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ISOLATION, CHARACTERIZATION AND IDENTIFICATION OF POTASSIUM SOLUBILIZING FUNGI FROM RHIZOSPHERE SOIL IN BANGALORE

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ABSTRACT

In this research, an attempt was made to find fungi that can solubilize the insoluble potassium from rhizosphere soil collected from Bangalore. Soil samples were collected and mixed with insoluble potassium (Feldspar) and incubated for 1 week at room temperature. After adaptation 1 gm of soil was inoculated in 100 ml liquid medium containing 1% glucose, 0.05%yeast extract and 0.5% feldspar and incubated at 37^0 C on 120 rpm for 1 week. Enriched samples were inoculated after serial dilution up to 10^{-6} on Aleksandrov agar. Colonies exhibiting clear zone of potassium solubilization were selected as potassium solubilizers from the 10^{-4}, 10^{-5} and 10^{-6} dilutions containing plates. Secondary screening was carried out on the basis of study of zone of activity of the different isolates by using Khandeparkar's selection ratio. Optimization experiment showed that solubilisation activity was more in glucose supplemented medium for (carbon source), urea (for nitrogen source), potassium chloride (for potassium source) and at the temperature of 30°C. One fungal isolate (*Aspergillus terreus*) showed good solubilizing activity.

Keywords: Isolation, Identification, Potassium, Solubilizing, Fungi

INTRODUCTION

Land (soil) remains a limited resource which cannot be expanded. Overtime, farmers have continued to cultivate this limited resource thereby depleting and exhausting it of the minerals. This has resulted in increasing demand for chemical fertilizers for cultivation of crops in developing countries. These chemical

fertilizers have in turn continued to degrade the soil in particular and the general environment subsequently endangering the plant, animal, microbial and human health [1].

Fixation of added nutrients/fertilizers in soil reduces the efficiency of applied potassium fertilizers and thus a large quantity of added fertilizers become unavailable to plants. Rhizosphere microorganisms contribute significantly in solubilization of bound form of soil minerals in the soil [15]. However, potassium nutrients are released slowly from the rock materials and their use as fertilizer often causes insignificant increases in the yield of crops [10].

It has therefore become expedient to explore better ways and methods to meet the nutritional needs of the crops in ways that reduce or eliminate the adverse effects. Ways that are eco-friendly and promote sustainable agriculture.

Potassium (K) is a major essential macronutrient for plant growth. The concentrations of soluble potassium in the soil are usually very low and more than 90% of potassium in the soil exists in the form of insoluble rocks and silicate minerals. Potassium (K), one of the seventeen chemical elements required for plant growth and reproduction, is often referred to as "the regulator" since it is involved with over 60 different enzyme systems in plants. Besides its potential to resist drought and disease [2, 3], it helps in the production of starch, controls root growth and regulates the stomata movement in plant cells and also contributes to quality.

Among Nitrogen (N), Phosphorus (P) and Potassium (K), Potassium is the third important plant nutrient. Potassium is essential macronutrient for plant growth and plays significant roles in activation of several metabolic processes including protein synthesis, photosynthesis, enzymes, as well as in resistance to diseases and insects etc. [4].

India totally depends on import of potassic fertilizers and farmers use very little or not of potassium in crop production [5].

Therefore, more research is needful to unearth more efficient and native strains of these potassium solubilizing microorganisms for use.

MATERIALS AND METHODS

Sample Collection

Rhizosphere soil samples were randomly collected from different locations in and around Bangalore. From each location, samples were collected from six different sites. The collected samples were pooled together to make the composite sample [6].

Adaptation and Enrichment

The composite soil samples collected were mixed with insoluble potassium (Feldspar) and incubated for 1 week at room temperature. After adaptation 1 gm of soil was inoculated in 100 ml liquid medium containing 1% glucose, 0.05% yeast extract and 0.5% feldspar and incubated at 37^0 C on 120 rpm for 1 week [7].

Isolation of Rhizofungi from the Rhizosphere Soil and Screening for potassium Solubilization

Enriched samples were inoculated after serial dilution up to 10^{-6} on Aleksandrov agar medium constituted as 1% glucose, 0.05% $MgSO_4.7H_2O$, 0.0005% $FeCl_3$, 0.01% $CaCO_3$, 0.2% $CaPO_4$ and 0.5% potassium aluminium silicate, agar 3 % at pH 6.5 [16] and incubated at 37^0C for 1 week. Fungal Colony exhibiting clear zone of potassium solubilization on Aleksandrov agar was selected as potassium solubilizer. Secondary Screening was carried out on the basis of study of zone of activity of the isolate by using Khandeparkar's selection ratio. Ratio = D/d = Diameter of zone of clearance / Diameter of growth [7].

Characterization of the isolate

Morphological and molecular identification of the isolate was done to ascertain what fungi it is. The isolate was grown in on czapeck dextrose agar medium and its colony characteristics were studied. The morphology of the isolate was studied under compound microscope using lactophenol cotton blue as stain. Molecular characterization of the isolate was done with help of 18S rRNA sequence analysis. Steps followed were: Genomic DNA was isolated from the fungal sample. The 600-650 bp ITS region was amplified using high–fidelity PCR polymerase. The PCR product was sequenced bidirectionally. The sequence data was analyzed to identify the culture and its closest neighbours [8]. Primers used were: ITS-Forward Primer 5' – GRAAGNAHADGTVGKAAYAWSG – 3'. ITS-Reverse Primer 5' – TCCTNCGYTKATKGVTADGH – 3'. The crude sequence was submitted and aligned to/by the National Centre for Biotechnology Information (NCBI) database. The accession number from NCBI is KX775949.

Solubilization Activity and Optimization Conditions for Efficient K Solubilization

The isolated K solubilizing fungus was tested for optimization its K solubilizing activity under varying conditions of carbon, nitrogen, potassium, temperature and pH sources used.

A loopful of 48 hour old grown fungi culture was inoculated into 25ml Aleksandrov medium broth in 50ml capacity flask containing either of different sugars: fructose, galactose, glucose and xylose with added flask for control which

was not inoculated. All the inoculated flasks plus the control were incubated at 28±2°C for 10 days. Same was done for nitrogen sources (beef extract, $NaNO_3$, peptone and urea), potassium sources (KCl and K_2SO_4), varying temperatures (25°C, 30°C, 35°C and 40°C) and varying pH (6.5, 7.0, 7.5and 8.0) [6].

Quantitative Estimation of Potassium Release

Different concentrations of KCl solution, ranging from 0 – 100 ppm, were used for preparation of standard curve. Sodium cobaltinitrite solution (5ml) was added slowly to each test tube containing varied concentrations of potassium and volume made up to 10ml by adding distilled water. The reaction mixture was incubated at 37°C for 45 minutes to precipitate the potassium and centrifuged at 13,000 rpm for 5 minutes to permit the precipitate to settle down in the tube. The supernatant was decanted, precipitate collected and washed twice with distilled water and once with absolute ethanol. After washing, 10ml of conc. HCl was added to the precipitate and incubated at 37°C for 15 - 20 minutes to develop the green colour and absorbance was measured at 600nm using the colorimeter [9].

Following the same procedure and conditions, potassium was estimated in 5ml of culture supernatant, with reference to the standard curve generated. Estimation for each parameter was carried out thrice to obtain the average which was now referenced to the standard curve to obtain the estimate of solubilization.

RESULTS AND DISCUSSION

Isolation and Solubilization Activity of Fungi

Of the colonies that were able to grow on Aleksandrov agar one of these isolated colonies was found to make a clearance zone indicating k-solubilization fig. 4 giving a Khandeparker's ratio of 2.33 Table 1.

Morphological and Biochemical Characters of Isolated Strain

The fungal isolate was circular, whitish, cotton-like colonies showing yellow pigmentation under fig. 1. It was identified to be *Aspergillus terreus* fig. 3. The isolate was found to be closest to *Aspergillus sp*. 7 BRO-2013 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, Sequence ID: gb|KF367546.1 fig. 2. The next closest homologue was found to be Uncultured fungus clone CMH583 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene,

Sequence ID: gb|KF800672.1|. This agrees with the finding of Rekha and Sreeramulu [11] who stated that most of the fungi noticed that were efficient potash solubilizers were *Aspergillus sp.*

1. K SOLUBILIZATION ACTIVITY

From (fig. 5) it is seen that the solubilization was best in glucose broth 26.15mg/l followed by galactose (22.31mg/l), then fructose (14.62mg/l) and the least was xylose (10.77mg/l) fig. 5. This agrees with the study by Barasso, C. B. et.al., [12], who stated that *Aspergillus* produces citric acid in medium supplement with carbon sources like glucose, galactose and the citric acid production enhances the solubilization of the insoluble nutrients.

As seen on fig. 6, the solubilization was most in urea (106.92mg/l), followed by $NaNO_3$ (103.08mg/l), then beef extract (91.54mg/l) and lastly peptone (64.62mg/l). Solubilization as observed on fig. 7 was more in KCl broth (476.15mg/l) than K_2SO_4 (372.31mg/l). This is in line with the fact that potassium nutrients are released slowly from the rock materials and their use as fertilizer often causes insignificant increases in the yield of crops [10].

Solubilization was best (49.23mg/l) at the temperature of 30^oC followed by 41.54mg/l at 25^oC, then the least were 41.54mg/l at 35^oC and at 40^oC fig. 8. Same temperature of 30^oC was found to be the temperature at which *Aspergillus* performed best by Maharani *et al*., [14].

At pH 7.5 solubilization was best yielding 87.69mg/l at pH 7.5, followed by 45.39mg/l at pH 6.5, next was 26.15mg/l at pH 7 and the least was 22.31mg/l at pH 8 fig. 9. This differs from findings of other researches that show best solubilization at lower pH of 3 to 4. The difference could be attributed to low quantity of solubilization as it has been reported by Trivedi, M. et. al., [13] that pH lowers with more quantity of potassium solubilized.

Table 1: K solubilization zone formation by isolates by Khandeparkar's ratio: D/d

Isolate	Diameter of Zone of clearance (D) in mm	Diameter of growth of Colony (d) in mm	D/d Ratio
Aspergillus terreus	14	06	2.33

D = Diameter of zone of clearance, d = Diameter of growth of isolate

Aligned sequence data: 2B (632bp)

TGGAAGTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTACCGAGTGCGGGTC
TTTATGGCCCAACCTCCCACCCGTGACTATTGTACCTTGTTGCTTCGGCGGGCCCGCCAGCGTTGCTGG
CCGCCGGGGGGCGACTCGCCCCCGGGCCCGTGCCCGCCGGAGACCCCAACATGAACCCTGTTCTGAAA
GCTTGCAGTCTGAGTGTGATTCTTTGCAATCAGTTAAAACTTTCAAAATGGATCTCTTGGTTCCGGCATC
GATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAATCATCGAGTCTTTGA
ACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCC
GGCTTGTGTGTTGGGCCCTCGTCCCCCGGCTCCCGGGGGACGGGCCCGAAAGGCAGCGGCGGCACCG
CGTCCGGTCCTCGAGCGTATGGGGCTTCGTCTTCCGCTCCGTAGGCCCGGCCGGCGCCCGCCGACGCA
TTTATTTGCAACTTGTTTTTTTCCAGGTTGACCTCGGATCAGGTAGGGATACCCGCTGAACTTAAGCATA
TCAATAAGCGGAG G

Fig. 2: Gene sequence of the isolate (*Aspergillus terreus*)

Fig. 3: Phylogenetic tree of *Aspergillus terreus*

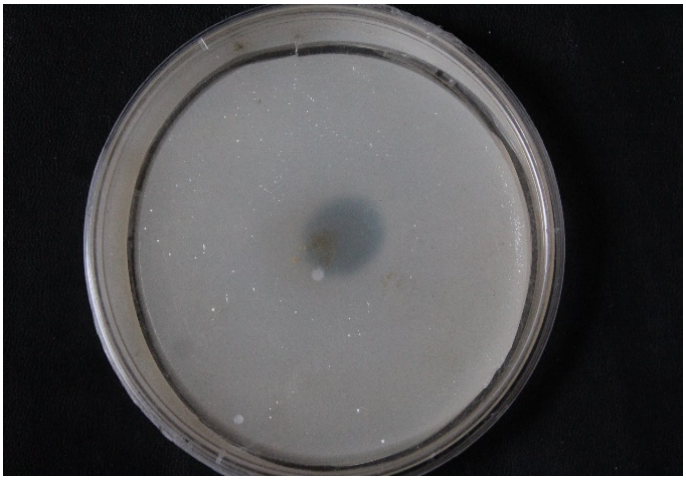

Fig. 4: *Aspergillus terreus* colony showing solubilisation zone on Aleksandrov medium

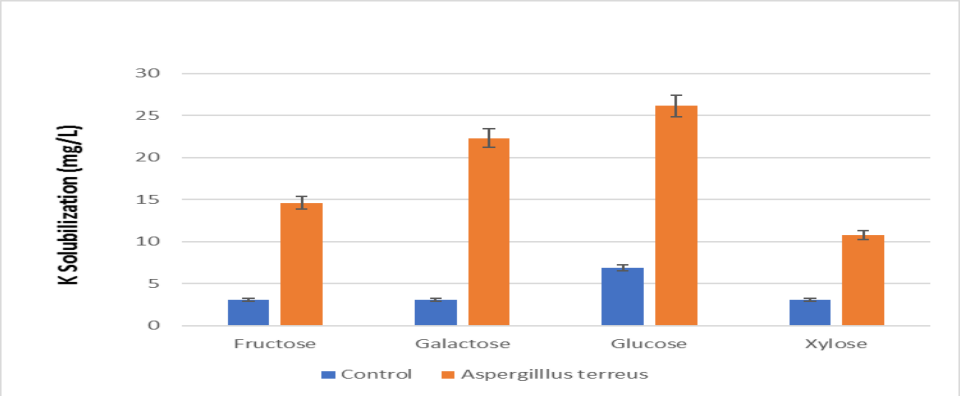

Fig. 5: K solubilization by the culture using different sugars (carbon sources)

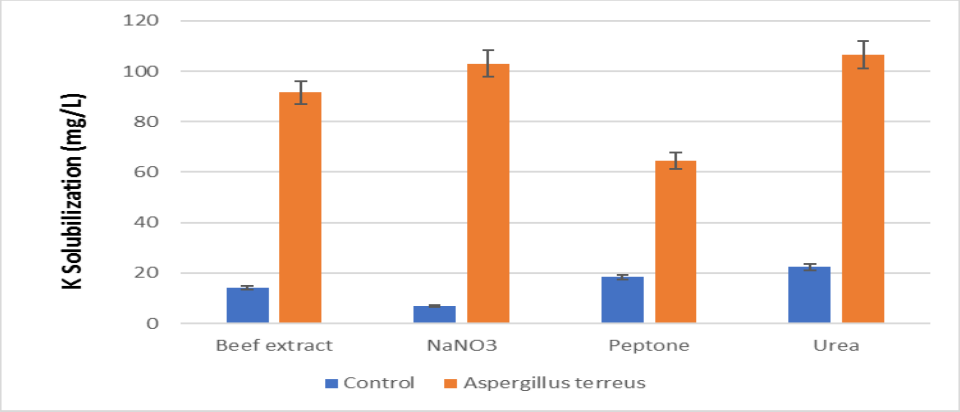

Fig. 6: K solubilization by the culture using different proteins (nitrogen sources)

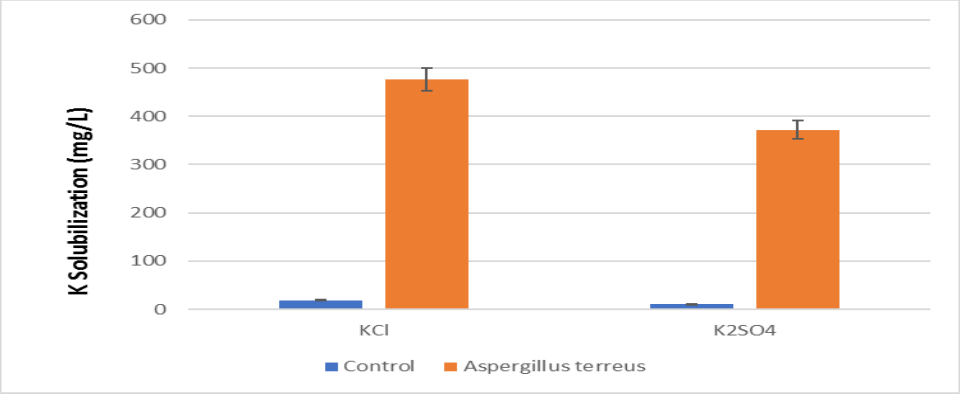

Fig.7: K solubilization by the culture using different potassium sources

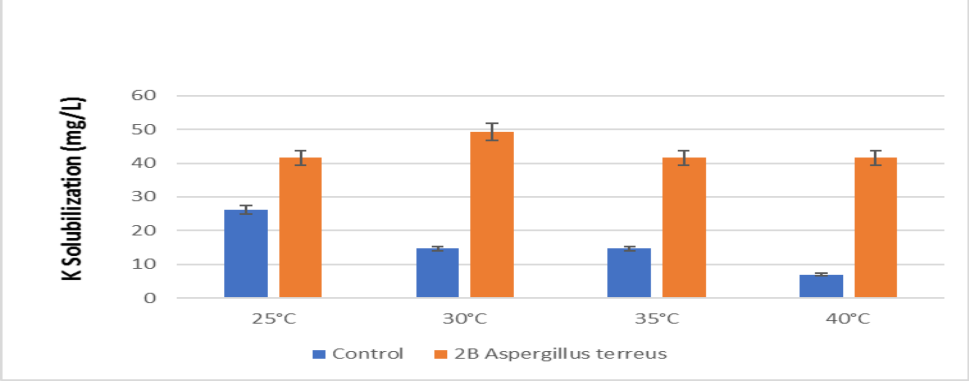

Fig. 8: K solubilization by the culture at different temperatures

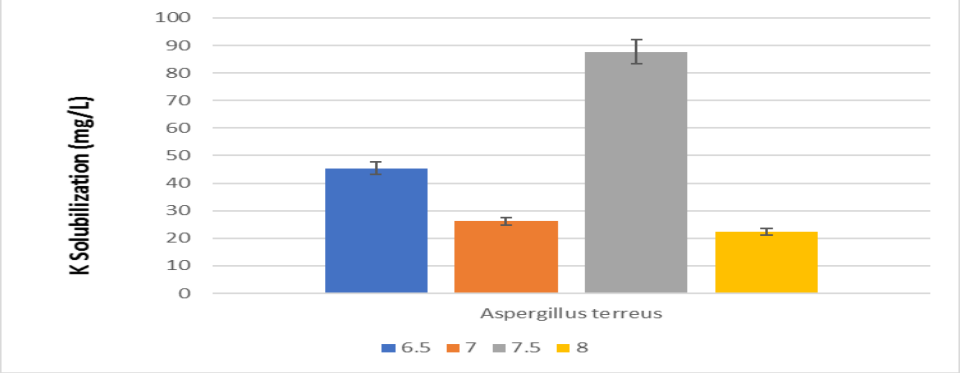

Fig. 9: K solubilization by the culture at different pH

CONCLUSION

This study attempted to find such fungal microorganisms that so contribute to the solubilization to the bound insoluble form of potassium in the soil.

One fungus was isolated which showed this ability to solubilize feldspar (an insoluble form of potassium) in Aleksandrov broth. The fungus was identified to be *Aspergillus terreus* and showed encouraging solubilization activity under varied parameters of carbon, nitrogen, potassium sources, temperature and pH. Thus, potassium solubilizing fungi isolated in this study could be a good candidate to test for use to supplement or replace potassium based fertilizers on potassium deficient soil. More concerted research efforts remain very valuable to continue to find these solubilizers for improved crop production and safety of the ecosystem.

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