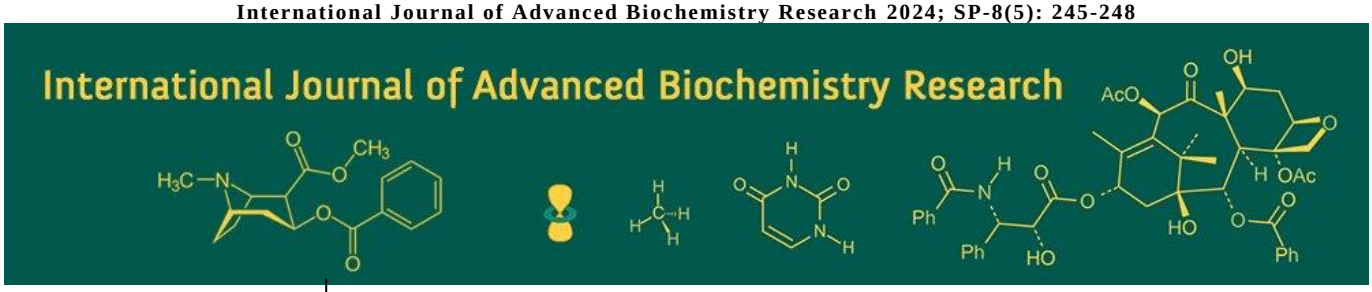


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# Evaluation and characterization of potassium solubilizing bacteria

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**Abstract**
Potassium is essential macronutrient for plant growth and plays significant role in activation of several metabolic processes including protein synthesis, photosynthesis, enzymes, as well as in resistance to diseases. Potassium solubilizing microorganisms present in the soil helps to convert complex potassium present in soil into simple form and make them available to plants. Soil microorganisms helps to break down complex minerals present in soil either by secreting acid or base and convert it into simple form which helps to maintain the soil fertility and iron cycle in soil. The soil sample were collected from rhizospheric soil. Isolation of KSB were carried out by using serial dilution technique on selective media i.e. Aleksandrov media. The further purification and maintenance was done on Aleksandrov media. Two isolates were extracted and named as KSB1 and KSB2. In respect of morphological characteristics, both isolates showed a negative Gram reaction, whitish to yellowish colony colour and colony appearance of most of the KSB isolates showed raised, smooth and glistening with short and long rod shape bacteria. The KSB isolates showed positive reaction to methyl red test, acid production, KOH test and catalase test. One isolate was positive to Simmons citrate test whereas one isolate showed negative reaction. Casein hydrolysis, Voges-proskauer test, starch hydrolysis, indole production test and H2S test showed negative reaction. Based on zone of solubilization, KSB1 showed maximum zone (20.67 mm) and KSB2 showed minimum zone of solubilization (17.67 mm).

**Keywords:** Potassium, rhizospheric soil, zone of solubilization, KSB

## 1. Introduction
The third most crucial nutrient for plants after nitrogen (N) and phosphorus (P) is potassium. Potassium is a macronutrient that is crucial for plant growth and is important for the activation of several metabolic processes, such as protein synthesis, photosynthesis, enzymes, and resistance to pathogens and insects, among others. Lack of potassium in the soil causes plants to grow slowly, generate smaller seeds with poorer yields, and develop their roots, shoots and branches poorly. India ranks fourth in the world for K-fertilizer usage and around 2.04 million tonnes are imported each year to meet the need. Numerous nutrient deficiencies, such as those in K, Zn and S, are present in Indian soils. The economic situation of a developing nation like India and its significant spending in importing potassium fertilizers have made it necessary to find alternative measures that are more environmentally and biologically friendly. Most of the time, soil has very little soluble potassium; instead, silicate minerals and insoluble rocks make up more than 98% of the soil's potassium content. Finding effective K-solubilizing bacterial isolates to reduce the reliance on chemical fertilizers would be a calculated move to address this problem (Singh *et al*., 2010) [16].
Mica and feldspar are frequently used as potassium supplements for soil. It is found in soil in the complexed or chelated insoluble mineral form in mica or illite. Potassium mobilization bacteria break down this material by releasing organic acid, which transforms the complex form into the simple form and makes K available to plants (Aleksandrov *et al.*, 1967; Bennett *et al.*, 1998) [1, 5]. The type of bacteria present and the state of the mineral in which they are living affect how well potassium is soluble. Consequently, applying biofertilizer containing potassium-rich minerals and K mobilizing bacteria will boost the yield of the crops. It has been discovered that silicate bacteria can remove aluminium, silicon, and silicon dioxide from insoluble materials. Recent studies have concentrated on their potential applications as biofertilizers or biocontrol agents for enhancing agriculture and safeguarding the environment.

Some bacteria have the ability to break down aluminium silicate, minerals, and release some of the potassium that is contained in aluminium silicate 2009 (Biswas and Basak) [3]. In the natural K cycle, microbes are crucial. Some rhizobacteria species have the ability to mobilize potassium in soils in an accessible form. K- solubilizing bacteria are widely distributed in the soil and rhizosphere. According to reports, silicate minerals make up the majority of the potassium in soil. When the minerals are slowly weathered or solubilized by the microbe, the potassium is made accessible to plants.

## 2. Materials and Methods

### 2.1 Soil Sampling

Twelve rhizospheric soil sample approximately one gram each were collected from different areas of Vidarbha regions of Maharashtra. Samples of the soil were collected 10 cm away from plants. Prior to collecting soil samples from the 6–10 cm soil layer, where roots were concentrated, the top 5 cm of the topsoil was removed. The rhizosphere is the zone of soil that is found between 0 and 2.5 mm from the root surface and is greatly impacted by living roots. The majority of the soil was manually separated from the rhizosphere dirt and roots. After being collected, all soil samples were used within eight hours and sealed in sterile ziplock bags, kept in an ice chest.

### 2.2 Isolation and screening of potassium solubilizing bacteria

For every section of soil sample, a series of tenfold dilutions were made, and 0.1 mL of each dilution was plated onto the Aleksandrov agar medium (pH 7.2). Briefly, the medium contained (per liter) 5 g glucose, 0.005 g MgSO4·7H2O, 0.1 g FeCl3, 2.0 g CaCO3, 2.0 g CaPO4, 20 g agar–agar and 3.0 g potash as insoluble K source per liter. The medium was sterilized by autoclaving at 15 psi. The inoculated Petri plates were sealed and incubated at 27 °C for 48-72 hours. After incubation, the K solubilizing ability of the bacterial isolates was qualitatively evaluated based on clear zone formation. Each bacterial strain forming clear zone was spotted on separate plates and incubated at 27 °C for 7 days. For further research, the bacterial isolates that could solubilize K were subcultured, purified, and kept at 4 °C in nutrient agar (0.3% beef extract, 0.5% peptone, 18% agar, pH 7.0–7.5).

### 2.3 Potassium solubilization and identification of best solubilizer

K solubilization efficiency was checked by zone assay method. By sprinkling 10 µl overnight-grown cultures on Aleksandrov's agar plates and incubation at 28-30°C for 2-3 days, the potassium solubilizing bacterial (KSB) strain's capacity to dissolve potash was examined. Potassium solubilizers were identified in isolates that had a distinct zone of potash solubilization surrounding the colony. In order to do the initial screening of the isolates, Khandeparkar's selection ratio was used to determine which ones had a better capacity for potassium solubilization (Prajapati and Modi, 2012b) [13].

$$\text{Khandeparkar's selection ratio} = \frac{Diameter\ of\ zone\ of\ clearance\ (D)}{Diameter\ of\ growth\ of\ bacteria\ (d)}$$

The diameter of zone of solubilization was measured and expressed in millimeter and also the selected isolates were preserved on agar slants for further studies.

### 2.4 Characterization of potassium solubilizing bacterial isolates

#### 2.4.1 Cultural characterization

All the selected isolates were examined for the colony color, shape of bacteria, colony appearance and Gram reaction (positive, negative) as per the standard procedures

#### 2.4.2 Biochemical characterization

The selected KSB isolates were subjected to biochemical characterization employing the standard procedures given by Seeley *et al.* (1991) [15] and Cappuccino and Sherman (2013) [6]. Starch Hydrolysis, Simmon's citrate test, Casein Hydrolysis test, Voges-proskauer test, KOH test, Acid and Gas production test, Indole test, Catalase test, Hydrogen sulphide production test and Methyl red test were performed.

## 3. Results and Discussion

### 3.1 Isolation of potassium solubilizing bacteria

The rhizosphere soil samples were collected from different locations of Vidarbha district and used for isolation of potassium solubilizing bacteria. Two KSB isolates were obtained from the rhizosphere of chickpea. These isolates named as KSB1 and KSB2 and were purified, identified and maintained for further studies. These results are in agreement with the findings of Norkina and Pumpynaskaya (1956) [11]; they also isolated two strains of *Bacillus spp.* and *Pseudomonas* from rhizosphere soil of various crop plants as mineral potassium solubilizers. Archana *et al*. (2008) [2] isolated potassium solubilizing bacteria, *Bacillus sp*. and *Pseudomonas sp.* from the rhizosphere soils of different crop plants from the places around Dharwad and Belgaum districts. Parmar and Sindhu (2013) [12] also obtained bacterial isolates (*Bacillus* and *Pseudomonas*) from wheat rhizosphere on modified Aleksandrov's medium containing mica powder as potassium source. Kumar (2014) [9] isolated nitrogen fixing (*Azotobacter*), phosphate solubilizing (*Bacillus*) and potash mobilizing bacterial (*Pseudomonas*) strains from the rhizosphere soil of agricultural lands.

### 3.2 K-solubilizing activity of isolates

K-solubilizing ability of isolates were analyzed on the basis of zone of solubilization produced by isolates on Aleksandrov media. Zone of solubilization produced by isolates range from 16 to 21 mm. The maximum zone was produced by KSB1 (20.67 mm) and minimum zone was produced by KSB2 (17.67 mm). Archana *et al*. (2008) [2] also screened thirty KSB strains for their ability to solubilize potassic mineral like muscovite mica in agar and broth medium. The zone of solubilization by all the mineral potassium solubilization strains ranged from 0.68 to 1.30 cm at 72 hours after incubation (HAI). Such observations were also made earlier that among the K bearing silicate minerals mica was found to be solubilized readily (Tandon and Sekhon, 1988; Mikhailouskaya and Tehernysh, 2005; Sugumaran and Janarthanam, 2007) [18, 10, 17]. The details are given in table 1.

**Table 1:** Value of potassium solubilization zone of bacterial isolates by Khandeparkars selection ratio

| Sr. No | Isolates | Clear zone diameter (D) mm | Growth diameter (d) mm | D/d (ratio) |
|---|---|---|---|---|
| 1 | KSB 1 | 20.67 | 10.33 | 2:1 |
| 2 | KSB 2 | 17.67 | 09.33 | 2:1 |

### 3.3 Cultural characteristics of KSB

Both isolates recorded whitish to yellowish colony color, colony appearance raised and smooth, Gram negative as bacteria did not retain the color of primary stain (crystal violet) during the staining and bacterial shape were short and long rod. The cultural characteristics of potassium solubilizing bacteria were shown in table 2. Results of the present studies are in accordance with the result of Rekha *et al*. (2010) [14] where it was reported that *Pseudomonas fluorescens* are gram-ve, cell size small, rod shaped, yellow green in colour and of large colony size.

**Table 2:** Cultural characteristics of potassium solubilizing bacteria

| Isolates | KSB 1 | KSB 2 |
|---|---|---|
| Colony color | Yellowish | White |
| Colony Appearance | Raised, smooth and Glistening | Slightly raised and smooth |
| Shape of bacteria | Rod | Rod |
| Gram Reaction | Negative | Negative |
| Probable genus | *Pseudomonas* | *Pseudomonas* |

### 3.4 Biochemical characteristics of KSB

Both the isolates of KSB were subjected to the biochemical tests for their identification, these tests were performed for comparison of the characteristics among isolates with respect of indole production, methyl red test, voges-proskauer test, starch hydrolysis, casein hydrolysis, simmon's citrate, KOH, acid production, catalase, H2S production test. The result are presented in the Table 3.

#### 3.4.1 Starch hydrolysis test

Both the isolates of KSB showed negative reaction to starch hydrolysis. This test was to check the ability of organism to produce a certain exoenzyme, including a-amylase and oligo-1, 6-glucosidase, that hydrolyze starch.

#### 3.4.2 Methyl red test

Both the isolates of KSB showed positive reaction to Methyl red test. The methyl red test detects the production of sufficient acid during the fermentation of glucose. Development of red colour upon addition of methyl red indicator showed glucose fermentation.

**3.4.3 Voges-proskauer test:** Both the isolates showed the negative reaction to the Vogesproskaur test. VP test used to determine the ability of bacteria to utilize glucose with the production of acetone as the neutral end product.

#### 3.4.4 Catalase test

Catalytic activities of both the isolates of KSB were found positive. It was performed to study an antioxidant enzyme responsible for eliminating molecules of hydrogen peroxide from the cells that are produced during respiration.

#### 3.4.5 Acid production test

Both isolates of KSB were tested for acid production and they found positive to produce acid. It is used to check the ability of bacteria to form an organic compounds by metabolizing certain carbohydrates.

#### 3.4.6 Casein hydrolysis

Both the isolates showed negative reaction to Casein hydrolysis. It is the test the digestion of casein by proteolytic exoenzyme that are secreted outside the cell.

#### 3.4.7 Simmon's citrate test

In a simmons citrate test one isolate showed a positive reaction and other isolate showed a negative reaction to the test. It was used to determine the ability of bacteria to utilize sodium citrates as its only carbon source.

#### 3.4.8 Hydrogen sulphide production test

Both the isolates of KSB were found negative on producing H2S gas. This test thus determines whether the microbe reduces sulfur containing compounds to sulfides during the process of metabolism.

#### 3.4.9 KOH test

Both the isolates of KSB showed positive reaction to KOH test. It is used to differentiate between Gram positive and Gram negative organism.

#### 3.4.10 Indole production test

Both the isolates of KSB did not produce indole hence showed negative reaction to indole production. The indole test screens for the ability of an organism to degrade the amino acid, tryptophan and produce indole.

Similar results were obtained by Parmar *et al*. (2013) [12] reported the biochemical characterization of the five selected isolates on the basis of sugar utilization, methyl red test, voges-proskauer (VP) test, urea hydrolysis, nitrate reduction test, gelatin hydrolysis test, catalase test, starch hydrolysis, casein hydrolysis and H2S production test and assigned them tentatively as *Bacillus* and *Pseudomonas* species. The results of the present studies are in agreement with the results of previous researchers Fatharani *et al*. (2018) [7] conducted catalase test, Ziehl-Nelseen test, methyl red and voges-proskauer test (MR-VP), urease test, nitrate reduction test, citrate utilization test to characterize potassium solubilizing bacteria. The results obtained in the present studies are in the full agreement with the results of previous workers. Bashir *et al*. (2018) [4] observed that all isolates had shown positive results for catalase, urease, casein hydrolysis, acid production tests and negative for Voges-proskauer and H2S tests.

**Table 3:** Biochemical characteristics of potassium solubilizing bacteria

| Biochemical Test | Isolates | |
|---|---|---|
| | KSB 1 | KSB 2 |
| Starch Hydrolysis | - | - |
| Methyl Red test | + | + |
| Voges-Proskauer test | - | - |
| Catalase test | + | + |
| Acid Production test | + | + |
| Casein Hydrolysis | - | - |
| Simmon's citrate test | - | + |
| Hydrogen sulphide production test | - | - |
| KOH test | + | + |
| Indole production test | - | - |

**Note-** (+) - Positive, (-) - Negative

### 3.5 Identification of KSB isolates

On the basis of biochemical characteristics, both the isolates were probably assumed to belong to Pseudomonas genus. These results were also suggested by Archana *et al*. (2008) [2] characterized the bacterial isolates as *Bacillus* and *Pseudomonas sp*. capable of solubilizing 119 potassic mineral by using cultural, morphological and biochemical characterization. All the above mentioned reviewed will strongly support the present study. Similar results reported by Jayswal *et al*. (1990) [8], characteristic main features of the genus *Pseudomonas* were Gram negative.

## 4. Conclusion

The isolation of potassium-solubilizing bacteria (KSB1 and KSB2) from rhizospheric soil highlights the presence of microorganisms capable of converting complex potassium compounds into a simpler form, potentially increasing the availability of this essential nutrient to plants. The morphological and biochemical characteristics of these isolates are consistent with known potassium-solubilizing bacteria. Their ability to solubilize potassium, as indicated by the zone of solubilization, suggests their potential use as biofertilizers to enhance plant growth and soil fertility. Further studies could focus on evaluating the effectiveness of these isolates in field conditions and their impact on crop yields.

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