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Myco-potash solubilizers

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This study was carried out to evaluate the efficacy of agriculturally beneficial fungi for potash solubilization and to develop myco-potash cultures for use in crop growth. In all six fungal cultures were utilized in the study, viz. *Paecilomyces lilacinus*, *Tricoderma harzianum*, *Aspergillus wentii*, *Emericella nidulans*, *Verticillium lecanii* and *Tricoderma viride*. Among them, *A. wentii* and *T. viride* were found to produce 3.3 and 3.65 mm solubilization index around the colony after 7 days of incubation (DAI) on Aleksandrov medium supplemented with mica as potash source. Whereas for agar medium supplemented with feldspar, maximum solubilization index was 2.5 mm (*A. wentii*), 2.55 mm (*T. viride*), 2.48 mm (*V. lecanii*) and 2.58 mm (*P. lilacinus*) 7 DAI. To reveal the mechanism of potash solubilization, *A. wentii*, *T. viride*, *T. harzianum* and *V. lecanii* were chosen for organic acid profiling using HPCL. *A. wentii* produced the highest amount of total organic acid (1847.775 µg/ml).

Keywords: Fungal cultures, myco-potash, organic acids, solubilization index.

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POTASSIUM (K) is one of 17 nutrients that are essential for plant growth. With the rapid development of agriculture in the world, available soil K levels have dropped due to crop removal, leaching, run-off and erosion. It thus becomes necessary to study the bio-activation of soil K reserves so as to alleviate the potash fertilizer shortage. Total soil K reserves are generally large, although the distribution of K forms differs from soil to soil as a function of the dominant soil minerals present[1]. Though potassium is present as an abundant element in the soil or is applied to the fields as natural or synthetic fertilizers only 1–2% of this is available to plants, the rest making complex with other minerals. The most common soil components of potassium, 90–98%, are feldspar and mica. For optimal nutrition of a crop, replenishment of a K-depleted soil solution is necessary which in turn is affected predominantly by the release of exchangeable K from clay minerals. Consequently, for maximal crop growth, soil solution and exchangeable K need to be replenished continually with K through release by weathering of K reserves (i.e. mica and feldspars)[2], or addition of K fertilizers. Many microorganisms in the soil are able to solubilize 'unavailable' forms of K-bearing minerals such as mica, illite and orthoclasses, by excreting organic acids which either directly dissolve rock K or chelate silicon ions to bring K into solution[3–6]. Therefore, application of K-solubilizing microorganisms (KSM)[7–9] is a promising approach for increasing K availability in KSM amended soils. Production of carboxylic acids like citric, tartaric and oxalic acids was associated with potash solubilization by microorganisms. A wide range of bacteria namely *Pseudomonas*, *Burkholderia*, *Enterobacter*, *Acidothiobacillus ferrooxidans*, *Bacillus mucilaginosus* and *Paenibacillus* sp. *Frateuria*, *Citrobacter*, etc. and some fungi strains are known such as *Aspergillus terreus* (KSF-1), *T. viride*, etc. has been reported to release potassium in accessible form from potassium-bearing minerals in soil[10–13]. Agriculturally beneficial fungi play a major role as biodegrader and biocontrol agent, but meagre efforts have been made till date to exploit fungi as biofertilizers.

Keeping in mind the above mentioned facts and needs, in the present study we have evaluated the potash-solubilizing efficiency and possible mechanism of potash solubilization of known biocontol fungi, viz. *T. harzianum*, *T. viride*, *P. lilacinus* and *V. lecanii* having phosphate solubilization capacity[14] and biodegrader fungi, viz. *A. wentii* and *E. nidulan*[15,16] to develop myco-potash cultures. The aim of the present study was to put a stepping stone to explore role of biocontrol and biodegrader fungi as biofertilizers.

Fungal strains, viz. *T. harzianum*, *T. viride*, *P. lilacinus* and *V. lecanii* as well as biodegrader fungi, viz. *A. wentii* and *E. nidulan*[15,16] were collected from culture depository of the Department of Agricultural Microbiology, B. A. College of Agriculture, Anand Agricultural Universty, Anand, Gujarat, India.

Individual fungal cultures were spot inoculated on Aleksandrov agar[17] supplemented with natural substrate of potash, viz. mica or feldspar @ 2 g/l. Plates were incubated at $28° \pm 2°C$ for seven days. Zone of clearance was checked around the fungal colonies at 48 h intervals. The zone of solubilization was measured and solubilization index was calculated, according to the ratio of total diameter (colony diameter + halo zone diameter) to the colony diameter.

Solubilization index (SI)

$$= \frac{\text{Colony diameter (mm)} + \text{halo zone diameter (mm)}}{\text{Colony diameter (mm)}}.$$

Erlenmeyer flasks (250 ml) containing 150 ml Aleksandrov medium supplemented with feldspar or mica (i.e. six flasks for each) were autoclaved at 121°C for 20 min. Sterilized media was inoculated with the respective fungal culture (10^8 spores/ml). For each test fungal culture, three flask were inoculated. The uninoculated flasks were treated as 'control'. Further the test samples were checked for potash release using flame photometry. One millilitre of sample was diluted with distilled water (1 : 9), mixed thoroughly and subjected to flame photometry for estimating and evaluating K content in the sample[18]. The amount of the free potash released by the respective test fungi culture was recorded.

Organic acid qualitative and quantitative analysis[19] was performed using high performance liquid chromatography (HPLC) technique[10].

Potash solubilization efficiency of the fungal cultures was tested on Alekzendrov medium containing mica and feldspar as the source of mineral potash. The standard SI and zone size for potash solubilization is to be minimum 5 mm as per Fertilizer Control Order, Government of India (2014). The highest SI in Aleksandrov medium supplemented with mica was recorded for test culture *T. viride* (3.65). This was then followed by test cultures *A. wentii* (3.3) and *E. nidulans* (2.25) (Table 1). The test cultures *V. lecanii*, *T. harzianum* and *P. lilacinus* were not able to form any zone on the respective plates. As a result, they were able to solubilize the mineral potash, i.e. mica.

SI of the test culture on feldspar ranged from 2.45 to 2.58. The highest SI in Aleksandrov medium supplemented with feldspar was recorded for test culture *P. lilacinus* (2.58) followed by *T. viride* (2.55), *A. wentii* (2.5), *E. nidulans* (2.45) (Table 1). The test cultures *E. nidulans* and *T. harzianum* were not able to form any zone around the growth on the respective plates.

Studies on agar plates revealed that the clear zones formed by potash solubilizing microorganisms are due to the production of organic acids in the surrounding medium. However, it has previously been reported that most of the potash-solubilizing micro-organisms lose their

Table 1. Solubilization index of fungal strains on Aleksandrov agar plate supplemented with mica

Organism	Aleksandrov medium with mica			Aleksandrov medium with feldspar		
	Colony diameter (mm)	Halo zone (mm)	SI	Colony diameter (mm)	Halo zone (mm)	SI
Aspergillus wentii	1	2.3	3.3	2	3	2.5
Tricoderma viride	2.2	3.2	3.65	2	3.1	2.55
Emericella nidulans	1.2	1.5	2.25	1.2	1.5	2.45
Verticillium lecanii	1.9	0	0	1.6	0	0
Tricoderma harzianum	1.7	0	0	1.9	0	0
Paecilomyces lilacinus	1.4	0	0	1.2	1.9	2.58

ability to form halo zone on the medium due to repeated sub-culturing. Generally, potash-solubilizing microorganisms are routinely screened based on SI. However, the reliability of this halo-based technique is being questioned as many isolates which do not produce any visible halo zone on agar plates could still solubilize various types of insoluble inorganic potash in liquid medium. A wide range of bacteria, namely *Pseudomonas*, *Burkholderia*, *Enterobacter*, *Acidothiobacillus ferrooxidans*, *Bacillus mucilaginosus*, *Paenibacillus* sp., *Frateuria*, *Citrobacter*, etc. and fungi strains such as *A. terreus* (KSF-1), *T. viride*, etc. have been reported to release potassium in accessible form from potassium-bearing minerals in soil[11,12,20,21].

After observing the positive SI of potential potash-solubilizing fungi on Aleksandrov agar plates supplemented with mica and feldspar respectively, the test cultures were further analysed in Aleksandrov liquid medium for K release from minerals feldspar and mica from 2 to 10 days of incubation (DAI).

In Aleksandrov medium supplemented with feldspar (Table 2), fungal strain *A. wentii* gave highest K release (3.7 µg ml⁻¹) at 10 DAI, which was found to be increasing from the second to the tenth day. It was considered as the best K solubilizer. Next was *T. viride*, which showed 2.9 µg ml⁻¹ of K release at 10 DAI, viz. *V. lecanii* showed 1.8 µg ml⁻¹ of K release at 10 DAI and *P. lilacinus* showed equal rate of solubilization, i.e. 1.1 µg ml⁻¹. Least K release was exhibited by *T. harzianum* which gave 0.75 µg ml⁻¹ at 10 DAI and solubilization was actually observed at 8 DAI.

Similarly, for Aleksandrov medium supplemented with mica, the test cultures were further analysed for K release with broth in the presence of mica from 2 to 10 DAI. Fungal strain *A. wentii* gave highest K release (2.5 µg ml⁻¹) at 10 DAI, which was found to be increasing from the second to tenth day. It was considered as the best K solubilizer. Next was *T. viride*, which showed 2.9 µg ml⁻¹ of K release at 10 DAI, *V. lecanii* and *E. nidulans* showed equal (0.75 µg ml⁻¹) K release at 10 DAI. *P. lilacinus* and *T. harzianum* showed, i.e. 1.4 and 1.1 µg ml⁻¹ respectively. Least K release was exhibited by *T. harzianum* (0.75 µg ml⁻¹) at 10 DAI and solubilization was actually observed at 8 DAI.

Some fungal strains are able to solubilize rock potassium and potassium aluminum silicate. Isolates of *Aspergillus* were selected from ceramic industry soils which showed high solubilization of potassium aluminum silicate. *A. terreus* showed more solubilization when grown in the presence of 1% rock potassium (feldspar) than *A. niger*[13]. Similarly, K release from muscovite mica by all isolates increased with increase in incubation time; it was maximum at 20 DAI ranging from 2.41 to 44.49 µg/ml. KSB11 released maximum amount of K from mica followed by KSB42, which gave 37.07 µg/ml. Out of the 30 isolates, 9 showed more than 20 µg/ml solubilization. Similarly, isolate MCRCp1 could solubilize a significant amount of K from muscovite mica (4.29 mg l⁻¹) than other potassium containing minerals like microcline (1.26 mg l⁻¹) and orthoclase (0.85 mg l⁻¹)[1,22].

Results of the experiment to the evaluate role of yeast *Torulaspora globosa*, isolated from the sugar cane rhizosphere, in the solubilization of potassium from alkaline ultramafic rock powder showed that as much as 38% of the total potassium in the rocks was released in the medium with yeast during a 15-day period of incubation. Acid production may be the mechanism by which yeast solubilizes potassium, because the total acidity increased during the sampling period[23].

Very few reports are available on optimization of potassium solubilizatiion by heterotrophic microorganisms. The results obtained from a study regarding[24], the optimization of feldspar solubilzation, 25 isolates were obtained from the rhizospheric soil of seasonal plants and ceramic industrial soil. Among 25 isolates, *Aspergillus* SDS7 was studied for optimum conditions for maximum solubilization of K. The amount of K released by the isolates ranged from 4.5 to 52.16 ppm. It was seen that pH 6.0 was the best for K solubilization and citric acid production, 25.16 ppm and 45.55 ppm respectively.

Organic matter after decomposition produces acids like citiric acid, formic acid, malic acid, oxalic acid. These organic acids enhance the dissolution of potassium compounds by supplying protons and by complexing Ca^{2+} ions. Previous work has shown organic compounds produced by microorganisms such as acetate, citrate and oxalate can increase mineral dissolution in soil[25]. Solubilization of potassium occurs by complex formation

Table 2. K release from feldspar and mica (potash mineral) in Aleksandrov broth

Organism	Aleksandrov medium with feldspar (µg/ml)					Aleksandrov medium with mica (µg/ml)				
	2 DAI	4 DAI	6 DAI	8 DAI	10 DAI	2 DAI	4 DAI	6 DAI	8 DAI	10 DAI
A. wentii	1.1	0.8	1.5	2.2	3.7	0.4	0.8	1.5	2.2	2.5
T. viride	0.75	1.4	1.8	2.2	2.9	0.75	1.1	1.1	1.4	1.8
V. lecanii	0.4	0.4	0.75	1.1	1.8	0	0	0.4	0.4	0.75
E. nidulans	0	0.4	0.75	0.75	1.1	0	0.4	0	0.4	0.75
T. harzianum	0	0	0	0.4	0.75	0	0	0.4	0.4	1.1
P. lilacinus	0.4	0.4	0.75	1.1	1.1	0.4	0.4	1.1	1.1	1.4

DAI, Days after incubation.

Table 3. Organic acid profile of promising potassium solubilizing test fungal cultures by HPLC at 7 days after inoculation

Organic acid	Organic acid production by fungal strains (µg/ml)			
	M 5 (T. harzianum)	M 7 (V. lecani)	M 9 (T. viride)	M 11 (A. wentii)
Oxalic acid	136.45	131.20	88.30	104.75
Gluconic acid	0.0	0.0	0.0	0.0
Tartaric acid	0.0	0.0	0.0	0.0
Pyruvic acid	0.0	0.0	0.0	0.0
Malic acid	0.0	0.0	0.0	0.0
Malonic acid	0.0	0.0	0.0	0.0
Lactic acid	275.10	0.0	68.46	1620.45
Acetic acid	68.58	66.15	0.0	122.58
Citric acid	0.0	0.0	0.0	0.0
2-Keto gluconic acid	0.0	0.0	0.0	0.0
Propionic acid	0.0	0.0	0.0	0.0
Butaric acid	0.0	0.0	0.0	0.0
Succinic acid	0.0	0.0	0.0	0.0
Total	480.1301	197.3548	156.76	1847.775
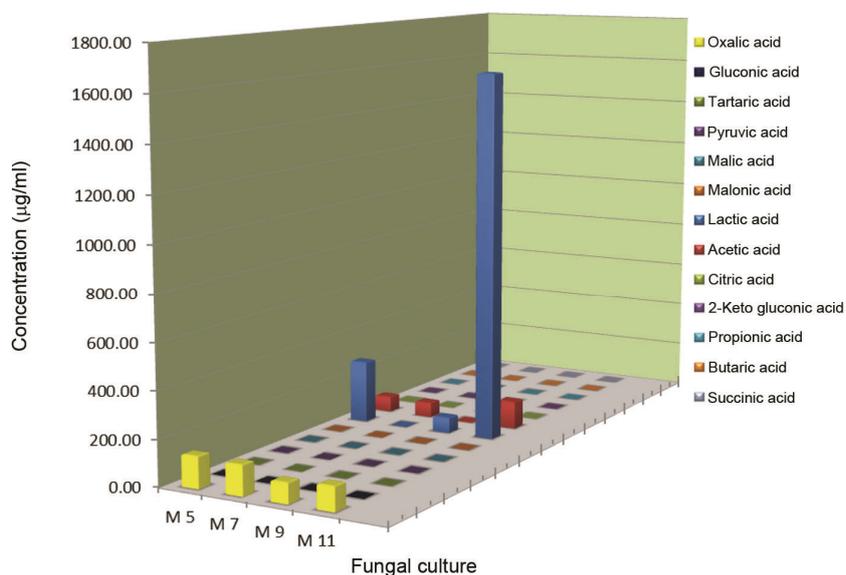
Figure 1. Organic acid production profile of selected test fungal cultures.

between organic acids and metal ions such as Fe^{2+}, Al^{3+} and Ca^{2+} (ref. 26).

HPLC analysis of culture filtrate was carried out to identify and quantify the organic acids produced during solubilization of mineral potash by *A. wentii*, *T. viride*, *T. harzianum* and *V. lecanii* at 7 DAI. During feldspar solubilization, it was found that the major organic acids produced by all the four fungi are lactic acid, acetic acid

and oxalic acid. It was observed that *A. wentii* produced maximum amount of lactic acid (1620.45 μg/ml) followed by acetic acid (122.58 μg/ml) and oxalic acid (104.75 μg/ml) at 7 DAI (Table 3). Test fungi *T. viride* showed organic acid production with major share of oxalic acid (88.30 μg/ml) and lactic acid (68.46 μg/ml). Table 3 shows organic acids which are produced in lower and higher concentration.

Total organic acid production was recorded as 1847.775 and 480.1301 μg/ml by *A. wentii* and *T. harzianum* respectively; *T. viride* and *V. lecanii* showed comparatively less overall organic acids production (197.3548 and 156.76 μg/ml respectively). Overall results show that lactic acid is the major organic acid produced by both the isolates followed by acetic acid and oxalic acid (Figure 1).

It has earlier been reported that potassium soluiblizing bacteria *B. mucilaginosus* are able to solubilize rock K mineral powder such as mica, illite and orthoclase through production and excretion of organic acids[5]. The weathering ability of bacteria involves production of protons, organic acids, siderophores and organic ligands. This was observed in *Cladosporoides*, *Cladosporium* and *Pencillium* sp. These fungal species isolated have the capacity to produce large amounts of oxalic, citric and gluconic acids in broth culture that leads to deterioration of clay silicates, mica and feldspar, and filamentous fungi can cause extensive weathering of stone due to organic acid excretion[27].

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