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Solubilization of Insoluble Potassium by Different Microbial Isolates *in vitro* Condition

P.P. Nihala Jabin* **and Syed Ismail**

Department of Soil Science and Agricultural Chemistry, Vasantrao Naik Marathwada Agricultural University, Parbhani 431 402, Maharashtra, India
**Corresponding author*

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A B S T R A C T

The efficacy of different microbial strains for potassium dissolution from insoluble soil mineral (mica) were evaluated in vitro (both plate and broth media assays). Nine microbial strains and 0.1% of chemical source (mica) in three replications were used. Colony and halo diameters were measured after incubating the plates for 72h in incubator. Further, potassium solubilizing ability of microbial strains was studied with mica solution in broth assay. Solubilization potential was assessed both qualitatively and quantitatively under in vitro conditions. The laboratory stock cultures (KSB-W1 (*Bacillus sp*), KSB-PD-3-A, (*Bacillus sp*), KSB-NP-3 (*Bacillus sp*), KSB-PD-1-A (*Pseudomonas sp*), KSB-M-1 (*Pseudomonas sp*), KSB-M-2 (*Pseudomonas sp*), KSB-PD (*Sinorhizobium metallidans*), KSB-PD-1-B (*Sinorhizobium metallidans*), KSB-M-3 (*Sinorhizobium metallidans*)) were obtained from All India Network Project on Soil Biodiversity-Biofertilizers, Parbhani and SKUAST, Kashmir and were selected on the basis of their potash solubilizing ability in laboratory condition. Results indicated that, by plate assay among the microbial strains *Pseudomonas sp* (KSB-PD-1-A) formed the highest colony diameter (1.17 cm) and halozone diameter (3 cm) with mica amended media. *Pseudomonas sp* (KSB-PD-1-A) formed significantly superior and greater solubilization index (3.57) and solubilization efficiency (257.32%) as compared to other microbial strains. In Broth culture assay, Maximum potassium solubilization was observed with the *Pseudomonas sp* (KSB-PD-1-A) in mica amended media compared to control. Maximum reduction in pH was also recorded in *Pseudomonas sp* (KSB-PD-1-A) with mica amended media compared to control.

Introduction

Potassium is involved in number of physiological process, protein synthesis and activation of enzymes. Recent studies showed declining status of K in Indian soils in most of the states from high to medium or medium to low status. It is considered that Indian soils are rich in K and seldom recommended K fertilizers to crops. High crop K removal than K addition by farmers and imbalanced use of NPK fertilizers are contributing to large-scale K mining leading to emergence of K deficiency in soils and crops. Red, lateritic and shallow black soils have under gone K fertility depletion. Deficiency of K is more sever in areas where intensive cropping systems are being followed (Naidu *et al.*, 2011). Nutrient K is less mobile in soils because of the strong affinity with some

exchange sites of clays. Large rates of K uptake can be attributed to its high mobility due to the large permeability of cell membranes to K-ions, which arise from the occurrence of a range of highly K selective, low and high affinity ion channels and transporters. The large K uptake rates achieved by roots result in a steep depletion of solution K in the rhizosphere. It has been well established that a significant proportion of plant needs of K is met from non-exchangeable fraction of soil K (Sreenivasarao *et al.,* 2010).

Exchangeable K and non-exchangeable K can thereby significantly be depleted and contribute a substantial proportion of plant uptake. Microbes can release soluble K from K-bearing minerals such as K-feldspar, mica, illite and from the non-exchangeable pools of soil. Data shows that the use of certain microbes in agricultural soils can assist the solubilization of K in addition to physical and chemical weathering of K minerals (Masood and Bano, 2016). These microbes release organic acid, which quickly dissolves rock and chelate silicon ions, releasing K ions in to the soil (Bennett *et al.,* 1998; Friedrich *et al.,* 2004). Using K-solubilizing microbes to increase the concentration of available K ions in the soil may mitigate K deficiency (Barker *et al.,* 1998).

In the context of unbalanced fertilization and /or lower potash application results in a significant depletion of soil potash reserves, yield loss and higher economic risk for farmers. Microbial inoculants that are able to dissolve potassium from minerals and rocks have influence on plant growth and have both economic and environmental advantage. Thus, keeping this in the view present study was undertaken to assess the solubilization potential of insoluble potassium source by using potassium solubilizing microorganisms in plate as well as in broth assay.

Materials and Methods

Microbial strains and culture conditions

The laboratory stock cultures (KSB-W1 *(Bacillus sp)* KSB-PD-3-A, *(Bacillus sp),*KSB-NP-3 *(Bacillus sp),* KSB-PD-1-A (Pseudomonas *sp),* KSB-M-1 (Pseudomonas *sp),* KSB-M-2 (Pseudomonas *sp),* KSB-*PD (Sinorhizobium metallidans),* KSB-PD-*1-B (Sinorhizobium metallidans),* KSB-*M-3 (Sinorhizobium metallidans)* from All India Network Project on Soil Biodiversity-Biofertilizers, VNMKV Parbhani and SKUAST, Kashmir were selected on the basis of their potash solubilizing ability in laboratory condition. The solubilization potential was evaluated both qualitatively and quantitatively under in-vitro condition.

Plate assay

To assess potassium solubilization ability of the strains, they were subjected on Aleksandrov medium (ingredients g l^{-1}), (Glucose-1%, $MgSO_4.7H_2O$-0.05%, $FeCl_3$-0.0005%, $CaCO_3$-0.01%, $CaPO_4$-0.2%, Potassium aluminum silicate-0.5%, Yeast extract-0.05%, Agar-3%, distilled water -1000 ml, pH 7.0) as given by (Shanware *et al.,* 2014), supplemented with 0.1% insoluble potassium source *i.e.,* mica.

After sterilization and plating freshly grown microbial cultures were spot inoculated in triplicates on the media using sterile tooth picks. The spotted plates were incubated at 28^0C for 3 days and the colonies exhibiting clear zones were selected and diameter of solubilization zone was calculated using following Khandeparkar's selection (Prajapathi and Modi, 2012).

$$SE = \frac{\text{Diameter of solubilization halo zone}}{\text{Diameter of colony}} \times 100$$

The solubilization index was calculated using following formula: (Sadiq *et al.*, 2014)

$$SI = \frac{\text{Colony diameter + halo zone diameter}}{\text{Colony diameter}} \times 100$$

Broth assay

The microbial isolates were inoculated separately to basal medium supplemented with 0.1% insoluble potassium compounds. 150 ml-Erlenmeyer flask containing 100 ml of liquid medium were supplemented with 0.1% mica added separately and were inoculated with one ml aliquot of each microbial culture (10^8CFU ml^{-1}). Aleksandrov medium supplemented with this mica but without microbial inoculation served as an un-inoculated control. Flasks (n=3 for each treatment) were incubated at 28^0C in an orbital shaker at 120 rpm for 10 days.

The samples were withdrawn at 3, 5, 7 and 10 days interval, centrifuged to remove the debris and cells. Subsequently aliquots of the medium were centrifuged at 6100×g for 10 min and filtered through Whatman No.42 filter paper. The culture supernatant was used to estimate available potash on flame photometer. The amount of potassium solubilized was obtained by subtracting the soluble potassium of the inoculated sample from the corresponding un-inoculated control and expressed as mg K ml^{-1} culture.

Determination of pH

The pH of the potassium solubilizing microbial culture filtrates and the un-inoculated samples were determined at 3, 5, 7and 10 days after inoculation. The culture was filtered using Whatman No.42 filter paper. The pH was measured using pH meter. The experiment was carried out in a factorial completely randomized design and the data obtained was subjected to statistical analysis of variance.

Statistical analysis

The results obtained were statistically analyzed and appropriately interpreted as per the method described in "Statistical Methods for Agricultural Workers" by Panse and Sukhatme, (1985). Appropriate Standard Error (S.E.) and critical differences (C.D.) at 1% level were worked out as and when necessary.

Results and Discussion

Potassium solubilization by microbial inoculants in solid medium

In plate assay, all the nine strains of microbial isolates produced a clear solubilization halo on Aleksandrov medium supplemented with insoluble K compound mica (Plate 1). The diameter of colony and solubilization halo produced by different microbial inoculants at 0.1% insoluble K is presented in Table 1. Solubilization of potassium compounds was higher in *KSB-PD-1-A (Pseudomonas sp)* than in other strains. *KSB-PD-1-A (Pseudomonas sp)* obtained the highest potential in mica containing medium, producing a colony diameter 1.17 cm and halozone diameter 3 cm. The solubilization efficiency and solubilization index of each isolate based on colony diameter and halozone is presented in Table 2. Results showed that *KSB-PD-1-A (Pseudomonas sp)* in mica amended media showed maximum solubilization efficiency and solubilization index (S.E. = 257.32 and S.I. = 3.57).

Potassium solubilization by microbial inoculants in liquid medium

The amount of K released from mica in a broth by the isolates was studied at 3, 5, 7 and

10 days after incubation (DAI). On evaluation under in vitro, inoculation of microbial strains produced substantially higher soluble potassium content in liquid broth as compared to uninoculated control (Fig. 1).

The results presented in Fig.1 indicated that the amount of K released by all strains increased with increase in incubation time, and was highest at 10 DAI. The K released from mica ranged from 3.08 ml to 15.18 ml (10 DAI), among the isolates, *Pseudomonas sp* (KSB-PD-1-A) released maximum amount of K from mica (15.18 ml), followed by *Pseudomonas sp* (KSB-M-2).

A significant pH change was observed as compared to uninoculated control as recorded in Fig. 2. The pH ranged from 3.90-5.24 at 10 DAI. Among all isolates, *Pseudomonas sp* (KSB-M-1) showed drop in pH to 4.10 from 5.24 (control) followed by *Pseudomonas sp* (KSB-PD-1-A) which showed pH 3.90 in mica supplemented cultures.

Table.1 Potassium solubilization activity of various microorganisms under insoluble potassium source in solid media (Aleksandrov media- Plate assay)

Treatment	**Colony diameter** (cm)	**Halozone diameter** (cm)
Microbial inoculants		
T_1: *Bacillus sp* (KSB-W1)	0.47	0.87
T_2: *Bacillus sp* (KSB-PD-3-A)	0.83	1.90
T_3: *Bacillus sp* (KSB-NP-3)	0.63	1.20
T_4: *Pseudomonas sp* (KSB-PD-1-A)	1.17	3.00
T_5: *Pseudomonas sp* (KSB-M-1)	1.07	2.70
T_6: *Pseudomonas sp* (KSB-M-2)	0.96	2.37
T_7: *Sinorhizobium metallidans* (KSB-PD)	0.73	1.53
T_8: *Sinorhizobium metallidans* (KSB-1-B)	0.83	1.83
T_9: *Sinorhizobium metallidans* (KSB-M-3)	0.67	1.33
SE±	0.03	0.07
CD at 1%	0.13	0.23

Table.2 Potassium solubilization activity of various microorganisms under insoluble potassium source in solid media

Treatment	**Solubilization Efficiency** (%)	**Solubilization Index**
Microbial inoculants		
T_1: *Bacillus sp* (KSB-W1)	185	2.85
T_2: *Bacillus sp* (KSB-PD-3-A)	228.24	3.28
T_3: *Bacillus sp* (KSB-NP-3)	189.68	2.89
T_4: *Pseudomonas sp* (KSB-PD-1-A)	257.32	3.57
T_5: *Pseudomonas sp* (KSB-M-1)	250.3	3.53
T_6: *Pseudomonas sp* (KSB-M-2)	244.81	3.44
T_7: *Sinorhizobium metallidans* (KSB-PD)	208.93	3.08
T_8: *Sinorhizobium metallidans* (KSB-1-B)	219.90	3.19
T_9: *Sinorhizobium metallidans* (KSB-M-3)	200.79	3.00
SE±	5.47	0.05
CD at 1%	17.01	0.16

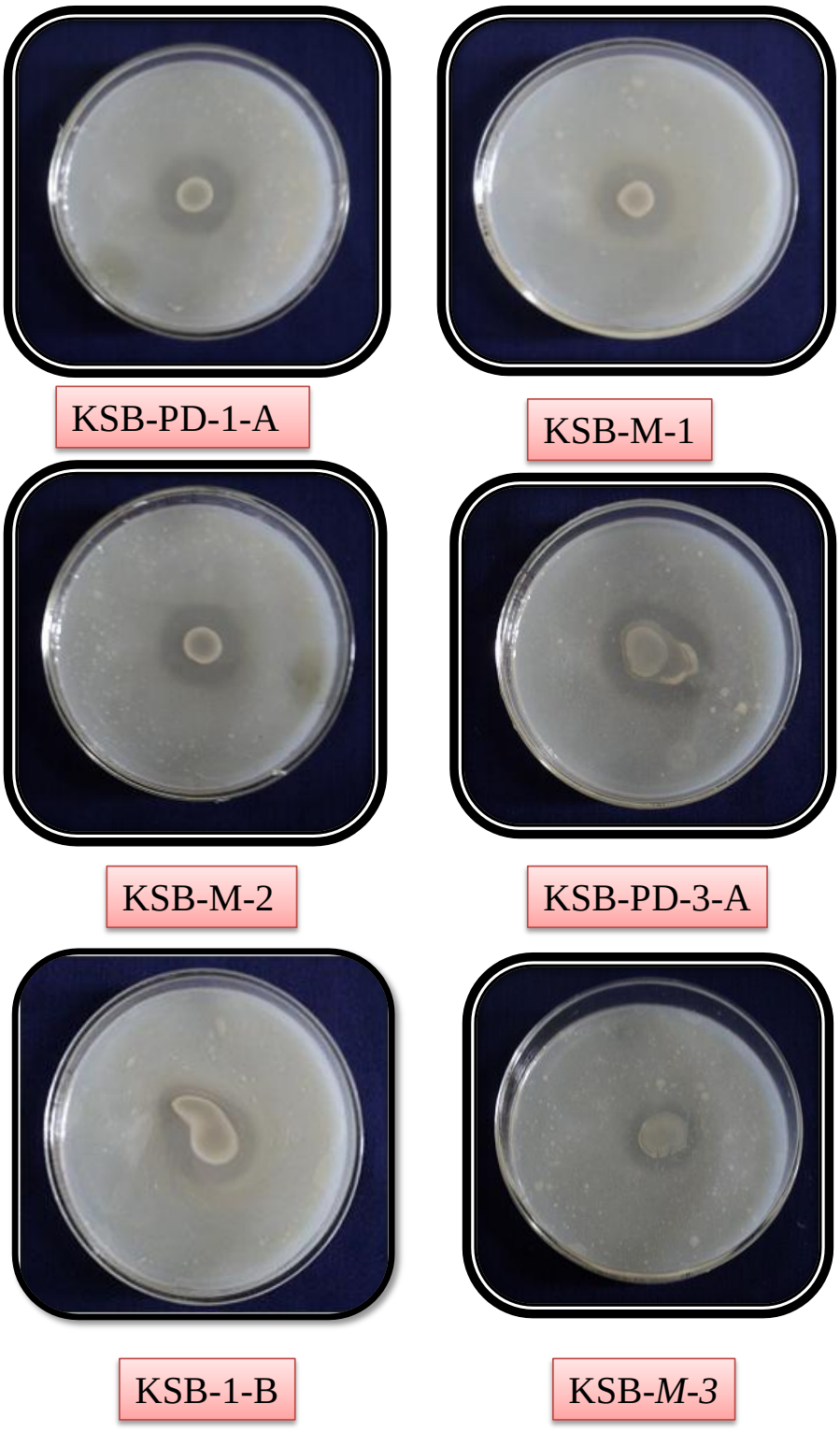


Plate 1 : Solubilization zone formed by different microorganisms on Aleksandrov medium.

Fig.1 Available K in broth containing mica

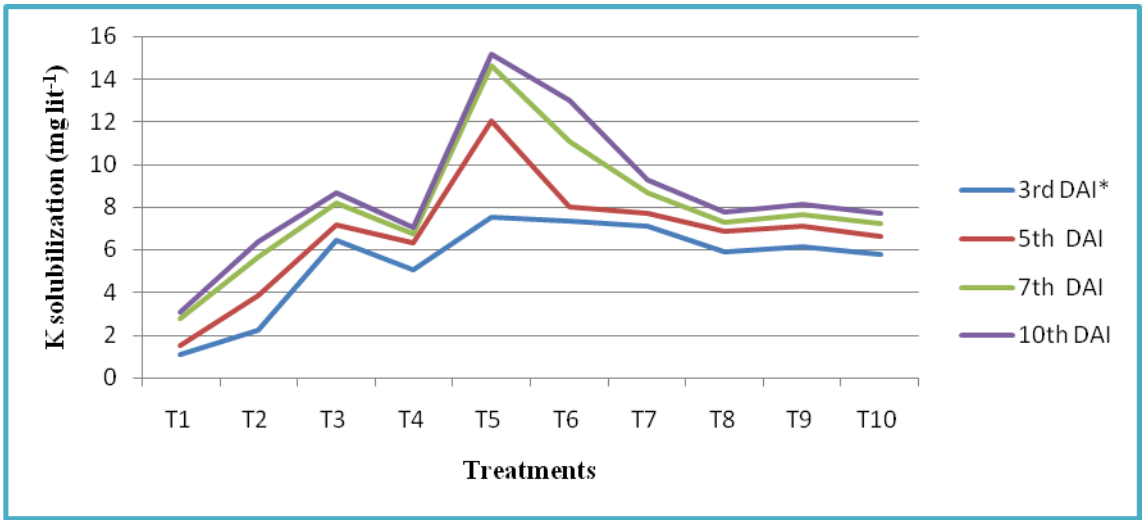
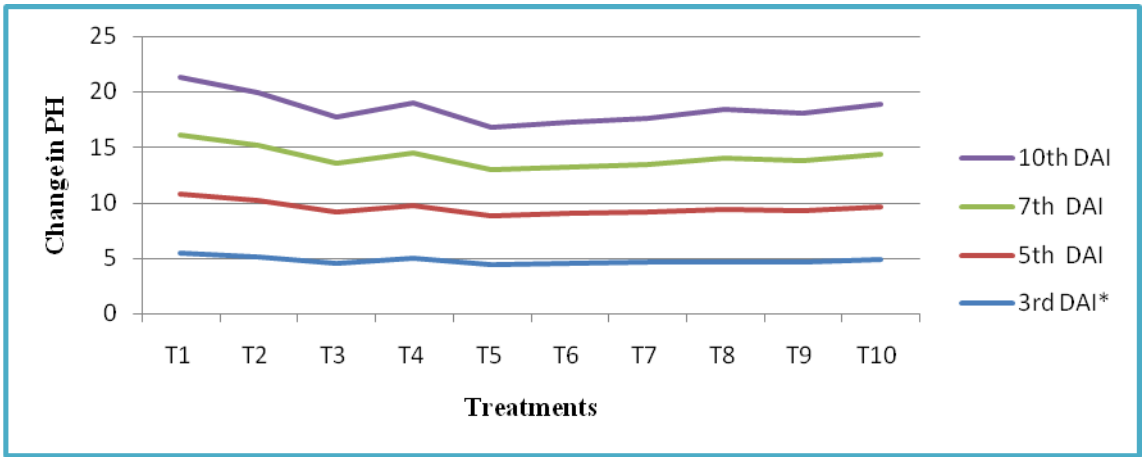


Fig.2 Change in pH in broth containing mica



The differential efficiency of bacteria to solubilize insoluble inorganic potassium could be due to differences in their ability to release organic acids. Our result showed that solubilization of potassium compounds was higher in *KSB-PD-1-A (Pseudomonas sp)* than in other strains. Mursyida *et al.*, (2015) stated that bacteria grown in solid Aleksandrov medium will solubilize K, characterized by clear zone around the colony. Our results are in agreement with the findings of Saiyad *et al.*, (2015) who recorded highest SI in Aleksandrov medium shown by isolate *B. coagulans.*

The amount of K released from mica in an Aleksandrov broth by the different strains increased with increase in the incubation time (Basavesha, 2013). Similar observation was seen in present study also. Findings are also in agreement with the findings of Pachaiyappan (2007) who reported that the isolate *Bacillus mucilaginosus* solubilize all the potassium containing minerals.

Further, Diep and Hieu, (2013) reported that all the bacterial strains used in the study showed significantly more solubilization of potassium than control. From the present

study, it was found that with increasing the inoculation time pH of the liquid culture start decreasing; most possible reason for this will be production of organic acids, as a result of this K content increased. Our results are in agreement with findings of Liu *et al.*, (2006) who reported that the bacterium produces copious amounts of organic acids that can form bidentate complexes with metal ions and which tend to be more effective in enhancing dissolution of minerals.

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