Isolation, *in vitro* screening and characterization of native phosphate and potash solubilizing bacterial isolates from Malnad region of Karnataka

Nandish MS, Suchitha Y and Reena Rosy Nelson Anthikat

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Abstract

In the present study as many as 45 soil samples were collected from different crop rhizosphere (banana, maize, ginger and tobacco) for isolation of phosphorous and potassium solubilizing bacteria and out of 45 samples collected eight potash and seven phosphorus solubilizing bacterial isolates were isolated by standard serial dilution pour plate technique and all the isolates were characterized based on the morphological and biochemical characteristics as *Bacillus* sp. Further when all the isolates screened for the potassium and phosphorous ability in the *in vitro* experiments. Out of 8 potassium solubilizing bacterial isolates the isolate number KSB – 6 showed the maximum zone of solubilization of mica on Alexandrove’s media (2.40cm) and also released 55.49 (mg/ml) of potassium at 10th day after inoculation. Similarly, out of seven phosphorus solubilizing bacterial isolates screened the isolate number PSB – 3 showed maximum zone of solubilization of phosphorus 2.20 cm on the Sperber’s media and released an amount of 9.50% of inorganic phosphorus at 10th days after inoculation. Scale of studies are required to develop and evaluate efficient potassium solubilizing *Bacillus* sp. KSB - 6 and phosphorous solubilizing PSB - 5 bacterial consortia green house and under field conditions using different crops.

Keywords: Isolation, screening, phosphorous, potassium, solubilizing *Bacillus* sp

Introduction

As we enter the third millennium with more than six billion people, we are confronted with a herculean task of providing environmental and food security to the expanding population particularly in the developing countries. This calls for the reorientation of strategies to minimize the use of external inputs in agriculture and depend more on eco-friendly approaches to sustain food production without causing disruption to the fragile agro-ecosystem. Scientific soil management strategy strives to attain twin objectives of higher crop productivity and soil health sustenance. Indian soils are of poor in fertility, as these have been consistently been depleted of their finite nutrient resources due to continuous cultivation for centuries, adoption of modern agricultural technologies, imbalanced and indiscriminate use of fertilizers and inadequate and irregular application of organic manures.

Plant nutrients and their fixation in soils

Soil is a very complicated natural ecosystem that acts as a pool for all the plant nutrients that are in fixed and available form. These nutrients are fixed in the soil by chemical reactions and ultimately influencing the non-availability of nutrients to the plants. On the other hand, the dynamic interactions between organic and inorganic soil components and soil microorganisms greatly influence the mobility and availability of mineral nutrients. Out of seventeen plant nutrients phosphorus is commonly deficient in most of the natural soils, since it is fixed as insoluble iron and aluminium phosphates in acidic soil. As a result of the phosphorus fixation some of the micronutrients are unavailable to the plants. Out of these micronutrients Iron (Fe), Manganese (Mn) Aluminium (Al) are the major one’s that form complexes with other nutrients and are unavailable to the plants and ultimately affect the yield parameters [15]. Numerous microorganisms especially those associated with roots have ability to increase the plant growth by solubilizing or releasing the unavailable mineral nutrients and also increase
soil fertility through atmospheric nitrogen fixation and weathering of soil minerals [13].

**Importance of microorganisms in agriculture**

Conceptual design is important in developing new technologies for utilization of phosphate and potash solubilizing microorganisms for sustainable production of different crops. The basis of conceptual design is simply to conceive an ideal or model and then to devise a strategy and method for achieving the reality. However, it is necessary to carefully coordinate the materials, environment, and the technologies constituting the method. Moreover, one should adopt a philosophical attitude in applying microbial consortial technologies to agricultural production and conservation systems. Hence, it is necessary to use consortial application of beneficial microorganisms in agriculture to attain the twin objective of both phosphorus and potassium availability to different crops.

Microorganisms play a vital role in the field of agriculture by converting the unavailable form of nutrient to available form to the plants by various mechanisms like solubilization and fixation of the nutrient element present in soil and atmosphere. However, they also play an important role in controlling many of the plant pathogens and insects. The present study concerns on the role of microorganisms on nutrient status of the soil. In ecosystem with low inputs and without any fertilization or soil amendments by humans, the nutrients available to plants come from atmospheric inputs and weathering of soil minerals [3].

Microbial inoculants that are able to dissolve potassium from mineral and rocks have influence on plant growth and have both economic and environmental advantage. Gaur et al., (1972) [16] reported that *Bacillus firmus* and *Bacillus polymyxa* play important role in plant nutrition through increase in phosphorus and potassium uptake by plants and thereby increasing crop yield.

Hu et al., (2006) [10] reported potassium solubilizing strains from the soil and they were phenotypically and phylogenetically characterized and were effectively dissolve mineral potassium when they grow on Alexandrov’s medium which were rod shaped spore formers with a large capsule and formed slimy and translucent colonies.

Based on the past work done by different researchers and in view of greater need for development of phosphate and potassium solubilizing bacterial consortia for different crop production the attempts were made and the experiment was conducted under Government of Karnataka funded project.

**Materials and Methods**

The present investigation was conducted in the Department of Agricultural Microbiology, College of Agriculture, Shivamogga. The details of materials and methodology followed during the course of investigation are highlighted herein.

**Collections of soil samples**

A total of 45 soil samples were collected from different crop and forest rhizosphere (banana, maize, ginger tobacco and different weeds) for isolation of phosphorous and potassium solubilizing bacteria.

**Isolation of Phosphate and Potassium solubilizing bacteria**

The phosphate solubilizing microorganisms were isolated from all the rhizosphere soil samples by dilution plate technique on Pikovskaya’s agar medium. The plates were incubated at 28° ± 2°C for seven days and colonies with clear zones around were counted. The representative colonies of each type of bacteria with clear zones around were purified, sub cultured and maintained on the slants of Pikovskaya’s agar [16]. Similarly, the same soil samples were used to isolate potassium solubilizing microorganisms using Alexandrove's media [10].

**Identification and characterization of Phosphate and Potassium solubilizing bacteria isolates**

The phosphorus and potassium solubilizing bacteria were identified and characterized based on various morphological and biochemical characteristics. Bacterial strains isolated were examined for colony morphology, pigmentation, cell shape and Gram’s staining as per the standard procedure given by [1, 2].

**In vitro screening of phosphorus solubilizing bacteria**

**Agar plate method**

All the phosphorus solubilizing bacterial isolates were spotted on Pikovskaya’s agar for analyzing the phosphate solubilizing potentiality of each isolates. Based on the zone of solubilization of phosphorus on the media the phosphate solubilizing potentiality was interpreted [8].

**Chemical method**

Isolates of the phosphate solubilizing bacteria (10 ml of the overnight culture were inoculated to 100 ml of Pikovskaya’s broth in 250 ml flask with equal number of uninoculated controls. The flasks were incubated on a mechanical shaker at 28°C for 10 days. The amount of pi released in the broth in flasks was estimated at 10 days after inoculation. The broth cultures of bacteria were centrifuged at 9000 rpm for 20 minutes in a centrifuge to separate the supernatant from the cell growth and insoluble phosphate. The available pi content in the supernatant/filtrate was estimated by phosphomolybdic blue colour method [11].

**In vitro screening of potassium solubilizing bacteria for K released from insoluble K bearing mineral**

**Agar plate method**

All the potassium solubilizing bacterial isolates were spotted on Alexandrov’s media containing mica for analyzing the potassium solubilizing potentiality of each isolates. Based on the zone of solubilization of potassium (mica) on the media the potassium solubilizing potentiality of the potassium solubilizing bacteria was interpreted.

**Chemical method**

The isolates showing zone of solubilization on Alexandrov’s agar were further examined for their ability to release K from broth media (supplemented with 1 per cent muscovite mica).

One ml of overnight culture of each isolate was inoculated to 25 ml of Alexandrov’s broth in replicates [10]. All the inoculated flasks were incubated for two weeks at 28±2°C. The amount of K released in the broth was estimated after 10th days of incubation from triplicate flasks at each stage in comparison with a set of uninoculated controls. The broth cultures were centrifuged at 10,000 rpm for 10 minutes in the microcentrifuge to separate the supernatant from the cell growth and insoluble potassium. The available K content in the supernatant was determined by flame photometry [11, 17].
Results and Discussion
Collection of Soil samples
In the present investigation as many as 45 samples (soil, compost and leaf litter samples) were collected from Shivamogga, Sagara, Thirthalli and Davanagere region of Karnataka (India) for the isolation of native phosphate and potassium solubilizing bacteria using suitable selective medium (Table 1 and Plate 1). The chances of isolating plant growth promoting rhizomicroorganisms are more in the rhizosphere soil of many crops \(^4\). With this view, 45 soil samples having the pH range from 6.2-7.6 were collected from the rhizosphere of different crops of Malnad region of Karnataka.

| S. No. | Nature of soil sample | Location |
|--------|-----------------------|----------|
| 1.     | Red soil              | UAHS, Shivamogga |
| 2.     | Compost sample        | UAHS, Shivamogga |
| 3.     | Red soil              | UAHS, Shivamogga |
| 4.     | Black soil            | Ablagere   |
| 5.     | Black soil            | Nyanthi    |
| 6.     | Red soil              | Honnalli   |
| 7.     | Clay soil             | Ablagere lake |
| 8.     | Red soil              | Belaguthi  |
| 9.     | Red soil              | Aynuru     |
| 10.    | Black soil            | Rippnpate  |
| 11.    | Sandy soil            | Humcha     |
| 12.    | Litter mixed soil     | Tirthahalli forest |
| 13.    | Forest soil           | Sagara forest |
| 14.    | Red soil              | Sagara     |
| 15.    | Forest soil           | Ripponpet forest |
| 16.    | Red soil              | Davangere  |
| 17.    | Clay soil             | Konanduru  |
| 18.    | Red soil              | Arsalu      |
| 19.    | Red soil              | Agumbe      |
| 20.    | Black soil            | Masthikatte |
| 21.    | Red soil              | Hosnagara  |
| 22.    | Clay soil             | Nagara     |
| 23.    | Litter mixed soil     | Koppa      |
| 24.    | Red soil              | NR pura    |
| 25.    | Black soil            | Shringeri  |
| 26.    | Red soil              | Araga       |
| 27.    | Clay soil             | Iruvaki    |
| 28.    | Red soil              | Anandapura |
| 29.    | Red soil              | Ulluru     |
| 30.    | Black soil            | Manchale   |
| 31.    | Red soil              | Garthikere |
| 32.    | Forest soil           | Guddekkopan |
| 33.    | Red soil              | Mugudhni   |
| 34.    | Red soil              | Lakkikopan |
| 35.    | Black soil            | Kannangi   |
| 36.    | Sandy loam soil       | Choradi    |
| 37.    | Clay soil             | Shikaripura |
| 38.    | Red soil              | Shiralkopan |
| 39.    | Red soil              | Devikopan  |
| 40.    | Black soil            | Issuru     |
| 41.    | Red soil              | Saluru     |
| 42.    | Clay soil             | Battemallappa |
| 43.    | Red soil              | Gajanuru   |
| 44.    | Red soil              | Mandagadde |
| 45.    | Black soil            | Chinmane   |

Table 1: Details of soil samples collected for isolation of native phosphate and potassium solubilizing bacterial isolates

Isolation of Phosphate and Potassium solubilizing bacteria isolates
Out of 45 soil samples collected, eight potash solubilizing bacteria capable of growing on Alexandrovne’s media and seven phosphorus solubilizing bacteria were isolated on Sperber’s by standard pour plate technique and all the KSB isolates were named as KSB – 1, KSB – 2, KSB – 3, KSB – 4, KSB – 5, KSB – 6, KSB – 7, KSB – 8 and PSB isolates were named as PSB-1, PSB-2, PSB-3, PSB-4, PSB-5, PSB-6 PSB-4, PSB-7 (Table 2 and Plate 2). The results are in agreement with the findings of \(^9\) who isolated three strains of Bacillus species from the soil samples of Mussoriee rock phosphate capable of solubilizing tri-calcium phosphate. Similarly \(^10\) also isolated potassium solubilizing microorganism from the different soils using Alexandrovne’s media.
Identification and characterization potassium and phosphate solubilizing bacterial isolates

The phosphorus and potassium solubilizing bacteria were identified and characterized based on various morphological and biochemical characteristics as *Bacillus* sp. (Table 3 and 4). The results are in agreement with the findings of [7], who isolated and characterized three strains of *Bacillus* species from soils of Mussoriee and Merton rock phosphate capable of solubilizing tricalcium phosphate. In support of [7, 14] isolated acid producing bacteria from rhizoplane, rhizosphere soils of oat plant for solubilization of phosphate mineral fertilizers and other related compounds.

Table 2: Phosphate and potassium solubilizing bacterial isolates obtained from soils samples collected form banana rhizosphere

| S. No | Potassium solubilizing bacterial isolates | Phosphorus solubilizing bacterial isolates |
|-------|------------------------------------------|------------------------------------------|
| 1     | KSB–1                                    | PSB –1                                   |
| 2     | KSB–2                                    | PSB –2                                   |
| 3     | KSB–3                                    | PSB –3                                   |
| 4     | KSB–4                                    | PSB –4                                   |
| 5     | KSB–5                                    | PSB –5                                   |
| 6     | KSB–6                                    | PSB –6                                   |
| 7     | KSB–7                                    | PSB –7                                   |
| 8     | KSB–8                                    |                                          |

Table 3: Biochemical characters of potassium solubilizing bacterial isolates

| Sl. No. | Isolates  | AG | H2S | NO3 | IP | MR | VP | CU | UA | CA | OA | GL | SH | PB          |
|---------|-----------|----|-----|-----|----|----|----|----|----|----|----|----|----|-------------|
| 1       | KSB - 1   | +  | -   | -   | -  | +  | +  | +  | +  | +  | +  | -  | -  | *Bacillus* sp. |
| 2       | KSB - 2   | +  | -   | +   | +  | +  | +  | +  | +  | +  | +  | -  | -  | *Bacillus* sp. |
| 3       | KSB - 3   | -  | +   | -   | -  | -  | -  | +  | +  | +  | +  | -  | -  | *Bacillus* sp. |
| 4       | KSB - 4   | -  | -   | -   | +  | +  | +  | +  | +  | +  | +  | -  | -  | *Bacillus* sp. |
| 5       | KSB - 5   | +  | -   | +   | -  | -  | +  | +  | +  | +  | +  | -  | -  | *Bacillus* sp. |
| 6       | KSB - 6   | +  | -   | +   | -  | -  | +  | +  | +  | +  | +  | -  | -  | *Bacillus* sp. |
| 7       | KSB - 7   | -  | +   | -   | -  | -  | -  | +  | +  | +  | +  | -  | -  | *Bacillus* sp. |
| 8       | KSB - 8   | -  | -   | -   | -  | -  | -  | +  | +  | +  | +  | -  | -  | *Bacillus* sp. |

Table 4: Biochemical characters of phosphorus solubilizing Bacteria

| Sl. No. | Isolates  | AG | H2S | NO3 | IP | MR | VP | CU | UA | CA | OA | GL | SH | PB          |
|---------|-----------|----|-----|-----|----|----|----|----|----|----|----|----|----|-------------|
| 1       | PSB - 1   | +  | -   | -   | -  | +  | +  | +  | +  | +  | +  | -  | -  | *Bacillus* sp. |
| 2       | PSB - 2   | +  | -   | +   | +  | +  | +  | +  | +  | +  | +  | -  | -  | *Bacillus* sp. |
| 3       | PSB - 3   | -  | +   | -   | -  | -  | -  | +  | +  | +  | +  | -  | -  | *Bacillus* sp. |
| 4       | PSB - 4   | -  | -   | -   | +  | +  | +  | +  | +  | +  | +  | -  | -  | *Bacillus* sp. |
| 5       | PSB - 5   | +  | -   | +   | -  | -  | +  | +  | +  | +  | +  | -  | -  | *Bacillus* sp. |
| 6       | PSB - 6   | +  | -   | +   | -  | -  | +  | +  | +  | +  | +  | -  | -  | *Bacillus* sp. |
| 7       | PSB - 7   | -  | +   | -   | -  | -  | -  | +  | +  | +  | +  | -  | -  | *Bacillus* sp. |

Note: AG = Acid and Gas Production (littmus reaction), H2S = Hydrogen Sulphide, NO3 = Nitrate reduction, IP = Indole production, MR= Methyl red test, VP= Voges proskauers test, CU = Citrate utilization, UA = Ureaese Activity, CA = Catalase activity, OA = Oxidase activity, GA = Gelatin liquefaction, SH=Starch hydrolysis. *VA* = Positive, *VA* = Negative, PB = Probable Genus

Table 5: Screening of potassium and phosphorus solubilization for their nutrient release into respective media

| S. No. | Potassium solubilizing bacterial isolates | Zone of Solubilization of Mica (cm) | Amount of potassium releasted (mg/ml) at 10th days after inoculation | Phosphorus solubilizing bacterial isolates | Zone of Solubilization of formed CaPO4 in Sperber’s media (cm) | Pi released (%) at 10th days after inoculation |
|--------|------------------------------------------|------------------------------------|----------------------------------------------------------------|------------------------------------------|------------------------------------------------|----------------------------------|
| 1      | Control                                  | 0.00                               | 0.08 (*)                                                          | Control                                  | 0.00                                           | 3.80 (**)                       |
| 2      | KSB–1                                    | 1.16                               | 36.02 (ef)                                                        | PSB –1                                    | 1.50                                           | 4.80 (**)                       |
| 3      | KSB–2                                    | 1.70                               | 48.09 (bc)                                                        | PSB –2                                    | 1.90                                           | 4.97 (**)                       |
| 4      | KSB–3                                    | 1.50                               | 45.00 (a)                                                         | PSB –3                                    | 2.20                                           | 9.50 (a)                        |
| 5      | KSB–4                                    | 1.60                               | 45.04 (d)                                                         | PSB –4                                    | 2.00                                           | 5.30 (b)                        |
| 6      | KSB–5                                    | 1.90                               | 51.06 (b)                                                         | PSB –5                                    | 2.00                                           | 5.36 (b)                        |
| 7      | KSB–6                                    | 2.40                               | 55.49 (a)                                                         | PSB –6                                    | 1.80                                           | 5.10 (c)                        |
| 8      | KSB–7                                    | 1.80                               | 49.07 (c)                                                         | PSB –7                                    | 2.00                                           | 5.40 (c)                        |
| 9      | KSB–8                                    | 1.20                               | 37.07 (e)                                                         | -                                        | -                                              | -                                |

Note: Means followed by the same letters do not differ significantly
In vitro screening of potassium and phosphate solubilizing bacterial isolates

In screening studies, out of 8 potassium solubilizing bacterial isolates screened, the *Bacillus* sp. KSB - 6 showed maximum zone of solubilization (2.40 cm) of potassium followed by *Bacillus* sp KSB – 5 (1.90 cm) and the same KSB – 6 also released the 55.49 mg/ml of potassium at 10th day after inoculation. Whereas, other KSB isolates showed less of potassium solubilization ability on the medium but comparatively less than the KSB - 6. On the other hand out of 7 PSB isolates screened for phosphorus solubilization tested, the PSB - 3 produced maximum zone of 2.20 cm on Pikovskaya’s agar and it released the maximum of 9.50% of inorganic phosphorus at 10th day after inoculation (Table 5). The findings are in agreement with the findings of [5] who isolated and screened *Bacillus megatherium, B.brevis, B. circulance, Bacillus sambitis* from rhizosphere of Oat and Arhar. Similarly, [12] also screened 17 *Bacillus* species for their potassium solubilizing ability. Scale of studies are required to develop and evaluate efficient potassium solubilizing *Bacillus sp KSB* - 6 and phosphorous solubilizing PSB - 5 bacterial consortia on different crops under greenhouse and field conditions”

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