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Nandish MS
Department of Agricultural Microbiology, College of Agriculture, UAHS, Shivamogga, Karnataka, India

Suchitha Y
Department of Agricultural Microbiology, College of Agriculture, UAHS, Shivamogga, Karnataka, India

Reena Rosy Nelson Anthikat
Department of Microbiology, PSG, College of arts and Science, Coimbatore, Tamil Nadu, India

Corresponding Author:
Nandish MS
Department of Agricultural Microbiology, College of Agriculture, UAHS, Shivamogga, Karnataka, India

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) [6] 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 Alexandrove'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 $28^0 \pm 2^0$ 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 28^0 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 10^{th} 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.

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

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	Nyamthi
6.	Red soil	Honnalli
7.	Clay soil	Ablagere lake
8.	Red soil	Belaguthi
9.	Red soil	Aynuru
10.	Black soil	Rippanpete
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	Iruvakki
28.	Red soil	Anandapura
29.	Red soil	Ulluru
30.	Black soil	Manchale
31.	Red soil	Garthikere
32.	Forest soil	Guddekoppa
33.	Red soil	Mugudthi
34.	Red soil	Lakkinkoppa
35.	Black soil	Kannangi
36.	Sandy loam soil	Choradi
37.	Clay soil	Shikaripura
38.	Red soil	Shiralkoppa
39.	Red soil	Devikoppa
40.	Black soil	Issuru
41.	Red soil	Saluru
42.	Clay soil	Battemallappa
43.	Red soil	Gajanuru
44.	Red soil	Mandagadde
45.	Black soil	Chinmane

Isolation of Phosphate and Potassium solubilizing bacteria isolates

Out of 45 soil samples collected, eight potash solubilizing bacteria capable of growing on Alexandrove'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 Alexandrove's media.

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	

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 Mussooriee 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 3: Biochemical characters of potassium solubilizing bacterial isolates

Sl. No.	Isolates	AG	H_2S	NO_3	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	H_2S	NO_3	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.*

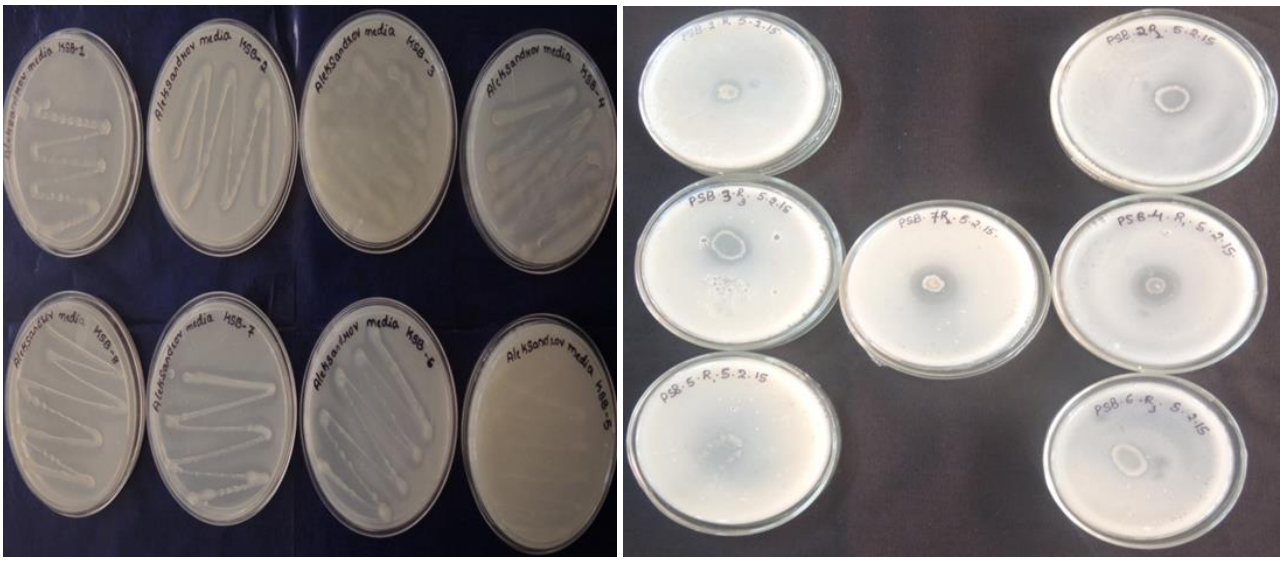
Note: AG = Acid and Gas Production (litmus reaction), H_2S = Hydrogen Sulphide, NO_3 = Nitrate reduction, IP = Indole production, MR= Methyl red test, VP= Voges proskauers test, CU = Citrate utilization, UA = Urease Activity, CA = Catalase activity, OA = Oxidase activity, GL= Gelatin liquefaction, SH=Starch hydrolysis. $^{+}$Ve = Positive, $^{-}$Ve = 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 rereleased (mg/ml) at 10th days after inoculation	Phosphorus solubilizing bacterial isolates	Zone of Solubilization of formed $CaPO_4$ in Sperber's media (cm)	Pi released (%) at 10th days after inoculation
1.	Control	0.00	0.08 $^{(g)}$	Control	0.00	3.80 $^{(e)}$
2.	KSB – 1	1.16	36.02 $^{(ef)}$	PSB – 1	1.50	4.80 $^{(d)}$
3.	KSB – 2	1.70	48.09 $^{(bc)}$	PSB – 2	1.90	4.97 $^{(d)}$
4.	KSB – 3	1.50	45.00 $^{(d)}$	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$^{(b)}$
9.	KSB – 8	1.20	37.07 $^{(e)}$	-	-	-
SEM±			0.15	-	-	0.42
CD @ 1%			0.48	-	-	1.26

Note: Means followed by the same letters do not differ significantly

Plate 1: Soil sampling and soil samples collected for isolation of native potassium and phosphate solubilizing bacteria



Growth of KSB on Alexandrove's media Growth of PSB on Pikovskaya's agar

Plate 2: Growth of potassium and phosphorus solubilizing bacterial isolates on specific media

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. cerculiance, Bacillus subtilis* 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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