Identification and Characterization of Potassium Solubilizing Bacteria Grown on Old Mica Heaps

D.L.K.L.M. Dassanayake *and* K.B. Wijesekara
Uva Wellassa University, Badulla, Sri Lanka

Introduction

Plants basically supplement nutrient requirements from soil. However, when cultivated in agricultural setup soil nutrient availability could be a limiting factor. In such situations it may be necessary to supplement nutrients additionally with agro fertilizers which incur large expenses to the farmer. Use of agro fertilizers in large amounts could results in environmental problems. Potassium is a key element that affects plant growth and potassium containing fertilizers are relatively expensive when compared to other forms of agro fertilizer. Mica is a rich natural source of potassium and mica processing, mainly for overseas market, produces large amount of decayed mica which is of no economic value. Mica could naturally release potassium to soil upon long term microbial degradation yet the process is low. Release of mica by microbial degradation could be expedited by the use of specific microbes that favor the release of potassium (Sugumaran, and Janarathanam, 2007; Archana, 2007). These microbes are naturally present associated with old and discarded mica heaps from which some virulent strains can be isolated (Basak and Biswas, 2008). These strains have the potential to be used as inoculants with decayed mica which could serve as a bio fertilizer. This study aims at isolation and characterization of potassium leaching bacteria associated with old and discarded mica heaps and to investigate their potassium leaching potential on Biotite and Phlogopite Mica.

Methodology

Mica samples were collected from more than two years old discarded heaps of decayed mica waste from Mathale, Kandy and Kuruwita areas. Samples are identified and separated in to groups as Biotite and Phlogopite and also as fresh and weathered. Samples were then washed thoroughly with 100 ml of sterile distilled water separately for each sample separately. Next, 1 ml of the washed liquid was taken and a dilution series of 10^{-6} was prepared. From the sixth dilution 1 ml was used to inoculate pour plates of pikovskaya medium, Aleksandrov medium and Potatoes Dextrose Agar (PDA) medium. Pure cultures were established from well isolated colonies appeared on the pour plates of the same three culture media mentioned above. Identification of bacterial strains was done by studying the colony morphology and also by Gram staining techniques. Identified strains, BSP1, BSP2 and Cocus, were used to prepare stock cultures from which samples were taken to inoculate Potato dextrose broth (PDB).

Mica samples were grounded and passed through a sieve to obtain 250 nm particles size and were autoclaved at 121 °C and 105 kPa for 30 min. 200 mg of autoclaved mica was introduced into a 250 ml PDB in a 500 ml conical flask. A bacterial cell suspension of 1 ml from overnight grown cultures of strains BSP1, BSP2 and Cocus was introduced separately into each of the conical flask. Conical flask with 250 ml PDB, 200 mg of media and no bacterial cells served as the control. All PDB cultures were incubated at 30 °C for seven days on an orbital shaker rotating at 90 rpm.

Daily samples of 5 ml were taken separately from each of the conical flask and centrifuged at 6500 rpm for 30 minutes to pellet the bacteria cells. The supernatant was used in analysis

by Atomic Absorption Spectrophotometer (AAS, Varian) for leached Potassium content. AAS was calibrated with standard Potassium solutions to obtain the standard curve.

Three experiments were done with all three bacterial strains and each treatment was replicated three times within an experiment. Three readings for Potassium leaching were taken for each of the samples analyzed by ASSA and expressed as average leached potassium for the sample.

Then potassium concentration was analyzed by daily leaching amount and the leached from each strain.

Results and Discussion

Three types of bacterial strains were identified from Mica surface, two bacillus types (BSP1, BSP2) and a Cocus. These three bacterial strains were the predominant types living associated with withered Mica thus concluded as the most responsible for Mica withering and releasing potassium. A previous study by Basak and Biswas (2008) also report that *Bacillus* stains are more effective in releasing potassium from waste Mica.

The amount of the potassium in the control was significantly ($P < 0.05$) less than the treated and the main reason for detecting potassium could be associated with agitation on the orbital shaker. However, the amount of potassium leached by agitation decreased gradually in the controls and also Biotite leached more K than Phlogopite.

Potassium leaching was significantly ($P < 0.05$) higher in all treatments than the controls. BSP2 bacterial strain showed the highest K leaching than the other two strains and the maximum leaching effect was observed after three days from initial inoculation.

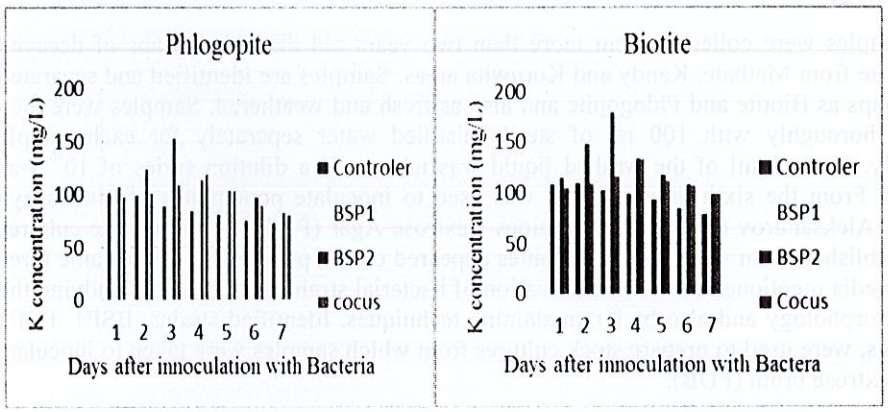

Figure 1a Figure 1b

Figure 1: Concentration of leached potassium from phlogopite Mica (Ia) and Biotite mica (Ib) during seven days incubation period with three bacterial strains BSP1, BSP2 and Cocus. Each data point represents the average of three AAS readings.

Table 1: K concentration (mg/L) of bacterial aided leached potassium for Biotite and Phlogopite mica during 7 days inoculation with three bacterial strains, BSP1, BSP2 and Cocus.

K concentration (mg/L)						
	Biotite			Phlogopite		
Days	BSP1	BSP2	Cocus	BSP1	BSP2	Cocus
1	103.11	114.64	104.53	99.58	107.3	98.19
2	109.44	147.36	108.94	109.05	124.92	101.72
3	123.11	179.47	115.78	117.64	154.08	109.75
4	144.05	133.97	132.64	135.08	114.58	120.14
5	114.44	117.44	111.78	97.8	103.78	103.97
6	107.36	107.78	106.53	93.42	98.33	90.83
7	96.44		98.78	91.22	83.67	82.33

Conclusion

This study identified three bacterial strains BSP1, BSP2 and Cocus directly involved in solubilizing and leaching potassium from Mica. BSP2 bacterial strain showed the highest leaching effect and has the potential of using as inoculums to facilitate the leaching process of fresh Mica. Identification of bacterial strains up to the species level needs further investigations. This study suggests a mixture of ground mica and bacterial strain could constitute a potassium fertilizer and used in both annuals and perennials crops.

References

Sugumaran, P. and Janarathanam, B., 2007. Solubilization of potassium containing minerals by bacteria and there effect on plant growth. *World J. of Agr. Sci.* 3(3), 350-355.

Basak, B.B. and Biswas, D.R., 2008. Influence of potassium solubilizing microorganism (*Bacillus mucilaginosus*) and waste mica on potassium up take dynamics by sudan grass (*Sorghum vulgare* Pers.) grown under two Alfisols. *Plant and Soil.* 317(1-2), 235-255.

Archana, D.S., 2007. Studies on Potassium Solubilizing Bacteria. Department of agricultural microbiology college of agriculture, Harwad University of agricultural sciences, Dharwad - 580 005.