

ISOLATION AND SCREENING OF PHOSPHATE AND POTASH SOLUBILIZING BACTERIA FROM RHIZOSPHERE SOIL OF MAIZE AND COTTON

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Abstract

The availability of soil fertility and growth of plants mainly depends on the interaction of microorganisms with soil nutrients. However, Phosphorus and potassium are indeed essential nutrients for plant growth, but they are often present in the soil as insoluble forms. A microorganism plays a critical role in nutrient mobilization, hence reducing the reliance on chemical fertilizers. The objective of this research is to isolate and screen Phosphate-Solubilizing Bacteria (PSB) and Potash-Solubilizing Bacteria (KSB) from the rhizospheric soil of maize (*Zea mays*) and cotton (*Gossypium herbaceum*). 38 different colonies of distinct bacteria were isolated and then screened using Pikovskaya's agar and Aleksandrow's agar. Through morphological identification and biochemical characterization, there were 14 isolates with the same morphology as well as 24 unique isolates. Out of them, 12 strains could solubilize phosphate, 10 could dissolve potassium, and 8 could perform both activities simultaneously. The two highest isolates for phosphate-solubilizing efficiency and the three best for potassium dissolution were found by measuring zones of solubilization on agar plates. Those isolates were characterized based on their 16S r RNA sequencing. This research clearly indicates that soil microorganisms play an important role in the availability of nutrients to plants, and provides a way for this dependence on chemical fertilizers to decrease.

Keywords: 16S RNA Sequencing; Soil Fertility; Biochemical Characterization; Microbial Solubilization; Nutrient Accessibility; Phosphate and Potash Bacteria; Rhizosphere Isolation

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Introduction

In the realm of plant growth and agriculture, phosphorus and potassium are two essential macrominerals that serve as cornerstones of healthy and productive crops. (White and Brown, 2010; Daroonkolaie *et al.*, 2022). These minerals are fundamental to various critical processes in plants, and their significance cannot be overstated. In particular, phosphorus is essential to plant development since it is a constituent of Adenosine Triphosphate (ATP), which is the main source of energy for plants (Ganesh Moorthy *et al.*, 2024). This molecule is the driving force behind fundamental processes such as photosynthesis, protein synthesis, nutrient uptake, and respiration. The influence of phosphorus extends even further, as it has been observed to stimulate root growth, accelerate early maturity, enhance crop quality, strengthen the stem, and fortify plants against diseases (Gram, 1884). As such, phosphorus is a linchpin for plant growth, development, and overall agricultural success. However, a considerable challenge arises from the fact that these vital nutrients are often found in soil in insoluble form, rendering them effectively inaccessible to plants. The conventional approach to address this issue has involved the extensive application of chemical fertilizers. While these fertilizers provide a quick remedy, they have raised concerns about environmental sustainability and long-term ecological impacts. There is an urgent need to investigate substitutes that lessen the dependency on chemical fertilizers, opening the door for a more ecologically conscious and sustainable future for agriculture in light of the increasing emphasis on sustainable agriculture and eco-friendly methods. In response to this challenge, the research is dedicated to the isolation of specific micro-organisms capable of solubilizing phosphate and potassium from the diverse bacterial community that resides in the soil. These remarkable micro-organisms play a pivotal role in converting insoluble form of potassium and phosphate into a state, that plants can readily absorb and utilize. It is aimed to provide a sustainable and environmentally friendly solution for improving the fertility of the soil and encouraging vigorous plant development by utilizing the special skills of these bacteria.

The benefits of microbial solubilizers are not only limited to agriculture but also give an in-depth understanding of the complex dynamics of soil microorganisms and their central role in maintaining soil health. This approach therefore provides a very practical and promising solution to the urgent need for more sustainable agricultural practices in this

rapidly changing world. From an agricultural perspective, microbial solubilizers can minimize the use of chemical fertilizers, reducing dependence on non-renewable resources and promoting organic farming. Ecologically, these microbes restore natural nutrient cycles, reduce pollution, and support sustainable agricultural systems globally. By enhancing crop productivity while lowering the ecological footprint of farming, this method aligns with the global emphasis on sustainability and addresses the dual challenge of food security and environmental conservation. This research has far-reaching implications and heralds a paradigm shift in agricultural practices. We can optimize benefits to crops and the environment alike by reducing dependence on chemical fertilizers for a more sustainable future. This discovery offers hope for feeding the world's growing population in an environmentally friendly manner. It will prove the ability of scientific innovation to overcome modern challenges for mankind while building a healthier and more sustainable landscape in agriculture. This research underscores the pivotal role of soil microbes, specifically Potassium and Phosphorus solubilizing micro-organisms. Potassium-solubilizing micro-organisms, maintain soil potassium balance. These microbes transform complex potassium compounds into plant-accessible forms by breaking down intricate minerals (Jacoby *et al.*, 2017). Biofertilizers with potassium-solubilizing bacteria enhance soil fertility, crop yields, disease resistance, and reduce chemical fertilizer usage (Sharma *et al.*, 2013). The use of microbial alternatives significantly reduces the environmental footprint of agriculture through improved nutrient availability and reduces reliance on synthetic fertilizers. These microbes reduce nutrient runoff, thereby preventing eutrophication in aquatic systems, and improving nutrient-use efficiency (Halder *et al.*, 1990). More importantly, replacing 20-30% of chemical fertilizers with microbial solutions could reduce global greenhouse gas emissions, saving millions of tons of CO₂ annually. In addition to these functions, microbial inoculants improve soil health by enhancing organic matter, structure, and carbon sequestration, which ensures sustainable farming. They reduce the costs of fertilizers by 15-25% without loss of yields. These are highly transformative steps in achieving environmentally sustainable agriculture. Potash-Solubilizing Bacteria (KSB) play a crucial role in making potassium (K) available to plants by secreting organic acids that decrease soil pH, leading to the

solubilization of various forms of K. This not only promotes sustainable crop production but also makes it an economically viable and environmentally friendly approach. Potassium can be efficiently released from insoluble minerals in soils by KSB, allowing plants to absorb it. Additionally, the utilization of organic manures containing KSB can further enhance crop growth. Overall, KSB contributes to improving soil health, increasing crop yield, and promoting sustainable agricultural practices. Similarly, phosphate-solubilizing micro-organisms, convert insoluble phosphates into plant-available forms, promoting plant growth and combating diseases. Despite their importance, their role in urban road verge ecosystems is not well-defined. Phosphate-Solubilizing Microorganisms (PSMs) offer several key advantages in agriculture and soil management (Vassilev and Vassileva, 2003; Bashan *et al.*, 2004). They are essential to improving phosphorus uptake by converting insoluble phosphate compounds into forms that plants can readily use (Pal, 1998; Bekele Mamo and Gebremedhin, 2022). This not only leads to improved plant growth and increased crop yields but also contributes to overall soil fertility (Römhild and Kirkby, 2010). Crucially, using PSMs can lessen the need for chemical phosphate fertilizers, making agriculture more economical and environmentally friendly (Rodríguez and Fraga, 1999; Cullen and MacIntyre, 2016). *The Klebsiella varicola* and *Bacillus tropicus*, actually produce nutrients that can be utilized by the plant. Such microbes can act as

biofertilizers or seed inoculants, leading to the solubilization of phosphate and potassium from soil minerals without chemical fertilizers. Besides that, the application of such microbes enhances the ability of the plant to cope with environmental stress, for instance, in a drought environment, salinity stress, or nutrient deficiencies. Intercropping, composting, and precision agriculture will promote nutrient cycling and soil health in addition to minimizing waste. They will, therefore promote climate change mitigation and sustainable agriculture by sequestering carbon and improving organic matter levels. These will be field-experiment demonstrations of increased yields realized by farmers, which will encourage them to adopt environmentally friendly farming practices. Additionally, certain PSM strains are useful for phytoremediation in contaminated soil because they can detoxify heavy metals.

Materials and Methods

Sample Collection

Soil samples were collected from the rhizospheres of maize and cotton at two distinct locations in Tamil Nadu: Tiruppur and Coimbatore. Cotton rhizosphere soil was gathered from Elur, Madukarai Taluk, Coimbatore District (PIN: 641032), Tamil Nadu (Latitude: 10° 54' 17.32" N, Longitude: 76° 57' 52.60" E). Maize rhizosphere soil was collected from Udumalpet, Tiruppur District. Samples were collected following standard protocols, (Darlene and Sabey, 1976), stored in sterile polythene bags, and refrigerated at 4°C to preserve the microbial integrity required for isolating phosphate-solubilizing and potash-solubilizing bacteria. Figure 1, depicts the roots of a maize plant along with the surrounding soil. Figure 2, shows the picture of the roots of the cotton plant and the soil collected from it.



Figure 1. Maize rhizosphere soil



Figure 2. Cotton rhizosphere soil

Serial dilution and plating of soil sample:

Dilution of the sample: To prepare the rhizosphere soil samples from maize and cotton plants for analysis, a series of steps was followed. First, the soil samples were soaked in sterile water to create a suspension. From this, a 10-gram portion of each sample was measured and placed in separate containers. This 10-gram sample was mixed with 90 ml of sterile water achieve a 10^{-1} dilution, which ensured accurate dilution. After the 10^{-1} dilution; numerous additional dilutions were carried out in a sterile environment to avoid contamination. These serial dilutions were intended to further decrease the sample concentration. Ultimately, this process resulted in a final dilution of 10^{-7} , which was

suitable for subsequent analysis and experimentation. The integrity and reliability of the study's findings depended heavily on the sterility and precision of the dilution process.

Bacterial counts: The enumeration of the total bacteria in the soil was conducted using the Standard Plate Count Method (Coico, 2005). Phosphate-solubilizing agar medium and potassium-solubilizing agar medium were used for this purpose. The total number of phosphate-solubilizing bacteria and potassium-solubilizing bacteria was determined by plating soil dilutions on the respective agar medium. This process involved using a plating enrichment and spread plate techniques. After that, the plated samples were incubated at 37°C for 24 hours. The dilution factor was multiplied by the number of cells per plate to calculate the total count of microorganisms. The formula used for this calculation is as follows:

$$\text{Bacteria per gram of soil} = \left(\frac{\text{Amount of bacteria}}{\text{Weight of soil}} \right) \times \text{Dilution}$$

Isolation of phosphate solubilizing bacteria and potassium solubilizing bacteria

Both phosphate and potassium solubilizers were identified in the rhizosphere soils using Pikovskaya agar and Aleksandrow agar media, which are suited for phosphate- and potassium-soluble bacteria, respectively, the discovered bacteria were isolated and evaluated (Rajawat *et al.*, 2019; Feng *et al.*, 2020). By using these media, it was possible to identify and enumerate the specific beneficial bacteria in the soil that aid in plant development and soil fertility. Several intrinsic and environmental factors could be responsible for differences in the performances of isolates regarding the efficiency of nutrient solubilization. Intrinsic factors range from genetic composition to increase in the production of specific enzymes, (Goldstein, 1994) such as phosphates and potassium-solubilizing enzymes necessary in breaking insoluble compounds (Bashan *et al.*, 2013; Sun *et al.*, 2020). Other contributory factors behind the differential abilities of the isolates include their metabolic activity and cellular mechanisms. Other environmental factors affecting the activity and productivity of such isolates are pH, temperature, availability of nutrients, and interactions with soil microbiomes. Some of these strains have additional secondary traits such as organic acids production or siderophores which enhance their capability in solubilizing their substrates. Figure 3, shows the isolated colonies by using Aleksandrow's agar and Pikovskayas agar. Figure 4 shows the visual examination of gram staining through a microscope.

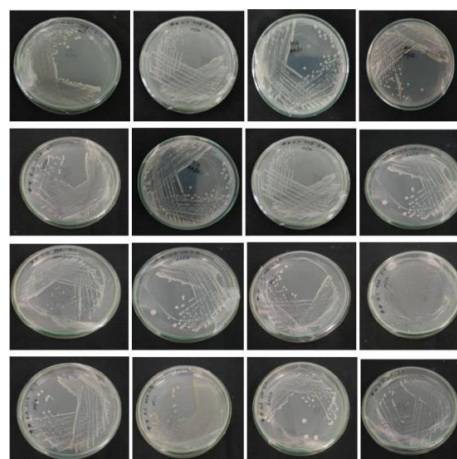


Figure 3. Purified isolated colonies

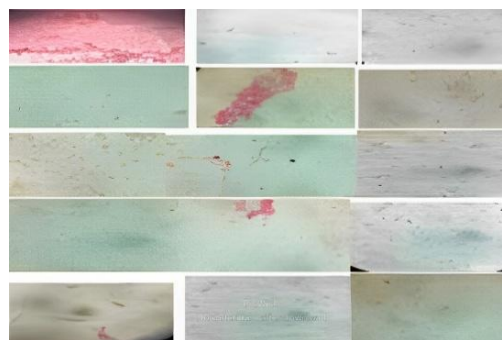


Figure 4. Microscopic examination by Gram staining

Microscopic examination and identification of gram staining

Bacteria isolated from soil samples underwent thorough testing, including microscopic analysis of their colony and cell morphology. Identification was further refined using the Gram staining method, first introduced by Gram in 1884 (Chabot *et al.*, 1993). This analysis revealed a range of bacterial shapes, such as gram-positive rods and cocci, as well as gram-negative rods and cocci (Bashan *et al.*, 2004; Mazhar *et al.*, 2020). A significant portion of the bacteria were identified as gram-negative cocci, which were found to have the ability to solubilize both phosphate and potash in the soil. This highlights the diverse and beneficial roles of these microbes play in the soil nitrogen cycle and in promoting plant growth.

Morphology examination of isolates:

Colony morphology of the bacterial isolates obtained from the rhizosphere of maize plant collected from Udumalpet, Tiruppur District: A selective media was employed for both the bacterial colonies which exhibited specific characteristics. The grown colonies were circular, raised in elevation, relatively small, and had a pink-like

Table 1. Colony morphology of maize rhizosphere soil isolates

Medium	Bacteria	Size	Shape	Elevation	Surface	Pigmentation
PSB medium	S1	Large	Circular	Flat	Smooth	Off white
PSB medium	S2	Medium	Circular	Flat	Shiny	Light yellow
PSB medium	S3	Medium	Irregular	Raised	Smooth	Off white
PSB medium	S4	Small	Round	Flat	Shiny	Off white
PSB medium	S5	Small	Round	Raised	Shiny	Light yellow
PSB medium	S6	Medium	Round	Raised	Smooth	Creamy white
PSB medium	S7	Small	Round	Flat	Rough	Pale yellow
KSB medium	S1	Small	Round	Raised	Smooth	Creamy white
KSB medium	S2	Small	Round	Flat	Smooth	Light yellow
KSB medium	S3	Small	Round	Flat	Smooth	Pale yellow
KSB medium	S4	Small	Round	Raised	Smooth	Light yellow
KSB medium	S5	Medium	Irregular	Raised	Smooth	Creamy white
KSB medium	S6	Medium	Round	Flat	Smooth	Light yellow
KSB medium	S7	Small	Round	Flat	Smooth	Off white

Table 2. Colony morphology of cotton rhizosphere soil isolates

Medium	Bacteria	Size	Shape	Elevation	Surface	Pigmentation
PSB medium	S1	Medium	Round	Flat	Slime	Light white
PSB medium	S2	Large	Irregular	Flat	Smooth	Off white
PSB medium	S3	Irregular	Irregular	Convex	Smooth	Creamy white

appearance, typically displaying a creamy white color as shown in Table 1. These colonies provide an extensive view of the bacterial morphology that plays a pivotal role in understanding of the unique properties and various functions of soil ecosystems.

Colony morphology of the bacterial isolates obtained from the rhizosphere of cotton plant collected from in Elur, Coimbatore District: The colonies of phosphate-solubilizing bacteria and potassium-solubilizing bacteria, cultivated on their respective selective media, displayed distinctive characteristics. These colonies were circular, raised in elevation, relatively small, and exhibited a pink-like appearance, with a creamy white coloration. This conformity with established characteristics aids in the accurate identification and differentiation of these microorganisms, contributing to our knowledge of their unique properties and roles within soil ecosystems (Kandi, 2015) as shown in Table 2.

Zone of Inhibition

The term “zone of inhibition” is especially important in microbiological studies because it describes the specific region around a bacterial colony where the growth of neighboring microorganisms is suppressed because the primary bacterial population releases bioactive substances or

metabolites into the surrounding area as shown in Figure 5. In the specialized context of Potash-Solubilizing Bacteria, these microorganisms are recognized for their unique capability to secrete substances with the biochemical capacity to dissolve potassium, commonly known as potash, within the soil matrix (Verma *et al.*, 2020; Daroonkolaei *et al.*, 2022). This increases the availability of potassium for plant uptake (Krauss, 2003). Similarly, Phosphate-Solubilizing Bacteria are capable of generating chemical compounds that act as catalysts in increasing the availability of phosphate for plant use. It is critical to stress that these microbial activities contribute to the development of well-defined zones within the surrounding soil milieu, characterized by the suppression or inhibition of competing microorganisms. This microbial phenomenon assumes paramount importance in the realm of plant growth and nutrient dynamics. The “zone of inhibition,” in its functional manifestation, profoundly enriches the chemical composition of the soil environment, thereby significantly contributing to the vitality and prosperity of the plant ecosystem. This dynamic interplay underscores the invaluable role of these specialized microbial communities in modulating the chemical landscape of their habitat, consequently rendering essential nutrients, such as potassium and

phosphate, more readily available for plant utilization. Consequently, the outcome leads to enhanced plant growth, effectively overcoming nutrient limitations that are commonly viewed as obstacles to their development. Figure 5 shows the zone of inhibition that occurred in Aleksandrov's agar medium.

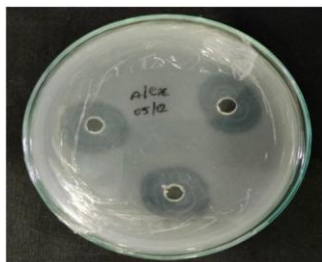


Figure 5. Zone of inhibition

Biochemical Tests

To characterize the bacterial isolates, a battery of biochemical tests was conducted, including the catalase test, methyl red test, starch hydrolysis test, citrate utilization test, casein hydrolysis test, indole test, urease test, and gelatin hydrolysis test (Aleksandrov *et al.*, 1967; Yassin *et al.*, 2021). These tests were conducted on bacterial isolates derived from Phosphate-Solubilizing Bacteria (PSB) and Potassium-Solubilizing Bacteria (KSB), which were cultured on media and collected from the rhizosphere soil of Udumalpet in Tirupur District, as well as cotton soil from Elur in Coimbatore District. To establish uniformity and dependability of the results yielded, each experiment was carried out in triplicates with corresponding controls. The results of these biochemical tests showed mostly negative results, except for a few isolated instances. This thorough characterization effort offers valuable insights into the metabolic and physiological characteristics of these bacteria, aiding in the identification of their unique roles within the soil microbial community (Deepak and Chayanika, 2018).

Catalase Test: In a test tube, 1-2 ml of hydrogen peroxide solution was first added. Then, a few colonies of the 18-24-hour-old test organism were carefully chosen and immersed in the hydrogen peroxide solution using a sterile wooden stick or a glass rod. Following this step, the observation was focused on the immediate reaction within the solution, specifically looking for the presence of bubbling as depicted in Figure 6. This method is frequently used to evaluate a microorganism's capacity to produce the catalase enzyme, which catalyzes the conversion of hydrogen peroxide into water and oxygen in the presence of the enzyme and causes bubbles to form.

Organisms capable of producing enzymes that break down the H_2O_2 and result in oxygen production indicate catalase positive. In addition to lacking the catalase enzyme, organisms lacking the cytochrome system are unable to decompose H_2O_2 into H_2O and O_2 are catalase-negative. Figure 6 illustrates the result of a catalase test performed on bacterial isolates.



Figure 6. Catalase Test

Citrate Test: The prepared Simon citrate-agar slant was streaked with a light inoculum that originated from the centre of a well-isolated colony of the bacterium. The slant was correctly labelled and then incubated for four to seven days in an aerobic atmosphere at 35 to 37°C. The tilt was then checked for any apparent colour shift. The surface of the slant turns blue instead of green when the microbe consumes citrate as it is only carbon source as shown in Figure 7. This shift in colour indicates that the microbe can metabolize citrate. The Figure 7 illustrate the result of a citrate utilization test performed on the bacterial isolates.



Figure 7. Citrate utilization Test

Starch utilization test: After preparation of the starch agar, each correctly labeled plate was inoculated with the target microorganism using a single streak method while closely adhering to sterile procedures. Following that, the inoculated plates were incubated at 37°C for 48 hours to promote bacterial growth and metabolism. Using a dropper, the plates' surface was gently flooded with an iodine solution after the incubation period, and this flooding was kept in place for exactly 30 seconds. After that, the extra iodine solution was poured off, and the plates were carefully examined. It was carefully determined whether there was a clear zone surrounding the bacterial growth line as shown in Figure 8, which provided significant details about the organism's capacity to produce amylase (Chaskes *et al.*, 2008). Figure 8 shows the starch utilization test performed on the bacterial isolate.

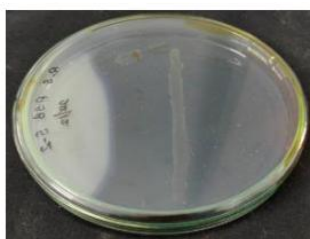


Figure 8. Starch utilization Test

Methyl red test: Cautiously prepared MRVP broth was allowed to equilibrate to room temperature prior to the inoculation stage. The medium was inoculated with a small number of organisms from a pure culture that was left for 18 to 24 hours. After that, an aerobic incubation was carried out for twenty-four hours at 37 degrees Celsius. 1 mL of the broth was carefully aliquoted into a sterile test tube following this incubation period. For additional analysis, the leftover broth was incubated for a further twenty-four hours. Two to three drops of methyl red indicator was added to the aliquot in order to measure the organisms' metabolic activity. Red colour was immediately observed. Figure: 9 illustrates the methyl red test conducted on bacterial isolates.

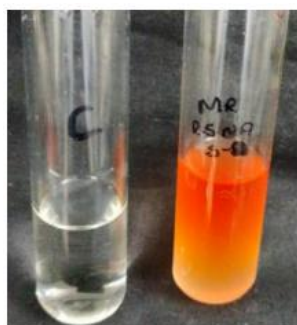


Figure 9. Methyl red Test

Casein hydrolysis test: By inoculating the particular organism on the agar plate in a straight or zigzag pattern, skim milk agar can potentially be made efficiently. After that, the agar plates were incubated at 25°C or 37°C, which created the perfect environment for bacterial growth. The milk agar plate cultures are next closely examined to ascertain whether each test bacterial organism has a distinct zone surrounding its growth, known as a zone of proteolysis (Chaskes *et al.*, 2008). This finding will provide insight into the organisms' ability to hydrolyze the medium's proteins and their proteolytic activity, as shown in Figure 10 which represents the casein hydrolysis test on the bacterial isolates.



Figure 10. Casein hydrolysis Test

Urease test: A batch of Christensen's Urea Agar was prepared for use. To guarantee uniform inoculum distribution, a portion of a well-isolated colony was streaked across the surface of urea agar. As an alternative, one or two drops from an overnight brain-heart infusion broth culture were used to inoculate the slant. The urea agar slant was incubated for a period of time ranging from 48 hours to 7 days at a temperature of 35-37°C in ambient air with the lid loosely sealed. During this time, the area was checked for the appearance of pink colour, which is a sign of urea hydrolysis. This stage is essential for determining the organisms' urease activity because it offers important information about their capacity to hydrolyze urea and release ammonia (Azizah *et al.*, 2020). The extended observation period is up to 7 days which allows for a comprehensive evaluation of urease production, contributing to the overall characterization of microbial traits. Figure: 11 represents the urease test on the bacterial isolates.

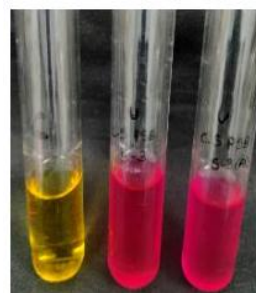


Figure 11. Urease test

Indole test: 4 ml of tryptophan was added into sterilized test tubes. The growth obtained from a 24-hour culture was introduced into the tubes using aseptic techniques. The test tubes were inoculated and then allowed to incubate for 24 hours at 37°C. Kovac's reagent (0.5 ml) was then added to the broth culture. A careful examination was done to determine whether a clear ring was present or not. This step is essential for identifying the production of indole, a metabolic by-product of some microorganisms' metabolism of tryptophan. After

adding Kovac's reagent, the presence of a ring in the medium indicates a positive indole test, demonstrating the bacterial culture's capacity to metabolize tryptophan into indole (Rodríguez and Fraga, 1999; He *et al.*, 2010). Figure 12 illustrates the indole test on the bacterial isolates.

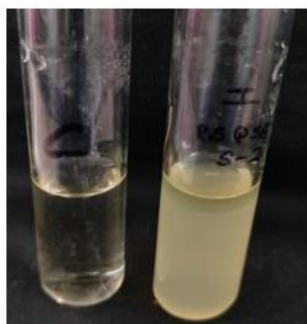


Figure 12. Indole Test

Gelatin hydrolysis Test: In the nutrient gelatin medium, 4-5 drops of a 24-hour broth culture was used, or an alternate technique that involved inoculating from a 24-hour-old colony by stabbing four or five times, 0.5 inches into the medium. The gelatin tubes were incubated for up to 14 days at 35-37°C in room temperature after being injected. The tubes were taken out of the incubator every day and placed at 4°C to gauge the gelatin's liquefaction. Carefully monitoring the liquefaction process, comparisons were done with an uninoculated control that was kept in the refrigerator. It is crucial to remember that liquefaction cannot be ascertained until the control has gelled or hardened. This process helps assess whether microbes can produce the enzyme gelatinase, which is capable of liquefying gelatin, and is essential for microbial characterization based on biochemical properties. Figure 13 depicts the gelatin hydrolysis test on the bacterial isolates.

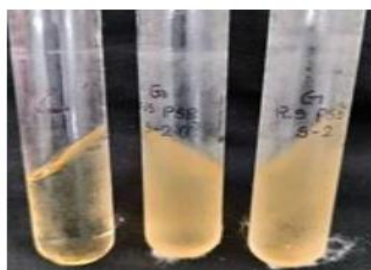


Figure 13. Gelatine hydrolysis Test

Result and Discussion

The soil samples collected from the rhizosphere of maize and cotton were subjected to solubilization

efficiency tests using Pikovskaya's agar and Aleksandrow agar respectively. A total of 38 colonies were isolated, purified, and characterized, with 14 colonies exhibiting similar morphological characteristics. Consequently, 24 unique bacterial colonies were identified for further analysis. Solubilization tests on Pikovskaya's agar and Aleksandrow's agar revealed that 11 isolates demonstrated phosphate solubilization (R.S PSB S-2, R.S PSB S-3, R.S PSB S-4, R.S PSB S-5, R.S KSB S-1, R.S KSB S-2, R.S KSB S-3, R.S KSB S-4, R.S KSB S-5, R.S KSB S-7, C.S PSB S-2), 12 isolates exhibited potassium solubilization (R.S PSB S-2, R.S PSB S-4, R.S PSB S-5, R.S PSB S-6, R.S KSB S-1, R.S KSB S-2, R.S KSB S-3, R.S KSB S-4, R.S KSB S-5, R.S KSB S-6, R.S KSB S-7, C.S PSB S-2), and 6 isolates (R.S PSB S-6, R.S KSB S-1, R.S PSB S-4, R.S KSB S-5, R.S KSB S-7, C.S PSB S-2) demonstrated the ability to solubilize both phosphate and potassium. These isolates were further characterized using various biochemical tests, including the catalase test, methyl red, starch hydrolysis, citrate utilization, casein hydrolysis, indole, urease, and gelatin hydrolysis tests. Notably, one isolate (R.S PSB S-6) exhibited significant phosphate solubilization, while another isolate (R.S KSB S-5) demonstrated remarkable potassium solubilization, both measured in millimetres. This comprehensive analysis provides insights into the solubilization potential and biochemical characteristics of the identified bacterial isolates, contributing to a better understanding of their role in soil nutrient dynamics. The findings align with those of Azizah *et al.* (2020), who reported the presence of efficient phosphate- and potassium-solubilizing endophytic bacteria in maize. Similarly, the isolates in the study exhibited significant solubilization potential, reinforcing their utility as biofertilizers (Archana *et al.*, 2012).

Biochemical characterization:

Biochemical characterization of bacterial isolates from maize soil in Udumalpet, Tiruppur District: The bacterial isolates were mostly gram-negative bacteria that solubilized potash and phosphate. The observations are in the Table 3 given below:

The Table 3 provides information on the biochemical characterization of bacterial isolates obtained from maize rhizosphere soil. It includes various biochemical tests performed on the isolates, such as gram staining, catalase test, methyl red test, starch hydrolysis test, citrate utilization test, casein hydrolysis test, indole test, urease test, and gelatin hydrolysis test. The results of these tests are indicated by plus (+) or minus (-) signs, representing positive or negative results, respectively, for each

Table 3. Biochemical characterization of maize rhizosphere soil isolates

Biochemical test	PSB S1	PSB S2	PSB S3	PSB S4	PSB S5	KSB S1	KSB S2	KSB S3	KSB S4	KSB S5	KSB S6	KSB S7
Gram staining	-	-	-	-	-	-	-	-	-	-	-	-
Bacterial shape	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci	Cocci
Catalase test	+	+	+	+	+	+	+	+	+	+	+	+
Methyl red test	-	-	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis test	-	-	-	-	-	-	-	-	-	-	-	-
Citrate hydrolysis test	+	+	+	+	+	+	+	+	+	+	+	-
Casein hydrolysis test	-	-	-	-	-	-	-	-	-	-	-	+
Indole test	-	-	-	-	-	-	-	-	-	-	-	+
Urease test	-	+	-	-	+	+	+	-	+	-	+	+
Gelatin hydrolysis test	-	-	-	-	-	-	-	-	-	-	-	+

Table 4. Biochemical characterization of cotton rhizosphere soil isolates

Biochemical test	PSB S1	PSB S2	PSB S3
Gram staining	+	-	-
Bacterial shape	Cocci	cocci	cocci
Catalase test	+	+	+
Methyl red test	-	-	-
Starch hydrolysis test	-	-	-
Citrate utilization test	+	+	+
Indole test	-	-	-
Urease test	-	+	+
Gelatin Hydrolysis test	+	-	+

isolate. The table also includes information on the bacterial shape observed for each isolate.

- Gram staining: Gram-negative bacteria were present in every isolate.
- Catalase test: Every isolate passed the test with a positive result.
- Methyl red test: The methyl red test yielded negative results for every isolate.
- Starch hydrolysis test: The starch hydrolysis test yielded negative results for every isolate.
- Citrate utilization test: Except KSB S7, all isolates yielded positive results.
- Casein hydrolysis test: All isolates, except KSB S3 and S7, had negative results.
- Indole test: All isolates, with the exception of KSB S7, had negative results.
- Urease test: While some isolates produced negative results, others produced positive results.
- The gelatin hydrolysis test produced positive results for a few isolates except KSB S2 & S7.

These results provide insights into the biochemical characteristics of the bacterial isolates from maize rhizosphere soil, indicating their ability to solubilize potash and phosphate

Biochemical characterization of bacterial isolates from cotton soil in Elur, Coimbatore district: In this region, there are a smaller number of bacteria in the soil that solubilize phosphate and potash. The observations are in the Table 4 given below:

The table provides information on the biochemical characterization of bacterial isolates from cotton rhizosphere soil. It includes the results of various biochemical tests conducted on the isolates.

- Gram staining: The isolates S2, and S3 are gram-negative, indicated by a negative result in the gram staining test, while S1 is gram-positive.
- Catalase test: All isolates show positive results, indicating the presence of the catalase enzyme.
- Methyl red test: All isolates show negative results, indicating the absence of the methyl red reaction.
- Starch hydrolysis test: All isolates show negative results, indicating the inability to hydrolyze starch.
- Citrate utilization test: All isolates show positive results, indicating the ability to utilize citrate.

- Casein hydrolysis test: All isolates show negative results, indicating the inability to hydrolyze casein.
- Indole test: All isolates show negative results, indicating the absence of indole production.
- Urease test: Isolate S1 shows a negative result, while isolates S2 and S3 show positive results, indicating the presence of the urease enzyme.
- Gelatin hydrolysis test: Isolate S1 shows a positive result, while isolates S2 and S3 show negative results, indicating the ability to hydrolyze gelatin.

These results provide insights into the biochemical characteristics of the bacterial isolates from cotton rhizosphere soil.

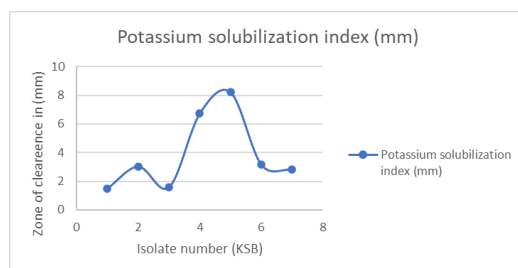


Figure 14. Scatter Plot of Potassium Solubilization Index for Different KSB isolates

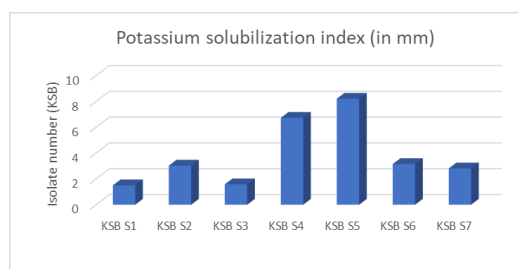


Figure 15. Potassium Solubilization Index of Different KSB isolates

Table 5 is plotted with the given data in Figure 14 which depicts scatter plot of the potassium solubilizing index for the samples varying from 1.5 mm (KSB S1) to 8.21 mm (KSB S5). KSB S5 exhibited the highest activity for potassium solubilization, and KSB S1 exhibited the lowest. Figure 15 Represents the bar graph of the potassium solubilizing index of varying samples as provided in Table 5.

Figure 16 Depicts the scatter plot of phosphate solubilizing index versus varying samples from the data provided in Table 6. Each point indicates a sample and its corresponding potassium solubilization index. KSB S5 exhibited the highest index (8.21 mm), while KSB S1 had the lowest index (1.5 mm). Figure 17 Represents the bar graph of phosphate solubilizing index versus varying samples.

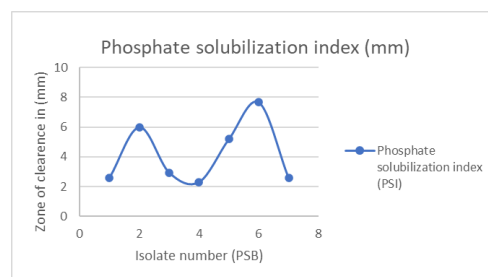


Figure 16. Scatter Plot of Phosphate Solubilization Index for Different PSB Samples

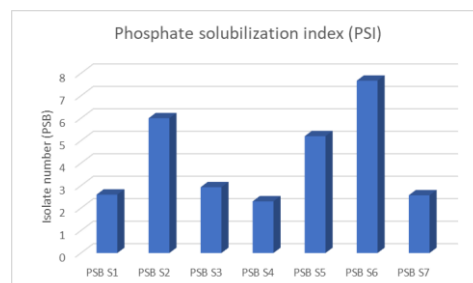


Figure 17. Phosphate Solubilization Index of Different PSB Samples

Table 5. Biochemical characterization of maize rhizosphere soil isolates

Sample ID	Potassium solubilization index (in mm)
KSB S1	1.5
KSB S2	3.03
KSB S3	1.58
KSB S4	6.73
KSB S5	8.21
KSB S6	3.15
KSB S7	2.81

Table 6. Phosphate Solubilization Index of Different PSB Samples

Sample ID	Potassium solubilization index (in mm)
PSB S1	2.6
PSB S2	6
PSB S3	2.93
PSB S4	2.3
PSB S5	5.2
PSB S6	7.67
PSB S7	2.57

16s RNA sequencing:

The outcome of the 16S rRNA sequencing procedure substantiated that R.S PSB S-6 corresponds to *Klebsiella variicola*, while R.S KSB S-5 is identified as *Bacillus tropicus*. The confirmation of these two bacterial species is noteworthy as both have demonstrated proficiency in solubilizing potash and phosphate minerals. This finding underscores the specific microbial capabilities of *Klebsiella variicola* in phosphate solubilization (Meena *et al.*, 2015) and *Bacillus tropicus* in potash solubilization, providing valuable insights into the functional potential of these bacterial strains in nutrient cycling within the soil ecosystem. Figure 18, the phylogenetic tree illustrates the evolutionary relationships among the bacterial isolates and reference sequences based on 16S rRNA gene analysis. The tree, constructed using the Neighbor-Joining method, reveals that

isolates A and B grouped closely with *Klebsiella variicola*. This grouping is strongly supported by a bootstrap value of 98%, indicating high confidence in the evolutionary relationship. These findings confirm the classification of these isolates as *Klebsiella variicola*, highlighting their potential role as biofertilizers (Zhang *et al.*, 2014). Figure 19, The phylogenetic tree, constructed on 16S rRNA gene analysis, illustrates the evolutionary relationships among the bacterial isolates of study and reference sequences. It shows, using a Neighbor-Joining method, that the isolates C and D are closely related to *Bacillus tropicus*. This is well supported by high bootstrap values and strengthens the confidence level in the phylogenetic relationship. The two observations point toward a common evolution-based origin for the isolates and *Bacillus tropicus*, and this, in turn, underlines their potential application as biofertilizers.

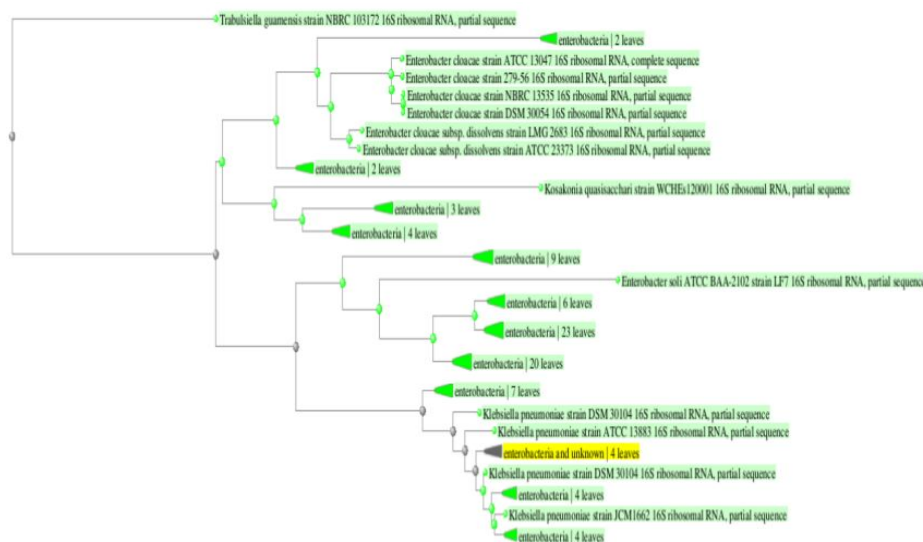


Figure 18. Phylogenetic tree analysis from sequencing data

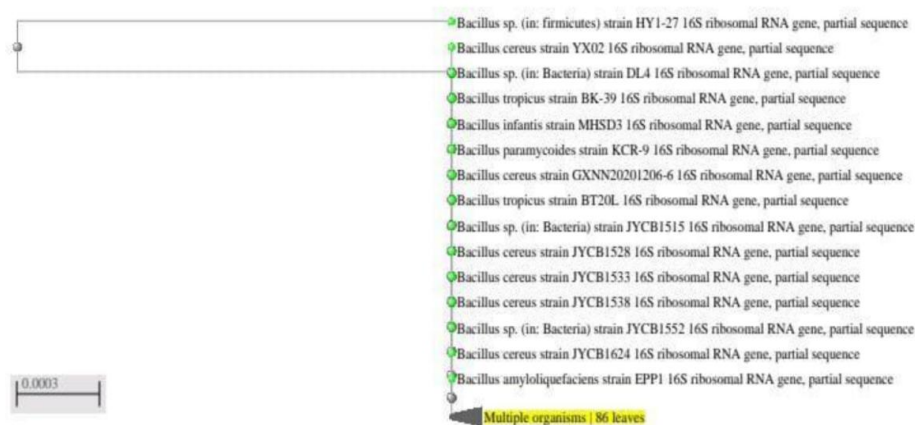


Figure 19. Phylogenetic tree for *Bacillus tropicus*

Conclusion

The current investigation has achieved successful isolation and characterization of phosphate solubilizing bacteria (PSB) and potash solubilizing bacteria (KSB) from soil samples collected in Tamil Nadu. The isolated bacterial strains, specifically identified as *Klebsiella variicola* and *Bacillus tropicus*, exhibited notable efficiency in solubilizing potash and phosphate minerals. These findings represent a significant contribution to our comprehension of microbial processes involved in mineral solubilization, particularly in the context of soil enhancement for agricultural purposes. The identified species, *Klebsiella variicola* and *Bacillus tropicus*, hold promise for their potential applications in agriculture by contributing to soil fertility improvement. This study lays the groundwork for future research endeavors aimed at exploring the practical implications of these microbial activities in enhancing overall soil health and, consequently, crop productivity.

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