



Isolation and characterization of mineral potassium solubilizing bacteria from rhizosphere soils

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Abstract: Attempts were made to isolate potassium solubilizing bacteria (KSB) from rhizosphere soil of different crops in Nadia district of West Bengal. A total of ten bacteria isolates were tested for K solubilization and characterized up to genus level based on morphological and biochemical characters. Among the ten isolates, six were gram positive rods belonging to genera *Bacillus* and remaining four isolates was gram negative rod belonging to genera *Pseudomonas*. The mechanisms involved in K solubilization and other agronomical beneficial traits were also analyzed for selected efficient strains. The diameter of zone of solubilization formed by the isolates on agar media supplemented with mica at 1 per cent ranged from 0.60 to 1.26 cm at 72 hours after incubation. *In vitro* K solubilization from mica powder by bacteria ranged from 3.23µg/ml to 41.20µg/ml at 20 days after inoculation. Organic acids production by the KSB isolates is the main reason for solubilization of potassium from mica powder. Results further suggested that the use of efficient potassium mobilizers can be a feasible option for meeting the K-requirement of crops.

Keywords: *In vitro* K-solubilization, Morphological and biochemical characters, Organic acids, Potassium solubilizing bacteria, Rhizosphere soil

INTRODUCTION

Potassium is one of the essential macronutrient absorbed most abundantly in cationic form by higher plants. Introduction of high yielding varieties and hybrids during green revolution and with the progressive intensification of agriculture resulted in depletion of reserve potassium from soils. As a consequence, potassium deficiency is becoming one of the major constraints in crop production, especially in coarse textured soils. Even though a soil is rich in potassium reserve, its amount in soil solution is very low and majority of it bound to mineral structure in soil (Das, 2011).

Microorganisms play a key role in the natural K cycle. Some species of rhizobacteria are capable of mobilizing potassium in accessible form in soils. There are considerable population of K solubilizing bacteria in soil and rhizosphere (Sperberg, 1958). Silicate bacteria were found to dissolve potassium, silicon and aluminium from insoluble minerals (Aleksandrov *et al.*, 1967). It has been reported that most of potassium in soil exists in the form of silicate minerals. The potassium is made available to plants when the minerals are slowly weathered or solubilized (Bertsch and Thomas, 1985). A wide range of bacteria namely *Pseudomonas*, *Burkholderia*, *Acidithiobacillus ferrooxidans*, *Bacillus mucilaginosus*, *B. edaphicus*, *B. circulans* and *Paenibacillus sp.* has been reported to release

potassium in accessible form from potassium-bearing minerals in soils (Sheng, 2005; Liu *et al.*, 2012). Potassium solubilizing bacteria are capable of solubilizing rock K, mineral powder such as mica, illite and orthoclase through production and excretion of organic acids which directly dissolve rock K or chelate silicon ion to bring K into soil solution (Friedrich *et al.*, 1991). Mineral potassium solubilization by microbes enhances crop growth and yield when applied with a cheaper source of rock potassium may be agronomically more useful and environmentally more feasible than soluble K (Rajan *et al.*, 1996).

Aleksandrov *et al.* (1967) isolated different bacterial species like silicate bacteria having the potential to dissolve potassium, silica and aluminium from insoluble minerals. Reports are also there that the slime producing bacterium *B. mucilaginosus* dissolves the silicates and also colonizes and develop in rhizosphere as well as non- rhizosphere soil (Lin *et al.*, 2002). Raj (2004) identified silicate solubilizing bacteria from rice ecosystem (SSB) in a medium containing 0.25 per cent insoluble magnesium trisilicate and also reported that *Bacillus sp.* found to solubilize silicate minerals more efficiently under *in vitro* conditions. Potassium solubilizing rhizobacteria were isolated from the roots of cereal crop by the use of specific potassium bearing minerals (Mikhailouskaya and Tchernysh, 2005). Potassium solubilizing strains were effectively able to

dissolve mineral potassium when they grown on Aleksandrov medium which are rod shaped spore formers with a large capsule and formed slimy and translucent colonies (Hu *et al.*, 2006).

Sheng and He (2006) reported that solubilization of illite and feldspar by microorganisms is due to the production of organic acids like oxalic acid and tartaric acids and also due to production of capsular polysaccharides which helps in dissolution of minerals to release potassium. Prajapati and Modi (2012) reported release of potassium from potassium aluminium silicate minerals by the selected bacterial strains by the action of different organic acids like Citric, Oxalic, Malic, succinic and Tartaric acid.

Soil potassium supplementation relies heavily on the use of chemical fertilizers. The mass application of fertilizer can increase costs, decrease the efficiency of K fertilizer (Zhang *et al.*, 2008), and damage the environment. An alternative to chemical K fertilizer is necessary for the sustainable development of agriculture. One possible alternative could be to fully exploit the reservoir of K in the soil. In this context, the use of potassium solubilizing bacteria for releasing the available K from the mineral K will be a feasible option.

However, the mechanism of mineral potassium solubilization has been a subject of study for a long time and is still a matter of curiosity. Although some works have been done on the biochemical and molecular mechanisms of mineral potassium solubilization by bacteria, no concerted efforts have been made in bacteria and other potassium solubilizers. Therefore the present investigation was carried out with the objective of isolation, identification and characterization of mineral potassium solubilizing bacteria from rhizosphere soils of different crops and their ability to solubilize insoluble potassic minerals and mechanism of solubilization.

MATERIALS AND METHODS

The present investigation was carried out at the Department of Agricultural Chemistry and Soil Science, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia. Twenty rhizosphere soils of different crop plants (approximately 500g from each) were collected from Jaguli, Mandauri and Gayeshpur farm of Nadia district (Table 1). The soil samples were processed for analysis of different soil physico-chemical properties.

Potassium solubilizing bacteria were isolated from collected soil samples by serial dilution plate count method using Aleksandrov medium (Glucose - 5.0 g, Magnesium sulphate - 0.005 g, FeCl₃ - 0.1 g, Calcium carbonate - 2.0 g, Potassium mineral - 2.0 g (mica), Calcium phosphate - 2.0 g, Distilled water - 1000 ml) which is a selective medium for isolation of potassium solubilizers. The plates were incubated at room temperature (30±1°C) for 3 days. Then the colonies exhibiting clear zones were selected and purified by four way streak plate method. The diameter of zone of solubilization (in cm) was measured and the selected

isolates were preserved on agar slants for further use. All the selected isolates were examined for the colony morphology, cell shape, gram reaction and ability to form spores as per the standard procedures given by Anonymous (1957) and Bartholomew and Mittewer (1950). The biochemical characterization of the isolates was carried out as per the procedures outlined by Cappuccino and Sherman (1996). Different tests viz., Starch and Casein hydrolysis, Acid (organic acids) and gas production (Seeley and Vandemark, 1970), Catalase activity (Blazevic and Ederer, 1975), Hydrogen sulphide production (Cowan and Steel, 1970), Urease test (James and Natalie Sherman, 1992), Gelatin liquefaction (Blazevic and Ederer, 1975), Oxidase test (Cappuccino and Sherman, 1996), Denitrification test, Methyl red, and Voges Proskauer tests (Seeley and Vandemark, 1970) were carried out.

Screening of the isolates for solubilization of mineral potassium was carried out through quantitative estimation of K released from insoluble K-bearing mineral (mica). The isolates showing zone of solubilization on Aleksandrov agar were 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 Aleksandrov broth (Hu *et al.*, 2006). All the inoculated flasks were incubated for three weeks at 28±2°C. The amount of K released in the broth was estimated at 7, 15 and 20 days of incubation at each stage in comparison with a set of uninoculated controls. The potassium so released was quantified by flame photometer (Sugumaran and Janarthanam, 2007). The bacterial cultures were tentatively identified upto genus level based on their morphological and biochemical characteristics.

Statistical analysis of data: Simple statistical analysis was carried out to justify the observations under this investigation.

RESULTS

In the present study, attempts were made to isolate potassium solubilizing bacteria from rhizosphere soil of different crop plants. The isolates were examined for their ability to solubilize insoluble potassic mineral. The selected isolates were characterized and tentatively identified upto genus level based on morphological and biochemical properties.

Isolation of potassium solubilizing bacteria (KSB) from rhizosphere soils of different crops: The rhizosphere soil samples of different crops were collected and used for the isolation of KSB. Rhizosphere soils of different crops collected from different locations and the types of potassium mobilizers isolated were furnished in Table 1. Out of the total KSB isolates, only ten were selected for screening of their potential for solubilization of mineral K. Table 2 showed that among ten isolates six were gram positive rods belonging to genera *Bacillus*

Table 1. Strain of potassium solubilizing bacteria isolated from different rhizosphere soils.

S. N.	Sampling site	District	Crops	KSB isolated
1	Mandauri Farm	Nadia	Coconut	KM1
2	Mandauri Farm	Nadia	Banana	KM1, KM2
3	Mandauri Farm	Nadia	Sugarcane	KM1, KM3
4	Mandauri Farm	Nadia	Sugarcane	KM2, KM3
5	Mandauri Farm	Nadia	Banana	KM1, KM2
6	Mandauri Farm	Nadia	Coconut	KM1
7	Mandauri Farm	Nadia	Coconut	KM3, KM4
8	Central Research Farm, Gayeshpur	Nadia	Banana	KM2, KM5
9	Central Research Farm, Gayeshpur	Nadia	Banana	KM5
10	Central Research Farm, Gayeshpur	Nadia	Coconut	KM6
11	Central Research Farm, Gayeshpur	Nadia	Coconut	KM1, KM5, KM6
12	Central Research Farm, Gayeshpur	Nadia	Coconut	KM1, KM6
13	Central Research Farm, Gayeshpur	Nadia	Banana	KM7
14	Central Research Farm, Gayeshpur	Nadia	Banana	KM2, KM5, KM7
15	Jaguli Farm	Nadia	Coconut	KM8
16	Jaguli Farm	Nadia	Coconut	KM1, KM8
17	Jaguli Farm	Nadia	Coconut	KM9
18	Jaguli Farm	Nadia	Banana	KM5, KM9
19	Jaguli Farm	Nadia	Banana	KM2, KM10
20	Jaguli Farm	Nadia	Banana	KM10

and remaining four isolates was gram negative rod belonging to genera *Pseudomonas*.

These potential isolates were tested for their K-solubilization ability in suitable growth media containing 1% muscovite mica as a source of mineral K. The solubilization of mineral K could be noticed from the zone of solubilization of the media. The diameter of zone of solubilization formed by the isolates ranged from 0.60 to 1.26 cm at 72 hours after incubation (table III). Among the isolates KM1 recorded maximum solubilization zone (1.26 cm diameter) followed by KM3 (1.20cm), KM7 (1.10cm) and KM8 (1.00 cm). However, the isolate KM4 showed the least solubilization zone of 0.60 cm diameter among all the isolates.

The amount of K released from muscovite mica in the broth by the isolates was studied at 7, 15, 20 days after inoculation (DAI). The amount of K released from muscovite mica by different strains ranged from 0.91µg/ml to 22.33µg/ml, 2.64µg/ml to 25.73µg/ml and 3.23µg/ml to 41.2µg/ml at 7, 15 and 20 days after inoculation respectively (table III). Among the isolates KM3 released maximum amount of K from mica (41.20µg/ml) followed by KM1 (31.95µg/ml) and KM7 (30.42µg/ml) at 20 DAI. Among all the ten isolates examined KM9 released least amount of K from Mica at 20 days after inoculation. Figure I. indicated that the amount of K released from mineral K by all strains increased with increase in incubation time and maximum at 20 days after inoculation.

DISCUSSION

Isolation of KSB from rhizosphere soils: The chances of isolating microbial isolates for solubilization of insoluble mineral nutrients are more in the rhizosphere soils of many crops (Altamare, 1999). With this view different bacterial isolates were isolated from the rhizosphere soils of different crop plants from

the places around BCKV and Nadia district. It was interesting to note that the bacteria isolated from rhizosphere soils were able to grow and solubilize the medium containing fixed or insoluble form of nutrients. In the present study we found that *Bacillus sp.* had potential in solubilizing the potassic mineral. Diep and Hieu (2013) also isolated seven different bacterial strains related with *B. megaterium* and *B. coagulans* on Aleksandrov medium of soils from weathered rocks. The results are in agreement with the findings of Hu *et al.* (2006) also who isolated two phosphate and potassium solubilizing *Bacillus sp.* from soils, in the modified medium containing phosphorite and potassium minerals like kaolinite and potassium feldspar.

Screening of KSB for release of mineral K: From the morphological and biochemical characters of the isolates, they were tentatively identified as *Bacillus sp.* and *Pseudomonas sp.* Then the organisms were inoculated to broth medium containing fixed mineral (mica powder) sources. Similarly Fang and Yan (2006) also isolated and characterized bacteria *B. mucilaginosus* capable of solubilizing two potassium bearing minerals like feldspar and illite.

The morphological characterization revealed that all potassium solubilizing bacteria were gram positive short to long rods spore formers, but differed in their physiology and nutrition. All the ten KSB strains performed differently in solubilizing potassic mineral like muscovite mica in agar and broth medium. The zone of solubilization by all the mineral potassium solubilizers ranged from 0.60 to 1.26 cm at 72 hours after incubation. Such observations were in line with the findings of Tandon and Sekhon (1988); Sugumaran and Janarthanam (2007); Mikhailouskaya and Tehernysh (2005); Archana *et al.* (2013); Parmar and Sindhu (2013). They postulated that among the K-bearing silicate minerals mica was much more susceptible to weather.

Table 2. Morphological and biochemical characteristics of the potassium solubilizers.

S. N.	Isolates	Morphological characters		Biochemical test												Carbon source utilization				Probable genus						
		Colony characters	Gram reaction and cell shape	Spore formation	1	2	3	4	5	6	7	8	9	10	11	12	a	b	c		d					
1	KM1	White smooth slimy large	+ve, rod	+	++	-	+	+	+++	+	+	+	-	-	++	++	+	+	+	-	-	-	-	-	-	<i>Bacillus</i>
2	KM2	Greyish white smooth widely spreading	-ve, rod	-	+	++	-	-	+	+	+	+	-	-	+	++	+	+	+	-	-	-	-	-	-	<i>Pseudomonas</i>
3	KM3	Large smooth opaque creamy white	+ve, rod	+	-	+++	+	+	++	+	+	+	-	-	+++	+	+	+	+	-	-	-	-	-	-	<i>Bacillus</i>
4	KM4	Large, smooth, opaque, creamy white	+ve, rod	+	++	-	-	-	++	+++	+	+	-	-	+	+++	+	+	+	-	-	-	-	-	-	<i>Bacillus</i>
5	KM5	Creamy, smooth, raised, large	-ve, rod	-	+	++	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-	-	-	-	-	<i>Pseudomonas</i>
6	KM6	White raised slimy	+ve, rod	+	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	-	-	-	-	-	-	<i>Bacillus</i>
7	KM7	White raised slimy, large	+ve, rod	+	+	+	+	+	+++	+	+	+	-	-	++	+	+	+	+	-	-	-	-	-	-	<i>Bacillus</i>
8	KM8	Creamy white small, slimy	-ve, rod	-	++	-	+	+	+	+	+	+	-	-	++	+	+	+	+	-	-	-	-	-	-	<i>Pseudomonas</i>
9	KM9	White, raised, circular	-ve, rod	-	+	++	-	+	+	+	+	+	-	-	+	+	+	+	+	-	-	-	-	-	-	<i>Pseudomonas</i>
10	KM10	Creamy white, smooth, large	+ve, rod	+	+	+	-	-	++	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	<i>Bacillus</i>

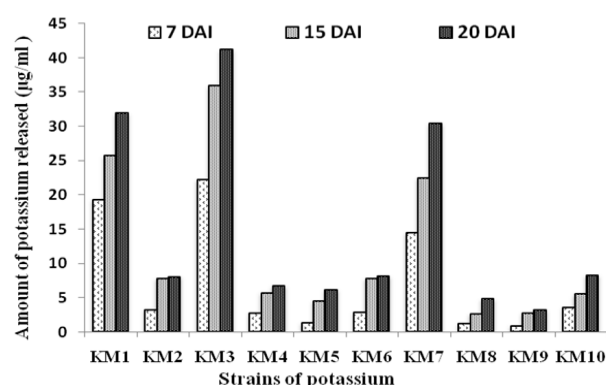
1 – Starch hydrolysis, 2 – Growth at N free media, 3 – Urea hydrolysis, 4 – Gelatin liquefaction, 5 – Catalase test, 6 – Acid production, 7 – Nitrate reduction test, 8 – Methyl red test, 9 – H₂S production, 10 – Gas production, 11 – Casein hydrolysis, 12 – Growth test, a – Mannitol, b – Sucrose, c – Maltose, d – Citric acid; + Positive, - Negative

Table 3. Details of zone of solubilization by different KSB isolates.

S.N.	Strains	Zone of Solubilization (cm)
1	KM1	1.26
2	KM2	0.92
3	KM3	1.20
4	KM4	0.60
5	KM5	0.90
6	KM6	0.75
7	KM7	1.10
8	KM8	1.00
9	KM9	0.72
10	KM10	0.83
	S.Em±	0.08
	CD @ 5%	0.39

The potassium solubilizing bacteria were subsequently tested for the ability to release K from muscovite mica in the external broth. The amount of K released from muscovite mica ranged from 3.23 µg/ml to 31.98 µg/ml at 20 days after inoculation. Among the isolates, *Bacillus* species KM3 showed the higher K release from the insoluble K source used. The findings are in agreement with the findings of Archana *et al.* (2013) who reported that three bacterial strain belongs to genera *Bacillus* was capable of solubilizing mica in appreciable amounts. The results also indicated great variation between the isolates to solubilize the same or different source of insoluble potassium minerals (Mikhailouskaya and Tehernysh, 2005; Liu *et al.*, 2006; Parmar and sindhu., 2013). Variations in the amount of potassium solubilization by the strains of same species of *Bacillus* and *Pseudomonas* were noticed. In contrast with the above Hu *et al.* (2006) reported that *Bacillus megatherium* and *B. mucilaginosus* were capable of solubilizing both rock phosphate and potassium. They also reported that co-inoculation of these two *Bacillus* species were potential in solubilizing potassium rocks. The present study thus indicated the *Pseudomonas sp.* was also capable of releasing some amount of potassium from mica (KM2: 8.01 µg/ml) but was comparatively less and the results compare well with the observations of Badr (2006). Greater release of K from muscovite has been documented by *Bacillus mucilaginosus* (Sugumaran and Janarthanam, 2007). The probable mechanism for solubilization of potassium bearing minerals was by the action of organic acids like oxalic acid and capsular polysaccharides.

The ability of bacteria to release K largely depends on the nature of the potassium mineral compounds (Yakhontova *et al.*, 1987). The variability among the bacteria indicated the importance of exploration of different mineral potassium solubilizing bacteria and understanding their solubilizing mechanisms. The release of K from insoluble potassic mineral by the isolated bacteria increased with incubation time up to 20 days. The results also indicated variability in the amount of K released by different isolates. The differential efficiency of bacteria to solubilize insoluble inorganic potassium could be due to differences in their ability to release organic acids.



KM- Potassium mobilizers, DAI- Days after inoculation

Fig. 1. Amount of potassium solubilized by different strains of potassium mobilizers.

Conclusion

Potassium availability to crop plants in soil is generally low since nearly 98 percent of total K in soil is in mineral form. Solubilization of soil mineral potassium by bacteria is well established. Rhizosphere micro-organisms contribute significantly in solubilization of bound form of minerals in the soil. It is clear from the present study that the rhizosphere bacteria *Bacillus* and *Pseudomonas* are efficient in solubilization of mineral K in soil by producing organic acids. The strain KM3 belongs to genera *Bacillus* showed significant potential in solubilizing the mineral K may be due to its capability to produce high amount of organic acids which release K to soil solution from K bearing minerals. The release of mineral K from the muscovite mica increased with the days of inoculation of specific strain of *Bacillus* and *Pseudomonas*. As the strains of *Bacillus* species KM1 and KM3 gave very good results, they can be now tested on the field to rate its performance as biofertilizer for the particular location. Further studies on the mechanism by which KSB solubilise muscovite mica and the effectiveness of their use in the fields is needed.

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