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ISOLATION, CHARACTERIZATION AND IDENTIFICATION OF EFFICIENT POTASH SOLUBILIZING BACTERIA AND FUNGI

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ABSTRACT
Potassium is the major cat ion among the trinity of nutrients required in large quantities by most of the crops. The availability of soluble form of potassium in soils is low and more than 90% of it exists as insoluble and in combined form with silicates. An attempt was made in this study to isolate efficient potash solubilizing microorganisms from rhizosphere soils of banana plantations enriched with different concentrations of potassium salts with muscovite. The most efficient potash solubilizing bacteria identified were *Bacillus mucilaginosus* (UASKB 102) and *Pseudomonas fluorescence* (UASKB 101). Similarly the two fungal isolates which were identified as efficient Potash solubilizers were *Aspergillus niger* (UASKF 102) and *Aspergillus terreus* (UASKF 101) and any one of this can be routinely used by farmers as a potash solubilizing biofertilizer in crop cultivation.

INTRODUCTION

Potassium is considered second only to nitrogen, when it comes to nutrients needed by the plants and is considered as the "Quality nutrient". It is the most essential macronutrient needed for the plant growth to obtain increased crop yields with quality produce (Romheld and Kirkby, 2010). Potassium has a direct effect on the plant shape, size, colour, taste and other growth attributes contributing to healthy produce. Plants absorb potassium in large amounts than other macronutrients. Potassium ion is present in soil mixed with other mineral salts and get absorbed in to the plant body from outside. Without adequate potassium, plants will have poorly developed roots, grow slowly, produce small seeds and have lower yields. Unlike other nutrients, potassium does not wind up in a plant chemical structure, instead, plants require it for ongoing processes important for their growth, reproduction and health. It is involved in many metabolic and physiological processes. Potassium helps to maintain turgidity, reduces water loss and wilting and helps in alleviating detrimental effects of abiotic stress in plants (Ismail, 2005). It helps in photosynthesis and food formation, reduces respiration and prevents energy losses. In photosynthesis, potassium regulates the opening and closing of stomata, thus regulates CO_2 uptake and play a major role in osmo-regulation. Potassium has an important role in the activation of many growth related enzymes in plants. Potassium triggers the activation of enzymes essential for the production of Adenosine Triphosphate (ATP). ATP is an important energy source for many chemical processes taking place in plant tissues. Potassium is essential at almost every step of the protein synthesis. In starch synthesis, the enzyme responsible for the process is activated by potassium and enhances the translocation of sugars and starch and help in producing grains rich in starch (Prajapati and Modi, 2012). Potassium helps to build cellulose in plants and reduces lodging. It retards crop diseases and known to improve drought resistance in plants. Potassium in soils is concentrated in minerals and they solubilize under appropriate conditions for plant uptake (Goldstein,1994). The availability of soluble form of potassium in soils are low and more than 90% of it exists as insoluble and in combined form with silicates. In recent years cultivated soils are getting more depleted with potassium at a faster rate and is becoming one of the major constraints in crop production. Identification of alternative indigenous sources of potassium and their efficient supply for plant growth is most needed. Soil microorganisms are said to play a key role in potassium mobilization for the benefit of plant growth (Perelomov and Kandeler,2006). In this study an attempt was made to isolate and characterize an efficient potash solubilizing bacteria and fungi for effective mobilization of potassium for the benefit of crop growth. Different bacteria have been reported to dissolve potassium, silica and aluminium from insoluble minerals (Aleksandrov *et al.,* 1967). Potassium solubilization by bacteria from insoluble silicates and release of K from muscovite has been reported by Sugumaran and Janarthanam (2007). The mechanism of solubilization of potash by production of organic acids like oxalic acid, tartaric acid, citric acid, acetate by potash solubilizing bacteria has also been reported by Ullman *et al.* (1996).

The information on efficient potash solubilizing bacteria and fungi, their mechanism of solubilization and their effect on plant growth is limited. In this context an attempt was made in this study to isolate and identify efficient potash solubilizing bacteria and fungal isolates for their routine usage as biofertilizer in crop production.

MATERIALS AND METHODS

Isolation of Bacteria and Fungi from crop rhizosphere soil

Isolation of efficient potash solubilizing bacteria and fungi were made through soil enrichment technique (Beijerinck, 1913). A pot experiment using rhizosphere soils of banana plantations enriched with 2 % muscovite mica with 1% MOP, SOP and with 0.5 % MOP and SOP was conducted in glass house of the Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK campus, Bangalore with onion as test crop. A separate set of sterilized and unsterilized set was maintained. The details of the pot experiment are as follows:

Experiment with sterilized soil

Sterilized soil enriched with mica and other K sources without crop

T1 : 500g rhizosphere soil + 2% muscovite mica (Control)

T2 : 500g rhizosphere soil + 2% muscovite mica + 1% MOP

T3 : 500g rhizosphere soil + 2% muscovite mica +1% SOP

T4 : 500g rhizosphere soil + 2% muscovite mica + 0.5% MOP + 0.5 % SOP

B. Sterilized soil enriched with mica and other K sources with onion as test crop

T5 : 500g rhizosphere soil + 2% muscovite mica + 1% MOP + Onion crop

T6 : 500g rhizosphere soil + 2% muscovite mica + 1% SOP + Onion crop

T7 : 500g rhizosphere soil + 2% muscovite mica + 05% MOP + 0.5% SOP + Onion crop

Experiment with unsterilized soil

A. Unsterilized soil enriched with mica other K sources without crop

T1 : 500g rhizosphere soil + 2% muscovite mica (Control)

T2 : 500g rhizosphere soil + 2% muscovite mica + 1% MOP

T3 : 500g rhizosphere soil + 2% muscovite mica + 1% SOP

T4 : 500g rhizosphere soil + 2% muscovite mica + 0.5 % MOP + 0.5 % SOP

Unsterilized soil enriched with mica and other K sources with onion as test crop

T5 : 500g rhizosphere soil + 2% muscovite mica + 1% MOP + Onion crop

T6 : 500g rhizosphere soil + 2% muscovite mica + 1% SOP + Onion crop

T7 : 500g rhizosphere soil + 2% muscovite mica + 05% MOP + 0.5% SOP + Onion crop

Soil samples from the experimental pots were drawn at 30, 60 and 90 days after sowing of onion seeds and microbial analysis was made by serial dilution plate count technique (Bunt and Rovira, 1955). The Potash solubilizing bacteria were enumerated on Aleksandraov medium (Hu *et al.*, 2006) containing Glucose 5.0 g , $MgSo_4$ 0.005 g , $FeCl_3$ 0.1 g, $CaCo_3$ 2.0g Potassium mineral 2.0g, $CaPO_4$, 2.0g , Agar 18g and distilled water1 liter). The Serial dilution of soil samples were made up to 10^5 and 1 mL suspension from 10^5 dilution was transferred to sterilized petri plates and poured approximately 18 to 20 mL of sterilized molten medium to each plate. Similarly Potash solubilizing fungi were isolated on Potato Dextrose Agar enriched with muscovite mica. The plates were incubated at 28±2°C in a BOD incubator for 4 days. The predominant colonies showing the typical characters of K solubilization were selected and were identified through morphological characters as outlined by Anon (1957) and biochemical characters as per the procedure of Blazevic and Ederer (1975).

Screening of bacteria and fungal isolates for K solubilizing efficiency

Potash solubilization capability of isolated bacteria and fungi were studied on Aleksandrov medium and on of PDA amended with muscovite mica respectively by spot application method. Spot inoculated plates were incubated at 28± 2°C for 4 days and zone of K solubilization was recorded.

RESULTS AND DISCUSSION

The bacteria and fungi developed in the soils of different treatments of sterilized and unsterilized sets of the pot experiment is presented in Table 1.

The results showed that in both the soils, enriched with potash minerals irrespective with crop or not have showed the growth of more number of bacterial colonies belonging to *Bacillus sp* and *Pseudomonas sp*. Similarly most of the fungi noticed

Table 1: Predominant bacteria and fungal isolates found in enrichment soil

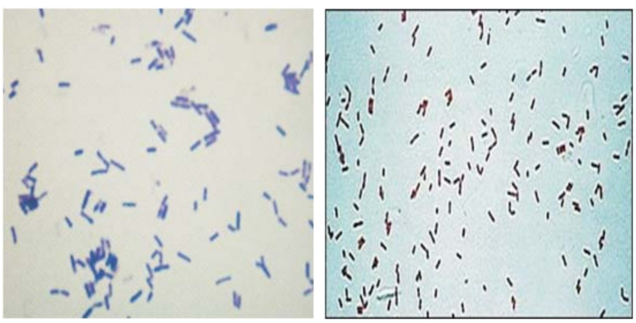
Unsterilized set	Predominant Bacteria	Predominant Fungi
I. Soil enrichment treatments		
T_1 : 500g rhizosphere soil + 2 % muscovite mica (control)	*Bacillus sp, Pseudomonas sp*	*Aspergillus sp, Fusarium sp*
T_2 : 500g rhizosphere soil + 2 % muscovite mica + 1% MOP	*Bacillus sp*	*Aspergillus sp*
T_3 : 500g rhizosphere soil + 2 % muscovite mica + 1% SOP	*Bacillus sp, Pseudomonas sp*	*Aspergillus sp*
T_4 : 500g rhizosphere soil + 2 % muscovite mica + 0.5 % MOP + 0.5 % SOP	*Bacillus sp , Pseudomonas sp*	*Aspergillus sp*
II. Treatments with onion as host crop		
T_5 : 500g rhizosphere soil + 2 % muscovite mica + 1 % MOP + Onion seeds	*Bacillus sp,*	*Aspergillus sp*
T_6 : 500g rhizosphere soil + 2 % muscovite mica + 1% SOP + Onion seeds	*Bacillus sp ,Pseudomonas sp*	*Aspergillus sp*
T_7 : 500g rhizosphere soil + 2% muscovite mica + 0.5% MOP + 0.5% SOP + Onion	*Bacillus sp , Pseudomonas sp*	*Aspergillus sp,*

Table 2: Morphological & biochemical characters of isolated bacterial strains

Strains	Colonymorphology	Gram's reaction& cell shape	Gelatin liquefaction	Starch hydrolysis	Casein hydrolysis	Catalase Hydrolysis	Acid	Gas	Simmon's Citrate test
Isolate •	MediumRoundCreamy &Slimy	-ve rods	-	+	-	+	+	+	+
Isolate , yellowish.	MediumRoundCreamy to	+ve rods	+	+	+	-	+	+	+

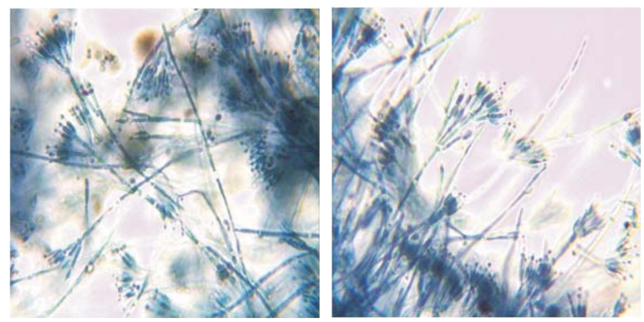
Table 3: Morphological characterization of fungal isolates

	Morphological characters					
Strains	Source	Shape	Colonymorphology	Mycelium	Fruiting body	Identified fungi
Isolate •	Rhizosphere soil	Circular	Cinnamon to brown coloured colonies	Septate	Conidiophore with vesicle like tip with flask shaped sterigmata and chains of conidia	*Aspergillus terreus*
Isolate ,	Rhizosphere soil	Circular	White cottony colonies with black coloured spores	Septate	Conidiophore with vesicle like tip with flask shaped sterigmata and chains of conidia	*Aspergillus niger*



Bacillus mucilaginosus ***Pseudomonas fluorescence***

Plate 1: Microscopic view of Potash solubilizing bacteria



Aspergillus niger ***Aspergillus terreus***

Plate 2: Microscopic view of Potash solubilizing fungi

Table 4: Potash solubilizing zones by different bacteria and fungal *Sp.*

Zone of solubilization	Bacterial Strains
+	UASKB 105
+ +	UASKB 104
+ + +	UASKB 103
+ + + +	UASKB 101, UASKB 102
-	UASKB 106, UASKB 107, UASKB 108, UASKB 109, UASKB 110
Zone of solubilization	Fungal strains
+	UASKF 105
+ +	UASKF 104
+ + +	UASKF 103
+ + + +	UASKF101, UASKF102
-	UASKF106, UASKF107

on PDA plates were *Aspergillus sp* and *Fusarium sp*. The bacteria and fungi colonies predominantly developed on the plates were isolated and further tested for their morphological and biochemical characters and the results are presented in Table 2.

The bacteria which were isolated and identified were further tested for their potash solubilizing capabilities by plating on Aleksandrov medium (Hu, *et al.,* 2006) supplemented with mica powder (2gL^{-1}) and the zone of solubilization was recorded.

In fungi identification has been made based on morphological characters like shape of the colony, colony colour, mycelium and fruiting body and have been identified as *Aspergillus terreus* and *Aspergillus niger* (Table 3 and Plate 2).

The K solubilization capabilities of isolated fungi were tested on PDA supplemented with mica powder (2gL^{-1}) and zone of solubilization was recorded.

The potash solubilization capabilities of different strains of bacteria and fungi were scored on the basis of clear zone formation around the colonies on the respective media plates after 4 days of incubation at 28$\pm$2°C and the results are presented in Table 4.

The highest K solubilization was recorded in the bacterial isolate UASKB101 *Pseudomonas fluorescence* and in UASKB102 *Bacillus mucilaginosus* while the highest K solubilization among the fungi was recorded in UASKF101 *Aspergillus terreus* and in UASKF102 *Aspergillus niger*. The results of the present study uphold the views of Duff and Webley (1959) who isolated 2-ketogluconic acid producing gram negative bacterium *Pseudomonas fluorescence* capable of dissolving the resistant natural phosphate and silicates in soil.

Das and Singh (2014) reported that manures with *Pseudomonas fluorescence* (PGPR) applied @ 5 t ha-1 was found superior among all the other manures tested for the cultivation of mungbean and obtained increased crop yields. Application of PGPR was found beneficial in improving the nutrient content of soil especially the phosphorus and potassium . Similarly Gaur *et al* (1973) reported that *Bacillus sp* play an important role in solubilization of phosphorus and potassium for the benefit of crop growth. In this study both *Pseudomonas fluorescence* and *Bacillus mucilaginosus* showed improved solubilization of potassium than other bacterial isolates which is in accordance with the findings of the above research workers. The findings of this study also uphold the views of Fang and Yan (2006) who reported that *Bacillus spp* are capable of solubilizing potassium bearing minerals like feldspar and illite. Similarly Dalal and Nandkar (2010) reported that maximum fruit yield of *Abelmoschus esculenlus* (L) was recorded in plants treated with *Pseudomonas striata* + *Azotobacter chroococcum*. They attributed increased yields were due to better availability of NPK to plants due to microbial inoculation. In the present study the bacteria *viz., Pseudomonas fluorescence* and *Bacillus mucilaginosus* and fungi *Aspergillus terreus* and *Aspergillus niger* have been identified as most efficient potash solubilizers and any one of this can be routinely used by farmers as a potash solubilizing biofertilizer in crop cultivation. The out come of the findings are of immense use to crops especially for the high potash demanding crops.

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