EXPLORING PROMISING POTASSIUM SOLUBILIZING FUNGI FOR PROSPECTIVE LIQUID BIOINOCULANT

ARTI SHANWARE, SUREKHA KALKAR, MINAL TRIVEDI

Abstract: Potassium is one of the major essential macronutrient required for the growth and development of plants. 90% of potassium in the soil exists in the form of insoluble rocks and silicate minerals and the concentrations of soluble potassium in the soil is very low .Rhizospheric microorganisms are known to dissolve potassium from insoluble K-bearing minerals. The aim of the present investigation was to isolate and screen potential potassium solublising fungi from Sugarcane rhizosphere from different sites in & around Nagpur region (Maharashtra). The isolates were screened on the basis of zone of solublisation on Aleksandrov medium containing mica powder as potassium source. Five isolates showed potassium solublisation activity. Quantitative estimation of potassium solublisation by these isolates was found in the range of 09mg/L -30mg/L .Isolate KSF 06, showed highest potassium solublisation, it was further identified based on colony morphology and 18s rRNA sequence analysis and was found to be *Aspergillus niger* .This potassium solubilizing fungus could be exploited for plant growth improvement and represents a budding contender for potash biofertilizer.

Keywords: Potassium Solubilization, Aleksandrov medium, Mica, Biofertilizer, *Aspergillus niger*.

Introduction: Use of synthetic fertilizers for nourishment of plant has destroyed microbial flora and caused the contamination of water and soil, hence using biofertilizers is an eco-friendly approach for nutrient nourishment to the plants. Currently carriers used in biofertilizers are solid based biofertilizers which have drawbacks such as low shelf life, less cell count and reduced efficiency. Hence there is need to develop biofertilizers with liquid carriers having longer shelf and higher efficiency. Potassium (K), one of the primary macronutrient required by plants plays vital role in numerous biochemical and physiological processes in plants like stomatal regulation where–K regulates the opening and closing of stomata and proper functioning of stomata is essential for photosynthesis. The plant transport system uses energy in the form of ATP. If K is inadequate, less ATP is available, and the transport system breaks down. Potassium plays a major role in the transport of water and nutrients in the plant. Potassium also activates starch synthetase responsible for synthesis of starch .Its role is known in improving shelf life of crops,disease resistance [1,2,3] .The Indian soils have 2% soluble K and 98% insoluble K .

In order to achieve optimum plant growth, Potassium is required in adequate and soluble form therefore we need to exploit more and more microorganisms that have ability to solublise potassium. Potassium solubilizing microorganisms play an important role in supplementing potassium to the plants by producing microbial organic acids and lowering pH of soil .The organisms with potassium solubilizing ability increase the availability of soluble potash and can enhance plant growth by production of plant growth promoting regulators [4]. Organic matter after microbial decomposition produces acids like citiric acid, formic acid, malic acid, oxalic acid. Solubilization of potassium occurs by complex formation between organic acids and metal ions such as $Fe^{2+,}$ Al^{3+} & Ca^{2+} .Microorganisms like *Paenibacillus glucanolyticus, Bacillus mucilaginosus, Aspergillus niger, Aspergillus terreus, Burkholderia* sp., *Pseudomonas* sp., *Bacillus megaterium* and *Bacillus coagulans are known* to posses potassium solublising ability [5] and were found to grow on muscovite, biotite, orthoclase microclase and mica *in vitro* [6].

Much work is done on effect of potassium solublisers on the crop production. Basak *et al*. found significant correlation between biomass yield, K uptake by Sudan grass in soil inoculated with *Bacillus mucilaginosus* [7]. In an experiment carried out by *El-Hadad et.al* the results indicated that bacterial biofertilizers were helpful in providing soil nutrients (nitrogen, phosphate and potassium) and for the biological control of *M. incognita* [8]. Prajapati *et al*. demonstrated inoculation with bacterial strain *Enterobacter hormaechei* increases root and shoot growth of Okra and was able to mobilize potassium efficiently in plant when feldspar was added to the soil [8].Solubilization of this soil mineral, by fungi and bacteria are well established, which reflects their use as competent biofertilizers [5]. Present investigation aims to isolate and characterize potassium solubilizing fungi from rhizospheric soil of *Saccharum officinarum* (Sugarcane) in and around Nagpur,(Maharashtra) and compare them on the basis of their potassium solubilizig abilities which can be employed in making prospective liquid biofertilizer.

Materials And Methods:

Collection of Sample: The soil samples were collected from depth of 15cm from the *Saccharum officinarum* (Sugarcane) rhizosphere from different sites like Kuhi, Hingn etc., i.e. adjoining areas of Nagpur (Maharashtra). The samples were mixed with mica as a source of insoluble potassium and incubated for 7 days at room temperature.

Isolation and Selection of Potassium Solubilizing Fungi (KSF) :

1 g of soil after adaptation was inoculated into 100mL of sterile distilled water .Serially diluted soil samples were spread on sterile Aleksandrov Agar medium containing 1% glucose, 0.05% yeast extract ,2% agar powder and 0.5% mica, pH-6.5 and incubated at 30ºC for 1 week [9].

Screening of potential Potassium Solubilizing Fungi:

Colonies that showed clear zone of potassium solubilization were selected as potassium solubilizers. Further screening of isolates was done by finding Khandeparkar's selection ratio [10].

Characterization of Fungi:

Selected fungal strains were grown on Potato Dextrose Agar (PDA) medium and their colony characteristics were studied, the cell morphologies of the isolates was studied under compound microscope using lactophenol cotton blue as stain [11]. The fungi were identified by extracting the DNA from the pure culture. The rDNA was amplified by using universal primers and identified by18s rRNA sequencing. The crude sequence was aligned with help of tools available in the National Center for Biotechnology Information (NCBI) database [12].

Quantitative Potassium Solubilization:

The fungal isolates were grown on 100 mL Aleksandrov broth ,incubated at 30ºC on 120 rpm for 7 days by using mica as insoluble source of potassium. The potassium released was determined by using Sodium cobaltinitrite and Folin- Ciocalteu Phenol reagent [13]. The pH value of the broth was also measured after each 24 hours.

Results And Discussion:

Five fungal colonies were obtained which showed potassium solubilisation on Aleksandrov agar, among them KSF 06 showed highest potassium solublisation (Fig.1). The amount of phosphate released in the Aleksandrovs broth by each of the isolates was quantitatively measured using Sodium cobaltinitrite and Folin- Ciocalteu Phenol reagent. In quantitative estimation, potassium solubilization was found between 09 mg/L to 30 mg/L (Table-1). Measurement of the pH was also carried out every day, and It gradually decreased with the increased incubation time. After a week, pH was decreased from 6.5 to 4.0 indicating that absence of soluble potassium in media stimulates the acid production (Fig.3).

Table 1. Potassium solubilization values of fungal isolates by Khandeparkar's selection ratio

Isolate Code	D	d	D/d ratio	Quantitative K Solublisation (in mg/L)
KSF 05	10	08	1.25	09
KSF 06	15	07	2.14	30
KSF 07	13	10	1.3	22
KSF 08	10	07	1.42	18
KSF 09	09	08	1.12	10
KSF 10	12	09	1.33	25

Key: D- Diameter of Zone in mm
d- Diameter of Colony in mm

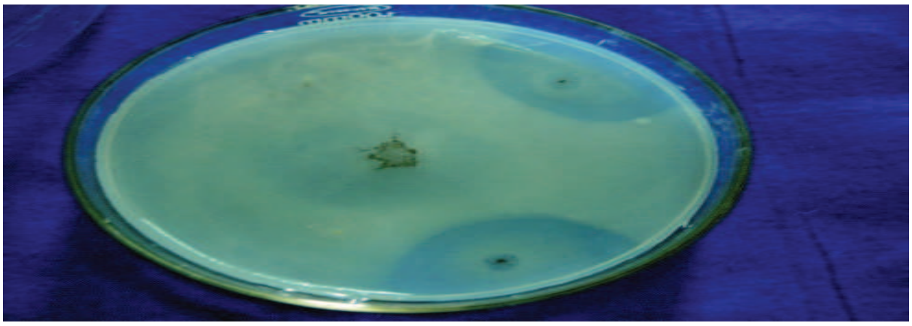

Fig.1. Zone of Potassium solublization by KSF 06

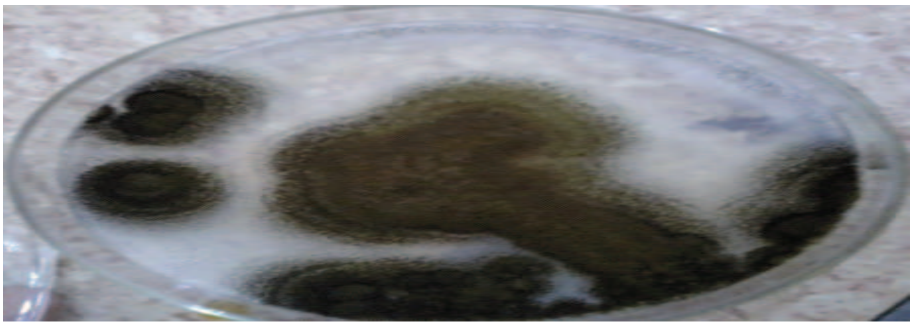

Fig.2. Growth of KSF 06 on PDA medium

Fungal strain KSF 06 showed black – brown colonies on Potato Dextrose agar (Fig.2) Conidia were brown, globose, dark with rough walls. Conidiophores were short smooth walled .Further genomic DNA was extracted from the pure culture and based on 18s rRNA analysis fungus was identified as *Aspergillus niger* (Table 2) & (Fig.4).

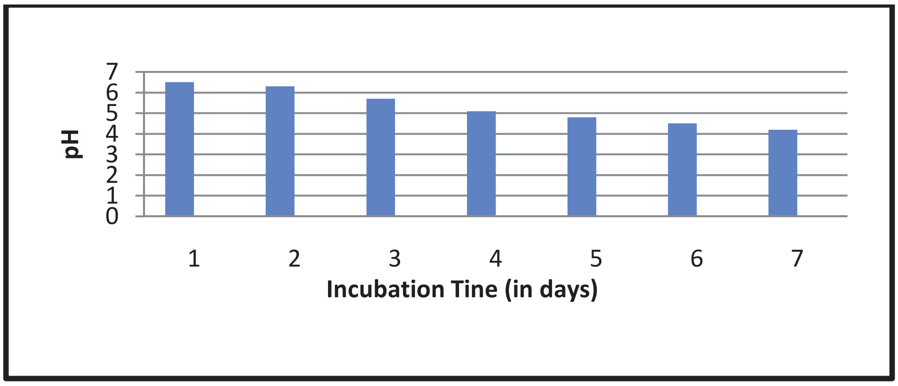

Fig.3 pH of KSF 06 in Aleksandrov Broth

Table 2. The aligned sequence data obtained for *Aspergillus niger* (approximately 622 bp)

5'GAAGGTGAAATCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAGACATTACCGAGTGCGGGTCC TTTGGGCCCAACCTCCCATCCGTGTCTATTGTACCCTGTTGCTTCGGCGGGCCCGCCGCTTGTCGG CCGCCGGGGGGGCGCCTTTGCCCCCCGGGCCCGTGCCCGCCGGAGACCCCAACACGAACACTGTC TGAAAGCGTGCAGTCTGAGTTGATTGAATGCAATCAGTTAAAACTTTCAACAATGGATCTCTTGG TTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAATGTGAATTGCAGAATTCAGTGAAT CATCGAGTCTTTGAACGCACATTGCGCCCCCTGGTATTCCGGGGGGCATGCCTGTCCGAGCGTCA TTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGCCGTCCCCCTCTCCGGGGGGACGGGCCCGA AAGGCAGCGGCGGCACCGCGTCCGATCCTCGAGCGTATGGGGCTTTGTCACATGCTCTGTAGGAT TGGCCGGCGCCTGCCGACGTTTTCCAACCATTTTTTCCAGGTTGACCTCGGATCAGGTAGGGATA CCCGCTGAACTTAAGCATATCAATAAGGCGGAGGAAA3'

Conclusion: In the present study five isolates showed zone of potassium solublisation that used mica as source. KSF 06 was found to be most efficient strain which solubilized insoluble potassium and based on morphology and molecular identification it was found to be *Aspergillus niger* . Such type of study is necessary as it promotes the use of potassium solublising fungi as potash biofertilizer and is an efficient approach to substitute chemical fertilizers. Apart from the identification of potash solublisers there is necessity for field demonstration studies of these isolates along with standardization of the dose so as to achieve optimum growth and also that the technology can be easily implemented by farmers for multiplication at their own level. Hence Isolation, characterization and development of liquid carriers for these potash solubilisers can be the means towards sustainable agriculture development

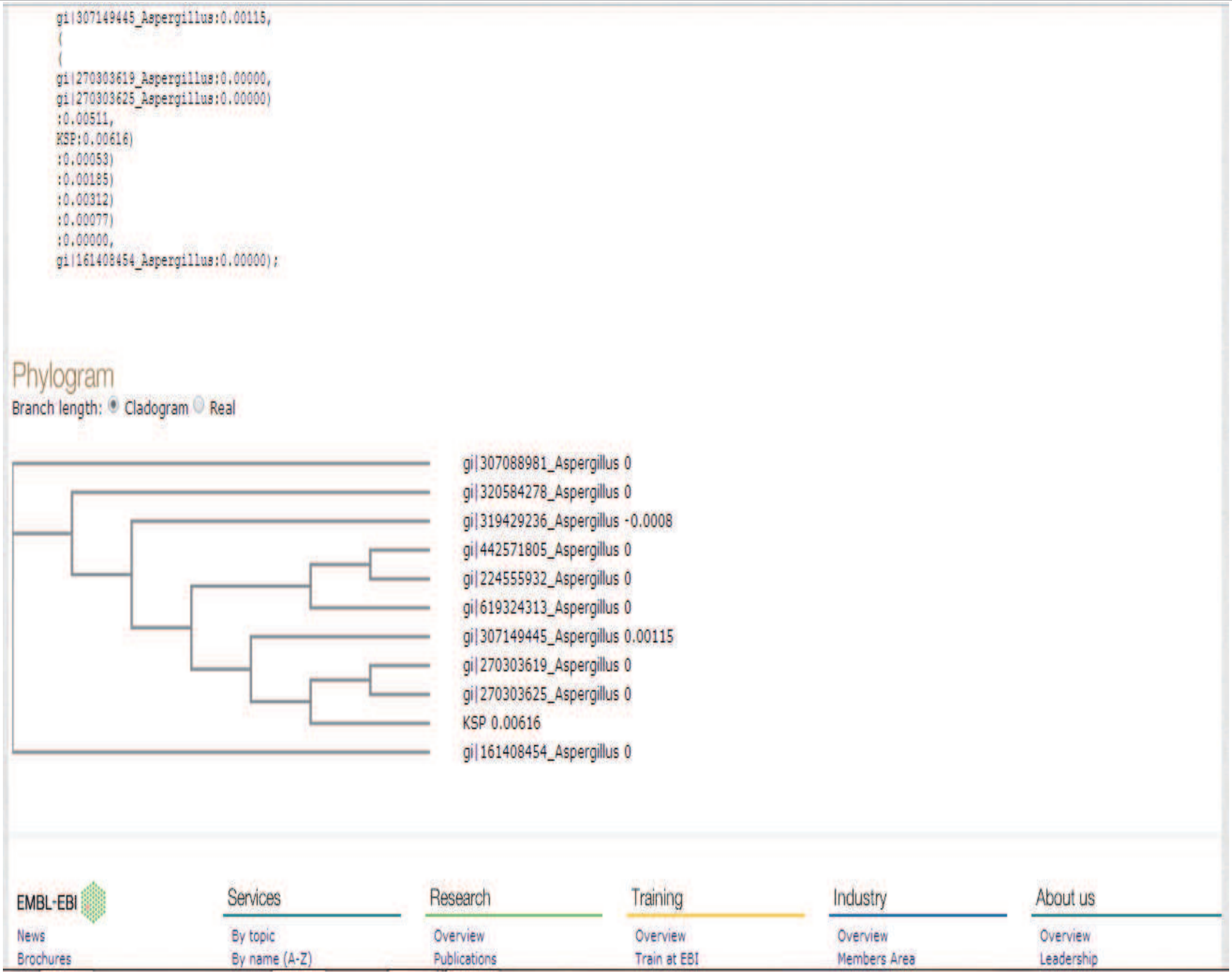

Fig 4. The phylogenetic tree of *A.niger* isolated from Nagpur soil with optimum potassium solubilizing capacity.

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