriculture, boosting the economy of Bhenda farmers while preserving environmental balance.

Keywords: Phosphate Solubilizing Bacteria, Biofertilizer, Sustainable Agriculture, Soil Health Improvement

Introduction

Introduction of Phosphate solubilization bacteria (PSB)

Phosphate-solubilizing bacteria (PSB) biofertilizer is a type of microbial inoculant that enhances the availability of phosphorus (P) to plants by solubilizing insoluble forms of phosphorus in the soil. PSB biofertilizers contain specific strains of bacteria capable of releasing phosphorus from organic and inorganic sources, making it accessible to plants for uptake.

Phosphate-Solubilizing Bacteria (PSB): The core component of PSB biofertilizer is the phosphate-solubilizing bacteria, which include various genera such as Bacillus, Pseudomonas, Enterobacter, and Rhizobium. Phosphate-solubilizing bacteria (PSB), associated with the plant rhizosphere, make mineral phosphorus more readily available for plant uptake by transforming insoluble P into forms that are available for the crop .The effect of PSB is considered a mechanism for enhancing plant growth. Until now, previously known PSB belong to numerous genera including Pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Micrococcus, Aereobacter, Flavobacterium and Erwinia . Inoculation with these phosphate dissolvers as biofertilizers has been reported to increase P uptake and promote plant growth. For example, treatment with PSB has increased the yield of wheat and promoted the growth of rice However, the ability of PSB to solubilize phosphate varies by bacterial species (Islam and Zafar, 2005; Saber and Shalaby, 2010).

Plants can uptake potassium through soil minerals, organic materials, and synthetic fertilizers. In India, the consumption of K exceeded 260 lakh tons over two years (2011–2012). To meet the demand for agricultural productivity, all K fertilizers were imported globally (FAI, 2013), leading to excessive application of K fertilizers. Potassium deficiency in the rhizosphere has become a key limiting factor for the sustainable development of evergreen agriculture in India (Naidu et al., 2011). In India, most K fertilizers are imported, which negatively impacts the agro-based economy. Continuous use of

chemical fertilizers has been proven to degrade soil texture and structure. Therefore, integrated nutrient management is essential to maintain soil fertility, productivity, and to minimize land degradation and environmental pollution for sustainable agriculture. After nitrogen (N) and phosphorus (P), potassium (K) is one of the most important plant nutrients, playing a key role in plant growth, metabolism, and development. In addition to enhancing resistance to diseases, pests, and abiotic stresses, K activates over 80 different enzymes responsible for vital plant processes such as energy metabolism, starch synthesis, nitrate reduction, photosynthesis, and sugar degradation (White and Karley, 2010; Almeida et al., 2015; Cecílio-Filho et al., 2015; Yang et al., 2015; Gallegos-Cedillo et al., 2016; and Hussain et al., 2016). K is the seventh most abundant element in the Earth's crust, with K content in soils ranging from 0.04% to 3% (http://www.phytojournal.com). However, despite its abundance, only 1% to 2% of soil K is available to plants (Sparks and Huang, 1985). The remainder is bound to other minerals and remains unavailable. Potassium reserves in soil are depleting at a rapid rate, making potassium deficiency a major constraint in crop production, particularly in coarse-textured soils. Even in fine-textured soils, the available fraction of K is low compared to total K, and crops respond positively to K fertilization. Excessive fertilizer use leads to nutrient leaching, contributing to environmental pollution without proportional increases in yield. A novel alternative to mitigate potassium limitations in the rhizosphere is the use of phosphate-solubilizing bacteria (PSB). These beneficial microbes enhance nutrient availability by solubilizing insoluble phosphorus compounds, thereby improving phosphorus uptake in plants. PSB plays a crucial role in maintaining a dynamic soil environment by mobilizing key nutrients from primary minerals and ores. These macronutrients support microbial populations in the rhizosphere and subsequently improve plant nutritional status (Sheng and He, 2006; Thangamani and Natarajan, 2008; Nishanth and Biswas, 2008; Abou-el-Seoud and Abdel-Megeed, 2012; Maurya et al., 2014; and Meena et al., 2014a). Inoculation with PSB has shown beneficial effects on plant growth across different crop species (Ahmad et al., 2016; Bakhshandeh et al., 2017; and Xiao et al., 2017). The integration of PSB into nutrient management strategies offers a sustainable approach to reducing dependency on chemical fertilizers while maintaining soil fertility and agricultural productivity.

Manilkara zapota, commonly known as sapodilla or chikoo, is a tropical evergreen fruit tree native to southern Mexico, the Caribbean, and Central America. It is highly valued for its sweet, granular, and fiber-free fruit, which is not only a popular delicacy but also rich in nutrients like vitamins (A, C), minerals (iron, calcium), and antioxidants. The sapodilla tree is also known for its timber and latex, used in various industries. The cultivation of sapodilla has expanded across several tropical and subtropical regions globally, including India, Thailand, Indonesia, and the Philippines, due to its adaptability to a wide range of climatic conditions. In these regions, it has become an essential crop for local economies, both for domestic consumption and export. Despite its hardiness and resistance to pests and diseases, the productivity of sapodilla is often constrained by nutrient deficiencies, particularly phosphorus, which plays a crucial role in plant growth, root development, and fruiting.

Phosphorus (P) is a vital macronutrient for plants, contributing to various essential physiological processes, including energy transfer (through ATP), photosynthesis, root development, flowering, and fruit production. However, phosphorus is often present in the soil in forms that are not readily available to plants, especially in acidic or alkaline soils, which limits plant growth and productivity. This is a common issue in many agricultural areas where soils are either naturally deficient in phosphorus or where chemical fertilizers have led to nutrient imbalances.

Phosphate fertilizers are commonly used to replenish phosphorus in the soil, but their excessive use can lead to environmental pollution, soil degradation, and long-term dependency on chemical inputs. Additionally, the high cost and unsustainable nature of chemical fertilizers have raised concerns about their widespread use in agricultural practices.

Phosphate-solubilizing bacteria (PSB) offer an eco-friendly and sustainable alternative to synthetic fertilizers. These microorganisms, including genera like *Pseudomonas*, *Bacillus*, *Enterobacter*, *Aspergillus*, and *Penicillium*, are capable of solubilizing insoluble forms of phosphorus in the soil and converting them into forms that are readily available for plant uptake. PSB play an important role in the soil ecosystem by breaking down complex organic matter and mineralizing phosphorus, thus improving the phosphorus status of the soil (Islam and Zafar, 2005; Saber and Shalaby, 2010).

The process of phosphate solubilization typically involves the secretion of organic acids (e.g., citric, oxalic, or gluconic acids) by the bacteria, which acidify the soil or dissolve phosphorus compounds like calcium phosphate, iron phosphate, and aluminum phosphate. By enhancing phosphorus availability, PSB not only boost plant growth but also increase the efficiency of fertilizers, reduce the need for chemical inputs, and improve the sustainability of agricultural practices.

Furthermore, PSB can provide additional benefits, such as the production of plant growth-promoting substances (auxins, cytokinin), nitrogen fixation, and the suppression of soil-borne plant pathogens. These traits make PSB a versatile component of biofertilizers, improving soil health and plant productivity in an environmentally friendly manner.

Biofertilizers containing PSB have been proven to improve plant growth, yield, and quality in various crops by enhancing nutrient uptake, particularly phosphorus. For *Manilkara zapota*, improving phosphorus availability in the soil through the use of PSB biofertilizers can significantly impact its growth and productivity. Phosphorus is particularly critical for fruit trees, as it enhances root development, flower and fruit formation, and overall plant vigor. In the case of sapodilla, phosphorus deficiency can result in poor fruit quality, low yields, and stunted growth, directly impacting the economic returns from sapodilla cultivation.

The use of locally isolated, efficient PSB strains in biofertilizers tailored for specific regions can provide a more effective solution than general-purpose biofertilizers. Local bacterial strains are likely better adapted to the regional soil types, climatic conditions, and plant needs, thus enhancing the overall effectiveness of the biofertilizer.

By promoting a sustainable approach to managing soil fertility, PSB biofertilizers can reduce reliance on chemical phosphorus fertilizers, which are costly, environmentally harmful, and contribute to the depletion of non-renewable phosphorus reserves. The use of PSB not only helps improve crop

yield and quality but also supports soil health, preventing nutrient imbalances and degradation over time. This study aims to isolate and identify efficient local phosphate-solubilizing bacteria (PSB) from soil samples in regions where *Manilkara zapota* is grown, with the goal of formulating a biofertilizer that enhances the productivity of sapodilla. The specific objectives of the study include:

- **Isolation and screening of PSB**: Isolate PSB strains from local soils and screen for their ability to solubilize phosphate and promote plant growth (Kumari, and Singh, 2015).
- **Characterization of efficient PSB strains**: Identify the most effective PSB strains based on solubilization efficiency, biochemical properties, and growth-promoting abilities.
- **Preparation of PSB-based biofertilizer**: Formulate the most efficient PSB strains into a biofertilizer using appropriate carrier materials to ensure its effectiveness and viability.
- **Field trials on *Manilkara zapota***: Assess the impact of PSB biofertilizer on sapodilla growth, yield, and fruit quality through controlled field trials.

The findings of this research could significantly improve the productivity and sustainability of *Manilkara zapota* cultivation. By optimizing the use of phosphate-solubilizing bacteria, farmers can reduce dependency on chemical fertilizers, lower input costs, and improve the nutritional value and quality of sapodilla fruits. This study also holds potential for contributing to the broader field of sustainable agriculture, offering a natural and environmentally friendly solution to soil fertility problems and enhancing food security in regions where phosphorus deficiency is a major constraint to agricultural productivity. Moreover, the study could open doors for the development of region-specific biofertilizers for other crops, enhancing the scope of using PSB in diverse agricultural systems.

Material and Methods

Total viability count (TVC)

Material:

Requirements :-

1. Glasswares: Petriplates, conical flask, measuring cylinder, pipette, Broth, beaker, bottles.
2. Material: Soil sample.
3. Chemicals: Ethanol, lignite powder, NaOH and HCI (to maintain pH)
4. Other: Loop, cotton, filter paper

Methods:

1. Collection of soil sample-
2. Choose a selected area for collect the soil sample
3. Dig up to 6 inches and collect the available soil
4. Remove the stone and other debris.

Here is a revised version of the procedure where citations are added directly within the paragraph text, referencing the appropriate research papers and preparation of PSB as shown in the Photoplates Fig. 1 – Fig. 9.

1. Soil Sample Collection

The first step in isolating phosphate-solubilizing bacteria (PSB) involves the collection of soil samples from the root zone of *Manilkara zapota* (sapota). Soil samples are collected using sterile sample bags to prevent contamination. The soil is transported to the laboratory for bacterial isolation. The rhizosphere (root zone) is particularly important because it is rich in microorganisms that interact with plant roots, and these microorganisms may have the ability to solubilize phosphorus, an essential nutrient for plants (Ali and Muneer, 2009; Nautiyal and Mehta 2000; Mehta and Nautiyal, 2001).

2. Soil Suspension Preparation

Once the soil samples are collected, 10 g of the air-dried, sieved soil is mixed with 100 mL of sterile distilled water (SDW) to prepare the soil suspension. The mixture is incubated at 28°C in an orbital shaking incubator for 30 minutes with periodic shaking at 150 rpm. This shaking helps disperse bacteria from the soil particles into the water. The suspension obtained contains a range of microorganisms, including potential phosphate-solubilizing bacteria (Chakraborty and Bandyopadhyay, 2013; Tiwari and Sharma, 2014).

3. Serial Dilution

To obtain individual bacterial colonies, 10 mL of the soil suspension is transferred to 90 mL of sterile distilled water (SDW), and the dilution process is repeated for 10 successive dilutions. This helps reduce the concentration of microorganisms, making it easier to isolate individual bacterial colonies. After dilution, 2 mL from the appropriate dilution is used to inoculate Pikovskaya agar plates (Vyas and Gulati, 2009; Khan et al., 2007).

4. Preparation of Pikovskaya Agar

Pikovskaya agar is specifically designed to promote the growth of phosphate-solubilizing bacteria. It contains a phosphorus source, typically in the form of tricalcium phosphate ($Ca_3(PO_4)_2$), along with other nutrients. The medium is prepared by dissolving ingredients such as D-glucose, tricalcium phosphate, ammonium sulfate, magnesium sulfate, sodium chloride, yeast extract, and agar in distilled water. The pH is adjusted to around 7.0, and the medium is sterilized by autoclaving at 121°C for 15 minutes. After cooling to approximately 50°C, the medium is poured into sterile Petri dishes for solidification (Pikovskaya, 1948; Zaidi et al., 2004).

Preparation of Pikovskays Agar Media

Sr.no	**Chemical**	**Weight**
1	Yest extract	0.500g
2	Dextrose (sucrose)	10.000g
3	Calcium phosphate	5.000g
4	Ammonium sulphate	0.500g
5	Potassium chloride	0.200g
6	Magnesium sulphate	0.100g
7	Magneses sulphate	0.0001g
8	Ferrous sulphate	0.0001g
9	Agar	9g

5. Inoculation of Pikovskaya Agar Plates

The soil suspension is inoculated onto the solidified Pikovskaya agar plates using a sterile loop or spreader. This inoculation allows bacterial colonies to grow and, if they possess phosphate-solubilizing properties, to break down the tricalcium phosphate in the medium. Clear zones around bacterial colonies will form, indicating phosphate solubilization (Goswami and Chawla, 2010; Bano and Musarrat, 2003; Vassilev and Vassileva, 2007).

6. Incubation

After inoculation, the plates are incubated at 28 ± 2°C for 5-7 days. During this period, phosphate-solubilizing bacteria will release organic acids, breaking down tricalcium phosphate and forming clear zones around bacterial colonies. These zones are an indicator of the bacterium's ability to solubilize phosphate (Zhao et al., 2014; Mehta and Nautiyal, 2001).

7. Observation for Colony Growth

After the incubation period, the plates are examined for colony growth. Bacteria that exhibit distinct clear zones are considered phosphate-solubilizing. The size of the clear zone is directly proportional to the bacterial efficiency in solubilizing phosphate (Rodrigues and Prakash, 2013; Molina and Mena, 2007).

8. Selection of Colonies

Once colonies with clear zones are identified, they are selected for further analysis. The colonies are picked and streaked onto fresh Pikovskaya agar plates to obtain pure cultures. This process ensures that only the phosphate-solubilizing bacteria are studied (Saharan and Nehra, 2011).

9. Purification of Isolates

To ensure pure cultures, the selected colonies are streaked onto fresh Pikovskaya agar plates and incubated. The presence of clear zones confirms the purity of the cultures, ensuring that no contamination has occurred (Khan and Zaidi, 2007; Kumar and Vaidya, 2012).

10. Confirm Phosphate Solubilization

The phosphate-solubilizing ability of the colonies is confirmed by measuring the clear zone diameter. A larger clear zone indicates a more effective phosphate solubilizer, which is desirable for biofertilizer applications (Chakraborty and Bandyopadhyay, 2013; Ghosh and Mazumdar, 2006; Ghosh, and Pati, 2013).

11. PSB Liquid Culture Production

The pure colonies of phosphate-solubilizing bacteria are transferred to Pikovskaya broth, where they are incubated for three days on a shaker. This liquid culture is then prepared as a mother culture for further biofertilizer production (Zaidi and Khan, 2004; Khan et al., 2013, Khan and Zaidi, 2006).

12. Preparation of Biofertilizers in Lignite Powder

Sterilized lignite powder is mixed with the prepared mother culture of PSB. The mixture is allowed to reduce moisture for about an hour before being packed for use as a biofertilizer (Zaidi et al., 2006; Ali et al., 2009).

13. Storage of Pure Cultures

Finally, the pure cultures of PSB are transferred to nutrient agar slants for long-term storage at 4°C. This helps maintain the bacterial activity while preserving their ability to solubilize phosphate for future use (Srinivasan and Radhakrishnan, 2007; Bano and Musarrat, 2003).

Photoplates Fig. 1 – Fig. 9 showing: PSB Biofertilizer Preparation

Fig.1: Sapota Garden

Fig.2: Soil Sample Collection

Fig.3: Soil Dilution

Fig.4: Pouring of PSB Inoculum

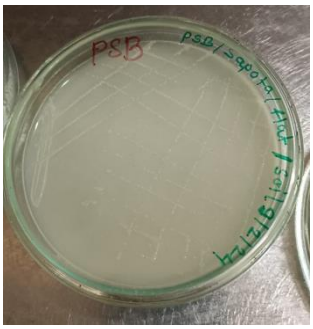

Fig.5: Colonygrowth after streaking

Fig.6: Liquid PSB culture

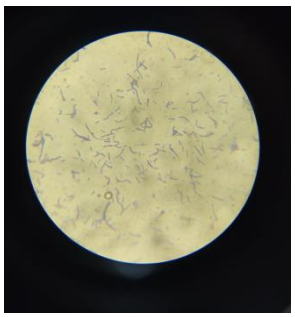

Fig.7: Gram staining

Fig.8: Liquid culture mixed in lignite

Fig.9: Packing of PSB Biofertilizer

Result and discussion:

The observed colony characteristics reveal a **pinpoint medium** size, approximately **1mm** in diameter. The shape of the colony is **bacillus**, indicating that the organism responsible for the colony formation is rod-shaped. The colony exhibits a **white** colour, which is typical for certain bacterial species, though it does not produce any pigments as evidenced by the **absence of pigment production**. In terms of texture, the colony shows a **smooth consistency**, and its **margin** is **entire**, meaning it has a well-defined, smooth border with no irregularities or lobes. The colony's **elevation** is observed to be **concave**, which means it curves inward from the edges to the center. Furthermore, the colony appears **opaque**, indicating that light does not pass through it easily, which is characteristic of many bacterial colonies.

Upon microscopic examination, the bacterium is confirmed to be **gram-positive**, as indicated by the staining characteristics. The morphology of the bacteria, consistent with the colony shape, is **rod-shaped (bacillus)**, confirming the type of microorganism present. The combination of these characteristics—rod shape, smooth colony consistency, and the lack of pigment production—suggests a specific group of bacteria, potentially of the genus *Bacillus* or a closely related genus. This description of colony and microscopic characteristics provides a useful starting point for identifying the organism and understanding its behavior in different growth conditions. Further biochemical and molecular tests would be needed to confirm the exact species or strain of the microorganism.

Conclusion

The present study underscores the significant role of Phosphate Solubilizing Bacteria (PSB) in enhancing phosphorus availability and promoting sustainable agricultural practices, particularly for sapota (*Manilkara zapota*) cultivation in the Bhenda region. Continuous reliance on chemical phosphate fertilizers has been detrimental to soil health, microbial biodiversity, and long-term crop productivity. In contrast, the use of indigenous PSB offers an eco-friendly, cost-effective, and sustainable alternative.

Through isolation and characterization of PSB strains from the sapota rhizosphere, an efficient bacterial isolate exhibiting high phosphate solubilization potential was identified and formulated into a lignite-based biofertilizer. The strain demonstrated strong solubilization efficiency with a viable count of 9×10^{8} CFU/g, indicating its potential for effective field application. The biofertilizer significantly improved phosphorus uptake, root development, crop yield, and fruit quality, reducing dependency on chemical inputs.

Morphological and microscopic examination revealed the bacterium to be a gram-positive, rod-shaped organism, potentially belonging to the *Bacillus* genus. The colony characteristics—smooth, white, opaque, concave, and medium-sized—further support this classification. These findings provide a foundational understanding of the PSB isolate's identity and behavior, paving the way for its further development and optimization.

Overall, this research validates the efficacy of region-specific PSB biofertilizers in improving soil fertility, crop productivity, and environmental sustainability. Adoption of such biofertilizers by local farming communities can not only reduce input costs but also enhance the nutritional quality of sapota fruits, contribute to long-term soil health, and bolster food security. Future work should focus on field trials across different agro-ecological zones and molecular identification of the isolate to further advance its application in broader agricultural contexts.

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