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# *In vitro* Screening of Potassium Solubilizing Potential (Efficiency) of Bacteria

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**The experiment was conducted to isolate KSB from 75 rhizosphere soils of different crop plants around Raichur district. Out of these, 28 isolates were selected as potential Potassium Solubilising Bacteria (KSB) based on zone of solubilisation and further these isolates were tested for morphological and biochemical characterization. Whereas five (KSBL-12, KSBR-28, KSBR-44, KSBD-55 and KSBD-72) isolates were identified as *Pseudomonas* and remaining 23 as *Bacillus.* Among the isolates KSBD-58 released maximum amount of K from muscovite mica (42.95 $\mu$g ml$^{-1}$) which was significantly higher than all other isolates including the reference strain (*Frateuria aurantia)* which released potassium of 38.95 $\mu$g ml$^{-1}$. All the isolates produced one or the other organic acids.**

**Keywords:** KSB, *Bacillus mucilagenosus*, *Frateuria aurantia*, *Bacillus edaphicus.*

Potassium exists in several forms in the soil, including mineral K, non-exchangeable K, and exchangeable K and dissolved or solution K (K+ ions). Plants can only directly take-up solution K. It exists in exchangeable, non changeable and in form of soil minerals. The potassium content of Indian soils varies from less than 0.5 %to 3.00 % (Mengel,1987).In Indian soil the soluble K form are present in approximately 2% and insoluble are present in range of 98% in form of minerals like biotite, feldspar, mica, muscovite, vermiculite (Goldstein,1994).

Potassium Solublizing Bacteria (KSB) would be a novel solution to convert insoluble form of soil potassium into soluble form. Potassium solubilizing bacteria such as *Frateuria aurantia, Bacillus mucilagenosus* and *Bacillus edaphicus* are example of microorganisms that are used as potassium biofertilizer. Potassium solubilizing bacteria are able to solubilize potassium rock through production and secretion of organic acids (Han and Lee, 2005). They can enhance mineral dissolution rate by producing and excreting metabolic by-products that interact with the mineral surface. Mineral potassium solubilization by microbes which 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). Potassium solubilizing bacteria are capable of solubilizing rock K, mineral powder such as mica, illite and orthoclases through production and excretion of organic acids (Friedrich *et al.*, 1991).

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Application of chemical fertilizer have side effects, such as leaching out, polluting water basins, destroying microorganisms and friendly insects, and making the crop more susceptible to the attack of diseases. Thus, biofertilizer is more favourable and encouraging to be used for its eco-friendly nature. Therefore, to increase the quality of biofertilizer at least as good as chemical fertilizer, inoculation of efficient KSB is necessary to increase potassium uptake on plants.

The major objective of the research was to determine the solubilization potential of selected isolates to solubilize insoluble soil minerals such as potassium *in vitro*.

## MATERIALS AND METHODS

In the present investigation 75 rhizosphere soil samples of different crop plants were collected from different locations around Raichur district. Among these 28 isolates were selected based on zone of potassium solubilization.The selected isolates were characterized and tentatively identified upto genus level based on morphological and biochemical properties. The efficient K solubilizers were further subjected for their ability to release K from potassium mineral.

**Screening of isolates for mineral potassium solubilization**

**Quantitative estimation of K released from insoluble K bearing mineral**

The isolates showing zone of solubilization on Aleksandrov agar were further examined for their ability to release K from broth medium (supplemented with 1 % muscovite mica). One ml of overnight culture of each isolate was inoculated to 25 ml of Aleksandrov broth (Hu *et al.*, 2006) in nine replications. The inoculated flasks were incubated for two weeks at 28±2 °C. The amount of K released in the broth was estimated at 7, 15, and 20 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 Remi micro centrifuge to separate the supernatant from the cell growth and insoluble potassium. The available K content in the supernatant was determined by flame photometry (Sugumaran and Janarthanam, 2007). One ml of the culture supernatant was taken in a 50 ml volumetric flask and the volume was made to 50 ml with the distilled water and mixed thoroughly. After that the solution was fed to flame photometer and compared using standards. Simultaneously, a standard curve was prepared using various concentrations from 2 ppm KCl solution. The amount of potassium solubilized by the isolates was calculated from the standard curve.

**Preparation of standard curve**

Potassium chloride was dried at 60 °C and 1.908 g of it was dissolved in distilled water and the volume made up to one litre. Ten ml of this was diluted further to 100 ml with distilled water to obtain two ppm solution and was used for preparation of standard curve.

**Organic acid production by K-solubilizing isolates**

One ml of 24 h old culture of each isolate was inoculated to 25 ml of Aleksandrov broth and incubated at 28±2 °C for 10 days. The broth culture was centrifuged at 10,000 rpm for 10 minutes. The supernatant so obtained was concentrated to nearly 1/10th of the original volume in a water bath at 60 °C. The concentrated material was then used for determination of organic acid by paper chromatography in comparison with standard organic acids (Gaur, 1990).

Pure organic acids were prepared at 20 g $ml^{-1}$ stock. About 10 µl of these standard acids and 15 µl of culture supernatants were spotted on Whatman No. 1 chromatographic paper and dried with a hair dryer. A descending chromatography was run using a solvent mixture of n-butanol, acetic acid and water in 12:3:5 ratios in a chromatographic chamber pre saturated with solvent for six hours. The chromatogram was run for six hours and air dried for three days. The air dried paper was sprayed with 0.04 per cent bromocresol green (40 g BCG in 1000 ml methanol with pH 7.0).

The paper was air dried at room temperature. The Rf (retardation factor) values and the intensity of yellow spots of organic acids developed on a blue background were measured and compared with the Rf values of the standard organic acids for identification.

$$\text{Rf value} = \frac{\text{Distance from base line travelled by solute}}{\text{Distance from base line travelled by solvent}}$$

## RESULTS AND DISCUSSION

### Quantitative estimation of K solubilizing ability of the isolates

The amount of K released from potassium mineral (muscovite mica) in broth by the isolates was studied at 7, 15, 20 days after incubation (DAI). The results (Table 1) indicated that the amount of K released from mineral K by all isolates increased with increase in incubation time and was maximum at 20 DAI. The K released from muscovite mica by the different isolates on 20 DAI ranged from 0.16 µg $ml^{-1}$to 42.95 µg $ml^{-1}$. Among the isolates KSBD-58 released maximum amount of K from muscovite mica (42.95 µg $ml^{-1}$) which was significantly superior over all other isolates including the reference strain

**Table 1.** *In vitro* K releasing potential of local isolates from potassium mineral

| S. No. | Isolate | K release (µg $ml^{-1}$) 7 DAI | 15 DAI | 20 DAI |
|---|---|---|---|---|
| 1 | Control | $0.05^{o}$ | $0.06^{r}$ | $0.06^{r}$ |
| 2 | KSBL-2 | $2.49^{k}$ | $5.76^{l}$ | $6.05^{o}$ |
| 3 | KSBL-5 | $10.09^{d}$ | $26.45^{d}$ | $31.65^{d}$ |
| 4 | KSBL – 8 | $2.44^{k}$ | $2.58^{p}$ | $6.71^{m}$ |
| 5 | KSBL – 9 | $5.88^{f}$ | $19.54^{f}$ | $29.62^{f}$ |
| 6 | KSBL – 12 | $3.58^{i}$ | $5.83^{l}$ | $6.80^{m}$ |
| 7 | KSBL – 13 | $3.27^{ij}$ | $8.14^{k}$ | $9.30^{k}$ |
| 8 | KSBL – 18 | $2.86^{j}$ | $4.70^{m}$ | $7.48^{mn}$ |
| 9 | KSBL – 23 | $1.92^{l}$ | $3.58^{n}$ | $8.21^{l}$ |
| 10 | KSBL – 25 | $2.87^{j}$ | $7.88^{k}$ | $9.11^{l}$ |
| 11 | KSBR – 28 | $0.16^{o}$ | $0.24^{qr}$ | $0.16^{r}$ |
| 12 | KSBR – 30 | $3.14^{j}$ | $7.98^{k}$ | $9.77^{k}$ |
| 13 | KSBR – 31 | $7.14^{e}$ | $17.62^{g}$ | $24.18^{g}$ |
| 14 | KSBR – 32 | $9.61^{d}$ | $23.42^{e}$ | $30.85^{e}$ |
| 15 | KSBR – 35 | $2.64^{j}$ | $7.78^{k}$ | $8.27^{l}$ |
| 16 | KSBR – 38 | $3.13^{j}$ | $9.44^{ij}$ | $10.64^{j}$ |
| 17 | KSBR – 41 | $22.16^{b}$ | $34.76^{b}$ | $38.73^{b}$ |
| 18 | KSBR – 42 | $4.60^{gh}$ | $9.11^{i}$ | $10.66^{j}$ |
| 19 | KSBR – 44 | $0.92^{mn}$ | $0.85^{q}$ | $2.64^{q}$ |
| 20 | KSBR – 46 | $4.88^{g}$ | $9.87^{hj}$ | $11.80^{i}$ |
| 21 | KSBR – 50 | $0.92^{mn}$ | $2.81^{o}$ | $2.99^{q}$ |
| 22 | KSBD – 51 | $2.86^{j}$ | $8.01^{k}$ | $11.26^{i}$ |
| 23 | KSBD – 55 | $3.13^{j}$ | $7.78^{k}$ | $9.29^{k}$ |
| 24 | KSBD – 58 | $23.12^{a}$ | $38.78^{a}$ | $42.95^{a}$ |
| 25 | KSBD - 63 | $1.18^{m}$ | $4.65^{m}$ | $6.64^{o}$ |
| 26 | KSBD - 64 | $4.15^{h}$ | $10.46^{h}$ | $13.36^{h}$ |
| 27 | KSBD - 67 | $11.36^{c}$ | $27.82^{c}$ | $33.48^{c}$ |
| 28 | KSBD - 72 | $0.72^{n}$ | $2.68^{p}$ | $5.59^{p}$ |
| 29 | KSBD - 74 | $4.88^{g}$ | $10.14^{hj}$ | $23.65^{g}$ |
| 30 | Reference strain | $22.25^{b}$ | $35.10^{b}$ | $38.95^{b}$ |
| | S.Em± | 0.14 | 0.21 | 0.21 |
| | CD at 1% | 0.52 | 0.77 | 0.79 |

DAI: Days After Incubation

(*Frateuriaaurantia)* which released potassium of 38.95 µg $ml^{-1}$. Out of 28 isolates examined, eight isolates showed more than 20 µg $ml^{-1}$ of potassium solubilizing ability. The results obtained in this study are in agreement with the findings of Parmar and Sindhu (2013) who reported that rhizosphere bacteriawere capable of solubilizing mica in appreciable amounts. Similar studies conducted by Hu *et al*. (2006) reported that *Bacillus megatherium*and *B. mucilaginosus*were capable of solubilizingboth rock phosphate and potassium. They also reported that co-inoculation of these two *Bacillus* spp were potential in solubilizing potassium rocks. Thus the present study indicates that the *Bacillus* spp. were effective in solubilizing the potassium compared to *Pseudomonas* spp. (9.09µg $ml^{-1}$broth). Similar results were obtained by Archana (2007) wherein she observed more K released (44.49 µg $ml^{-1}$) by *Bacillus* spp. compared to *Pseudomonas* spp. (10.72 µg $ml^{-1}$) in liquid broth. The variation in K release among same genera may be attributed to species variation.

**Organic acid production potential of the local isolates**

The organic acid profile of the isolates of potassium solubilizers was analysed by paper chromatography and the results are presented in Table 2. All the isolates were found to produce one or the other organic acid tested for. It was found that the citric acid and oxalic acid were the most common organic acids produced by all the 28 isolates including the reference strain (*Frateuriaaurantia)*. In addition to citric acid and oxalic acid production, the potassium solubilizing

**Table 2.** *In vitro* organic acid production potential by local isolates of potassium solubilizing bacteria

| S. No. | Isolate | Oxalic acid | Citric acid | Malic acid | Tartaric acid |
|---|---|---|---|---|---|
| 1 | KSBL - 2 | + | + | - | - |
| 2 | KSBL - 5 | + | + | - | + |
| 3 | KSBL - 8 | + | + | + | - |
| 4 | KSBL - 9 | + | + | - | - |
| 5 | KSBL - 12 | + | + | - | - |
| 6 | KSBL - 13 | + | + | - | - |
| 7 | KSBL - 18 | + | + | + | - |
| 8 | KSBL - 23 | + | + | - | - |
| 9 | KSBL - 25 | + | + | - | - |
| 10 | KSBR - 28 | + | + | - | - |
| 11 | KSBR - 30 | + | + | - | - |
| 12 | KSBR - 31 | + | + | - | - |
| 13 | KSBR - 32 | + | + | - | + |
| 14 | KSBR - 35 | + | + | - | - |
| 15 | KSBR - 38 | + | + | - | - |
| 16 | KSBR - 41 | + | + | - | - |
| 17 | KSBR - 42 | + | + | - | - |
| 18 | KSBR - 44 | + | + | - | - |
| 19 | KSBR - 46 | + | + | + | - |
| 20 | KSBR - 50 | + | + | - | - |
| 21 | KSBD - 51 | + | + | - | + |
| 22 | KSBD - 55 | + | + | - | - |
| 23 | KSBD – 58 | + | + | + | - |
| 24 | KSBD – 63 | + | + | - | - |
| 25 | KSBD – 64 | + | + | - | + |
| 26 | KSBD – 67 | + | + | - | - |
| 27 | KSBD – 72 | + | + | + | - |
| 28 | KSBD – 74 | + | + | - | - |
| 29 | Reference strain | + | | - | + |

isolates *viz.*, KSBL-8, KSBL-18, KSBR-46, KSBD-58 and KSBD-72 also produced malic acid, whereas KSBL-5, KSBR-32, KSBD-51, KSBD-64 and the reference strain were shown to produce tartaric acid.The production of organic acids like oxalic acid, tartaric acid, citric acid, acetate by potassium solubilizing bacteria have been reported earlier by various workers (Girgis *et al*., 2008; Sheng and He, 2006; Liu *et al*., 2006)

## REFERENCES


1. Archana, D. S., Studies on Potassium solubilising bacteria. *M. Sc. (Agri.) Thesis,Univ. Agric. Sci.*, Dharwad, India, 2007; p. 71.
2. Friedrich, S. N. P., Platonova, G. I., Karavaiko, E., Stichel and Glombitza, F., Chemical and microbiological solubilization of silicates. *Acta. Biotech*., 1991; **11**: 187-196.
3. Gaur, A. C., Phosphate solubilizing microorganisms as biofertilizers. Omega Scientific Publishers, New Delhi, 1990; p. 176.
4. Girgis, M. G. Z., Khalil, H. M. A. and Sharaf, M. S., *In Vitro* evaluation of rock phosphate and potassium solubilizing potential of some *Bacillus* Strains. *Australian J. Basic Appl. Sci.*, 2008; **2**(1): 68-81.
5. Goldstein, A.H., Involvement of the quino protein glucose dehydrogenase in the solubilization of exogeneous mineral phosphates by gram negative bacteria. In phosphate in micro-organisms: *Cell. Mol. Biol*., Eds., 1994; pp.197-203.
6. Han, H. S. and Lee, K. D., Phosphate and potassium solubilizing bacteria effect on mineral uptake, soil availability and growth of eggplant. *Res. J. Agric. Biol. Sci.*, 2005; **1**(2): 176-180.
7. Hu, X. F., Chen, J. and Guo, J. F., Two phosphate and potassium solubilizing bacteria isolated from Tiannu Mountain, Zhejiang, China. *World J. Micro. Biotech.*, 2006; **22**: 983-990.
8. Liu, W., Xu, X., Wu, S., Yang, Q., Luo, Y. and Christie, P., Decomposition of silicate minerals by *Bacillus mucilaginosus* in liquid culture. *Environ. Geochem. Health*, 2006; **28**: 133-140.
9. Mengel, K. and Kirkby, E. A., Principles of Plant Nutrition, International Potash inst. Bern, Switzerland; 1987; pp. 200-210
10. Parmar, P. and Sindhu, S. S., Potassium solubilization by rhizosphere bacteria: Influence of nutritional and environmental conditions. *J. Micro. Res*., 2013; **3**(1): 25-31.
11. Rajan, S. S. S., Watkinson, J. H., Sinclair, A. G., Phosphate rock for direct application to soils. *Adv. Agron.*, 1996; **57**: 77-159.
12. Sheng, X. F. and He, L. Y., Solubilization of potassium bearing minerals by a wild type strain of *Bacillus edaphicus* and its mutants and increased potassium uptake by wheat. *Canadian J. Microbiol.*, 2006; **52**(1): 66-72.
13. Sugumaran, P. and Janarthanam B., Solubilization of potassium containing minerals by bacteria and their effect on plant growth, *World J. Agric. Sci.*, 2007; **3**(3) 350-355.