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Solubilization of Potassium Containing Minerals by Bacteria and Their Effect on Plant Growth

P. Sugumaran and B. Janarthanam

Shri AMM Murugappa Chettiar Research Center, Taramani, Chennai-600 113, India

Abstract: Potassium solubilizing bacteria, isolated from soil, rock and mineral samples and their effects on solubilization from microcline,orthoclase and muscovite mica minerals and groundnut plant growth were examined. One of the bacteria *Bacillus mucilaginosus* MCRCp1, had particularly strong ability to form slime. The maximum potassium solubilization (4.29 mg L^{-1}) was found in muscovite mica. MCRCp1 bacterium could colonize and developed in soil than the control. Total number of bacteria increased due to inoculation from 8.4×10^3 cfu g^{-1} to 9.6×10^6 cfu g^{-1} respectively. Phosphorus (P) and Potassium (K) nutritional status in the soil were markedly improved through inoculation of this bacterium. The results showed that available P and K is increased from 6.24 to 9.28 mg kg^{-1} and 86.57 to 99.60 mg kg^{-1} in soil. Groundnut plant dry matter increased by 125% and the oil content 35.41% were increased through inoculation of MCRCp1 bacterium.

Key words: Microcline · orthoclase · muscovite mica · solubilization · groundnut

INTRODUCTION

Soil is a dynamic natural body on earth crust. There are several minerals available in soil but most important minerals are Nitrogen (N) Phosphorus (P) and potassium (K). Potassium is a third important plant nutrient. Ghosh and Hasan [1] have been documented the state wise available potassium status in India and categorized that the 21 percent districts are in low, 51 percent are medium and 28 percent are high.

According to the Buchholz and Brown [2] more than 98% of potassium in soil exists in the form of silicate minerals (microcline, muscovite, orthoclase biotite, Feldspars, etc.). Potassium and other metal elements can be released when these minerals slowly weathered. Earlier in this century, scientists found that some microorganisms in soil could damage the silicate crystal and release the Si from quartz [3, 4]. Feldspar are igneous minerals that include the alkali feldspar albite, orthoclase and plagioclase as end members. They are readily weathered through the protonation although complexation by some of organic acids and acidic polysaccharides of microbial origin can also play a role. The source of the acids may be biotic or abiotic, but more frequently the former. The acids may be organic or inorganic. Many kinds of organic acids with a potential for weathering feldspars are produced anaerobically through bacterial fermentationv [5]. The slime forming microorganisms to remove the iron from quartz and aluminum from bauxite ores [6]. The quartz degrading activity mainly related to the intensity of slime secretion [3]. The slime forming *Bacillus mucilaginosus* produced nuclease, endoglucanase, cellobiase, protease, ribonuclease, deoxyribonuclease and phosphomonesterase [7]. The partial oxidation of glucose to organic acids as the predominant mechanism by which the non-proliferating bacteria used in our study enhanced feldspar dissolution at neutral pH [8]. Organic acids can directly enhance dissolution by either a proton- or ligand-mediated mechanism. They can also indirectly enhance dissolution by the formation of complexes in solution with reaction products and as a consequence increase the chemical affinity for the overall dissolution reaction [9].

Several laboratory studies have suggested that acid-producing microorganisms can enhance the dissolution of various types of minerals. However, none of these studies identified un equivocally the mechanisms underlying the process of enhanced dissolution.

There is a variety of possible mechanisms for both the enhancement and inhibition of mineral solubilization by bacteria, they may be involved direct (bacterial cell) or indirect (bacterial exoproducts) affect on solubilization. Despite the difficulties associated with attempting to

Corresponding Author: Dr. P. Sugumaran, Young Scientist-Shri AMM MCRC 2/19, Kamarajar Street, Palavakkam, Chennai - 600 041 Tamilnadu, India

interpret complex processes occurring in the natural environment based on simple laboratory experiments, it is still possible to glean information about the basic principle of bacterially mediated dissolution or solubilization from experimental work.

In the present study, the availability of potassium solubilizing bacteria isolated from different resources release potassium from different ores as well as their effect on groundnut grown and their role in improving crop nutrition were studied.

MATERIALS AND METHODS

Sample collection: Potassium containing minerals such as microcline,orthoclase and muscovite mica were gifted from C.V.C. Mining Company, Gudur andhra prdesh, India and soil samples were collected from our MCRC campus.

Media (Lin Qi-mei *et al.*, [10])

A. Starch 10g, $Na_2HPO_4 .12H_2O$ 2 g, $FeCl_3$ 0.005g, $MgSO_4 .7H_2O$ 0.5 g, $CaCO_3$ 0.1 g, Yeast Extract 1g, agar 20 g, distilled water 1000 ml, pH 7.4.
B. Sucrose 5 g, $FeCl_3$ 0.005 g, $MgSO_4$. $7H_2O$ 0.5 g, $CaCO_3$ 0.1 g, Yeast Extract 1 g, distilled water 1000 ml, pH 7.4.
C. Sucrose 5 g, $FeCl_3$ 0.005 g, $MgSO_4$. $7H_2O$ 0.5 g, $CaCO_3$ 0.1 g, acid leached soil 2 g, distilled water 1000 ml, pH 7.4.
D. Sucrose 5 g, $FeCl_3$ 0.005 g, $MgSO_4$ $7H_2O$ 0.5 g, $CaCO_3$ 0.1 g, mineral ores 2 g, distilled water 1000 ml, pH 7.0-7.4.
E. Peptone 5 g, beef broth 3g, NaCl 5 g, agar 20 g, distilled water 1000 ml, pH 7.4.

Isolation and identification of potassium solubilizing bacteria: Soil samples were taken from MCRC campus Taramani, rock and mineral samples (microcline, orthoclase and muscovite mica) gifted from C.V.C. Mining Company. A series of suspension were made and poured onto medium A. After incubation for 3 days at 28°C, the colonies with heavy slime forming bacteria were selected and inoculated onto medium B. The culture was purified and then selected for further tests.

The purified bacteria isolates grown on medium E at 28°C for 48 h were made into suspension (10^8 cfu ml^{-1}). Then inoculated 1 ml into 50ml medium C (acid leached soil) and then incubated for 5 days at 28°C. The culture solution was centrifuged at 9000 rpm for 30 min. Potassium content in the supernatant was determined by flame photometry.

A volume of 0.5 ml of bacterial suspensions was individually spread onto medium A plates and then incubated at 28°C for 3 days. Green gram (*Vigna radiata)* seeds washed with 0.1% $HgCl_2$ solution for 4 min and then washed with sterilized distilled water at least 5 times, were left to germinate at 28°C to an embryo size of 1 cm, 10 germinated seeds were put onto 500 ml conical flask prepared above and then incubated at 28°C. Then sterilized water was added to keep a high humidity during incubation. Plant dry weight, height and un-germinated seed number were enumerated after 14 days incubation. Three replicates were made for each isolate.

The isolates with highest activity in potassium released from acid leached soil and improving green gram seedlings growth were identified according to the Krieg *et al.* [11] and Knudsen *et al.* [12].

Measurement of bacterial abilities to solubilize the different potassium containing minerals: The selected isolates were inoculated into medium D (5 ml per 100 ml medium) after additions of potassium containing minerals such as microcline, orthoclase and muscovite mica (<100 mesh). After 4 days incubation at 28°C, the culture solution was centrifuged at 9000 rpm for 30 min. The potassium content in the supernatant was estimated as described above. Five replicates were made for each treatment. A control without inoculation was also included.

Pot experiment: Pot experiment were carried out in the selected isolate MCRCp1 and control treatments. Surface soil was collected, air-dried and sieved < 2 mm. The soil contained organic matter 9.6 g kg^{-1}, Available P 6.24 mg kg^{-1} and K 88.57 mg kg^{-1}.

The selected isolate MCRCp1 was inoculated into the soil (1.27×10^6 g^{-1} soil). A control treatment was prepared with same volume of sterilized medium E. Three groundnut seeds were shown into mud pot and after the germination only one plant were maintained into each pot. Three replicates were made. After 90 days of incubation total and slime forming bacteria, available P and K in soil, plant dry matter, root length, No. of pods, pod weight and oil content were measured. Total potassium solubilizing bacteria were estimated by using medium E and medium A respectively. Available P and K were estimated according to Olsen *et al.* [13] and Knudsen *et al.* [12].

RESULTS

Isolation and identification of potassium solubilizing bacteria: Thirty isolates were obtained from medium A. Five isolates were selected according to their greatest slime production yield. They are numbered as MCRCp1,

Fig. 1: Groundnut plant control pots

Fig. 2: Groundnut MCRCp1bacterium inoculated pots

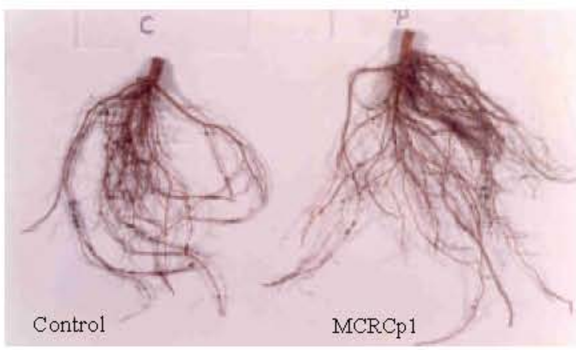

Fig. 3: Groundnut plant root growth

Fig. 4: Groundnut plant seeds

Table 1: Effect of slime forming bacterial isolates on green gram germination and growth

Isolate no.	Total un-germinated seed no.	Ave. seedlings height (cm)	Total dry dry weight (g)	Total dry weight (g)
MCRCp1	1	18.8	0.016	0.40
MCRCp2	3	15.6	0.012	0.32
MCRCp3	3	15.1	0.011	0.36
MCRCp4	2	15.8	0.012	0.31
MCRCp5	4	14.2	0.010	0.32
Control	3	14.4	0.014	0.36

Results represent mean±SD of three replicates

Table 2: Potassium released from 3 minerals by MCRCp1 after 4 days incubation at 28°C (mg L^{-1})

Treatments	Muscovite mica	Microcline	Orthoclase
Control	3.18±0.01	0.19±0.05	0.29±0.01
Inoculation	4.29±0.03	1.26±0.03	0.85±0.02

Results represent mean±SD of three replicates

Table 3: Effect of inoculating MCRCp1 on groundnut soil

Characters	Control	Inoculation
Total bacterial number	10.20±0.02	28.40±0.3
Slime forming bacterial number (10^4 cfu g^{-1})	0.74±0.05	4960.00±260
Available P (mg kg^{-1})	6.24±0.3	9.28±0.1
Available K (mg kg^{-1})	86.57±7.5	99.60±1.3

Results represent mean±SD of three replicates

Table 4: Effect of inoculating MCRCp1 on groundnut plant

Characters	Control	Inoculation
Plant dry weight (g)	0.49±0.01	1.10±0.01
No. of branches (nos)	7.10±0.01	13.40±0.02
No of pods (nos)	19.00±0.01	24.33±0.02
Oil content (%)	26.00±0.01	35.40±0.07
Root length (c.m)	16.25±0.02	35.00±0.01

Results represent mean±SD of three replicates

p2, p3, p4 and p5. These isolates were tested for their solubilizing capacity from acid leached soil. The maximum potassium content in the culture solutions was in the order of MCRCp1(2.26 mg L^{-1})>p2 (2.18 mg L^{-1})>p3 (2.06 mg L^{-1})>p4 (1.95 mg L^{-1}) and p5 (1.90 mg L^{-1}). MCRCp1had significantly higher activity to dissolve the silicate than others. (p = 0.05).

By incubating these bacteria together with green gram, the number of un-germinated seeds significantly decreased in all of the inoculated treatments (Table 1). Plant average height was grater than the control. However, plant dry weight was lower than the control except the treatment of MCRCp1. Therefore MCRCp1was selected for further tests.

According to the Avakyan *et al*. [14] and Li [15] the isolate MCRCp1was identified as a strain of *Bacillus mucilaginosus*. It had the characteristics as followed: gram positive, rod shaped, 4-7 μm long, 1.2-1.5 μm wide, immobility, having ellipse sporangium at middle, smooth, trim edge and transparent, no color produced in liquid culture and having activities of catalase, lecithinase and amylase, but no V.P. reaction.

Solubilizing capacity in potassium containing (silicate) minerals: The isolate MCRCp1 solubilize all the potassium containing minerals. A significant amount of potassium was released from the minerals during 4 days incubation. The solubilizing capacity was not related to the minerals solubility in the medium. The amounts of potassium released in muscovite mica 4.29 mg L^{-1}, microcline mineral 1.26 mg L^{-1} and orthoclase 0.85 mg L^{-1} were observed. (Table 2).

Effect of MCRCp1 on Groundnut plant growth: The inoculated soil contained 3.5 times more bacteria colonized than the un-inoculated soil pots. Inoculating *Bacillus mucilaginosus* (MCRCp1) did not cause a significant increase in the total bacterial number in the inoculated soil (Table 3).The solubilizing bacteria were increased to about 10^6 and 10^7 cfu g^{-1} soil respectively at 90 days of inoculating with the bacterium. The count of potassium solubilizing bacteria were only occurred 10^3 cfu g^{-1} in the control soil.

The available phosphorus and potassium were increased from 6.24 to 9.28 mg kg^{-1} and 88.57 to 99.60 mg kg^{-1} in the bacteria inoculated soil. Inoculation with MCRCp1 increased the groundnut plant dry matter by 125%. 0.49 g pot^{-1} and 1.10 g pot^{-1} for the control and inoculation treatments respectively. The oil content is 35.41% and root length 35cm was respectively increased compare than the control (Table 4).

DISCUSSION

Bertsch *et al*. [16] reported that most of the potassium in soil exists in the form of silicate minerals. The potassium can be available to plant when the minerals are slowly weathered or solubilized. Schrocder [17] pointed out that due to their different lattice structure of potassium and other minerals are released with different speeds from the silicate minerals. Our results showed that potassium in the muscovite mica minerals was most easily released than that of other potassium containing minerals (silicates). The mechanisms of potassium released from the minerals is still not clear. The bacteria may be production of bacterial acids, alkalies or chelants to enhance the release of elements form potassium containing minerals. Lin Qi-mei *et al.* [10] pointed out and reported for the same.

Belkanova *et al.* [3] reported that *Bacillus mucillaginosus* did not obtain energy from hydrolysis of silicate bond. Degrading activity relied on slime formation. In our study, the slime forming bacterium *Bacillus mucillaginosus* could grow in the medium without nitrogen (N) and potassium (K). Also the pH of the culture solutions did not change during the incubation (unpublished data) and indicated that acids were not excreted. Groudev [6] reported that production of exopolysaccharides seems to be the main mechanisms of releasing of potassium from silicates although their precise role remains unclear.

Lin Qi-mei *et al.* [10] results showed silicate dissolving bacteria increased 70% in rhizosphere soil and 20% in non-rhizosphere soil respectively . Our results showed that number of potassium solublizing bacteria increased by more than 500 times. The proportion of these bacteria as percentage of the total also increased by 50% in the soil respectively. The bacterium MCRCp1 clearly indicating to develop and colonize in the soil. The ground nut plant treated with MCRCp1increased oil percentage 35%, due to the number of pods yield and kernal size. The results indicated similar to the Lin Qi-mei. Ghosh and Hasan [1] reported that most of the soil is nutrient deficit in India. However, inoculating MCRCp1 significantly improved the nutrient availability of Phosphorus (P) and potassium (K) in the inoculated soil. There was no nutrient deficient zone is close to the root.

Laboratory studies provide evidence to show that microorganisms can substantially enhance the dissolution of large array of rock-forming minerals. However, the actual mechanism by which microorganisms enhance the dissolution of minerals is still unclear.

CONCLUSIONS

The present study clearly indicate there was a small amount of indigenous potassium solubilizing bacteria in soil. The bacterium MCRCp1could colonize and develop in the soil and released the large amount of potassium from different minerals and increased the groundnut crop production.

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