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Isolation and purification of potassium solubilizing bacteria from different regions of India and its effect on crop's yield

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Abstract

Potassium is one of the important component of plant nutrients to meet the requirement of plant's growth, crop yield and quality of product. Isolates of KSB was carried out using mica (insoluble potassium) from the soil sample of different regions of India basically U.P and M.P on Aleksandrow's agar media. Out off 14 isolated bacterial isolates 7 bacterial isolates MPS1C2, MPS2C5, MPS2C4, MPS5C1, UPS1C1, UPS2C1, UPS3C1 showed highest D/d ratio 3.13, 3.50, 2.0, 3.22, 5.00, 4.13, 3.75. Optimum pH and temperature was pH-7 and 28±2°C. Maximum K solubilization was achieved in KCl and K_2SO_4. The isolated bacterial strain was identified as *Micrococcus varians* and *Corynebacterium kutscheri*. KSB treated Groundnut plant showed maximum pods of 16 and seeds 72 per plant as compared to control having pods 7 and seeds 21.

Keywords: Potassium solubilizing bacteria (KSB), modified Aleksandrov media, dilution plating, trial plots, germination rate, Khandeparkar's selection ratio.

Introduction

Potassium (K) is one the important component for the plant growth, metabolism and development of crop. Lack of potassium in the soil leads to poor development of roots, shoots, less branches, slow growth, produce smaller seed size with lower yield. Feldspar and mica are the common potassium supplement for soil. (McAfee, 2008). With emerge of hybrid seeds and GMO plants and continues use of soil for agriculture, there is depletion of K in soils. Moreover, due to intense application of chemical fertilizer, there is lack of complete utilization of minerals by plants and excess of minerals are wasted away through rain water or excessive irrigation, this leads into shortage of potassium availability to plants in agricultural crop production. In order to resolve these problems an alternate method of potassium availability to plants and maintain K level in soil has to be followed in order to achieve higher yield without disturbing soil texture (Han, 2006).

In recent report soil microbes have played an important role in maintaining Potassium balance in soil. Potassium Solubilizing Microorganisms present in the soil helps to convert complex potassium present in soil into simple form and make them available to plants (Groudev, 1987, Rogers et al 1998]. Soil microorganisms helps to break down complex minerals present in soil either by secreting acid or base and convert it into simple form which helps to maintain the soil fertility and Iron cycle in soil (Bin Lian*et al*., 2010). Alexander (1985), found that certain Silicate bacteria are able to break down Potassium, Silicon and Aluminum from complex insoluble minerals into simple form. Biswas and Basak (2009) also reported that some bacteria are able to decompose Aluminosilicate and release potassium out of it.

Some bacteria like Bacillus, Thiobacillus, Pseudomonas, Acidothiobacillus, has been found to simplify and secret potassium from potassium-bearing complex minerals in soils [Sheng, 2005, Liu, 2012]. Potassium is present in soil either in the complexed or chelated insoluble mineral form in mica or illite, this is dissolved by Potassium Solubilizing Bacteria by releasing organic acid to convert complex form into simple form and make K available to plant (Aleksandrov, 1967, Bennett, 1998). With the application of biofertilizer containing KSB, it not only improves fertility of soil but also increases yield of crops, protects from harmfull diseases and reduce application of other chemical fertilizers (Sheng et al., 2003, Sindhu et al., 2010). Sheng and Huang (2002), reported that it is pH, oxygen and bacterial strain that helps to release potassium from minerals.

The potassium solubilization efficiency depends upon the nature of bacteria and condition of the mineral where it is serviving. Therefore, the yield of the crops can be increased by applying biofertilizer possessing Potassium containing minerals along with K-solubilizing bacteria. Thus, it is important to identify microbial strains which proves to be beneficial over damaged soil and repair its texture along with good fertility of soil lessen the environmental pollution caused by heavy application of chemical fertilizers. Biofertilizer containing KSB has shown good results on crops like cotton and rape (Sheng 2005), pepper and cucumber (Han 2006), sorghum (Badr 2006), wheat (Sheng et al., 2006), Sudan grass [Basak 2008, 2010), maize and wheat plants (Singh 2010). Xie (1998), reported that KSB has been used in Korea and China as biofertilizer in the area where K is deficient.

In this study efforts are made to isolate and identify wild strain of potassium solubilizing bacteria which can withstand different temperature and pH without loosing its prime character. Different field trial will be done to find out maximum yield in crop and maintain soil texture.

Materials and Methods

Sample collection: Soil samples were collected from different rhizospheric region of different zones of India such as "Adalpura, Mirzapur (U.P.), Sarnathchirgoan block Varansi (U.P.) and Namipura (M.P), Rangwasa (M.P), Rau (M.P). The sample were collected in aseptic bags and transported to lab for further process.

Adaptation and Enrichment: Soil samples collected from different regions of India were mixed with Mica and kept at room temperature for one week. Withdraw 1gm of soil sample and inoculate in 90 ml liquid media containing 0.95% glucose, 0.045% yeast extract and 0.45% Mica make final volume to 100ml and incubated at room temperature at 120 rpm for 7 days.

Purification and Isolation of Potassium Solubilizing Bacteria: The enriched soil samples were serial diluted up to 10^{-12} and inoculated on modified Aleksandrov agar medium which constitute 0.5% glucose, 0.05% MgSo4.7H2O, 0.0006% FeCl3, 0.06% CaCO3, 0.2% $CaPO_4$, 0.3% Mica, 3% agar pH 6.5 and phenol red dye as indicator, plates were incubated at 27 ± 2^0 C for 7 days. Potassium solubilizing colonies were selected from 10^{-8}, 10^{-9} and 10^{-10} dilution containing plates. Isolation of colonies was done on the basis of zone formed around the colonies only those isolates were selected whose diameter showed more D/d ratio according to Khandeparkar's selection ratio.

Ratio = D/d = Diameter of zone of clearance/ Diameter of growth

Study of Cultural, Morphological and Biochemical nature of KSB: In order to study the cultural morphology and biochemical characteristic of isolates modified Aleksandrove's agar media was used to grow isolates. Colony characteristics such as size, shape, texture, colour, opacity and consistency were examined. Gram staining, endospore sand capsule staining was carried out for the purified isolates. Indole test, Methyl red test, Starch hydrolysis test, Simmons Citrate test, Triple Sugar Iron test, Vogesproskauer test, Casein hydrolysis test, H_2S production were determined.

Study of impact of pH on the growth of KSB: The purified isolates were studied for their effect on different pH and their ability to solubilize K. This study was done using different pH rang (5, 6, 7, 8, 9). pH was adjusted using 0.1 M HCl and 1N NaOH. 10 ml of Aleksandrove broth adjusted with different pH was inoculated with 1 loopful of previously activated bacterial culture and incubated at 28±2°C for 24, 48 and 72 hours. The growth was checked through dilution plating.

Study impact of Temperatures on the growth of KSB: The effect of temperature on the growth of bacteria and its K solubilization was studied at different temperature ranging from 25, 30, 35, 40, 45, 50 degree Celsius. A loopful of previously activated bacterial culture was inoculated in 10ml of Aleksandrov broth and incubated for 24 hrs. The growth was checked through serial dilution.

Study of different Substrates on the growth of KSB: Bacteria shows different behavior on different substrate to understand this phenomena, Aleksandrov's broth was prepared using different types of minerals as substitute of potassium i.e., KCl, K_2SO_4 and $AlK(SO_4)_2.12H_2O$, 3.0 g each. Out of 14 isolates 6 bacterial isolates were selected and incubated at 28±2℃ for 10 days. After 10 days of incubation, released K was determined by measuring zone of solubilization in plate.

Study of Mica on Growth of KSB: The study of Mica on the growth and K solubilization was done by using Aleksandrov medium with different Mica concentration (1, 3, 5, 7, 9, 11, 13) in gram. Media was inoculated with 1 loopful of previously activated bacterial culture and incubated at 28±2˚C for 72 hours. The growth was checked using dilution plating.

Identification of isolated bacteria: The identification of purified KSB bacteria was done on the basis of its Biochemical study and *Bergeys Manual of Systematic Bacteriology.*

Field trial: The efficacy test of identified KSB bacteria was done on field trial basis. There is an increasing evidence to show that microbial inoculation of seeds may benefit plant growth through a number of mechanisms. Basically farmers apply bacterial products in three forms, Soil, Root and Seed treatment. Beneficial bacteria are mixed with some suitable carrier which helps them to survive for more then 6-8 months. These carriers have neutral pH and non-hazardous to humans. In this experiment we have used talc as carrier material.

Groundnut seeds were pre coated with sticky material and then mixed with talc based KSB culture. These KSB treated seeds were allowed to dry for 20-30 min. in shade before sowing. Seeds were sown in trial plots along with control and allowed to germinate. Germination rate, growth rate, number of pods in each plant, number of grains per plant was noted.

Results

Isolation and Screening of Potassium Solubilizing Microbes: The purified bacteria grown on both modified Alkendrove media containing phenol red dye and without dye showed clear zone around colonies were selected, only those colonies were selected which showed distinct morphology. Out of so many colonies only 14 bacterial isolates which showed clear zone around colonies were selected. (Table 1 and 2). From the 14 bacterial isolates only seven bacterial isolates, MPS1C2, MPS2C5, MPS2C4, MPS5C1, UPS1C1, UPS2C1, UP53C1 exhibited higher zone of solubilization. The ratio of solubilization zone was measured according to Khandeparkar's selection ratio as listed in Table 1 and 2.

Table 1: Value of Potassium solubilization zone of bacterial isolates (modified Alkendrove media containing phenol red dye) by Khandeparkar's selection ratio

Bacterial isolates	Clear zone diameter (D) mm	Growth diameter(d) mm	D/d (ratio)
MPSIC2	29	8	3.63
MPS2C5	18	4	4.50
MPS2C4	09	3	3.00
MPS4C1	11	7	1.57
MPS4C2	18	8	2.25
MPS4C3	15	9	1.67
MPS4C4	2.5	9	2.78
MPS5C3	35	13	2.69
MPS5C4	15	8	1.88
MPS5C2	14	6	2.33
MPS5C1	34	9	3.78
UPS1C1	32	6	5.30
UPS2C1	38	8	4.70
UPS3C1	37	8	4.60

Table 2: Potassium solubilization values of bacterial isolates (Alkendrove media without dye) by Khandeparkar's selection ratio

Isolates	Diameter of zone of clearance (D) mm	Diameter of growth (d) mm	D/d (ratio)
MPSIC2	18	8	3.13
MPS2C5	14	4	3.50
MPS2C4	6	3	2.00
MPS4C1	10	7	1.43
MPS4C2	15	8	1.88
MPS4C3	13	9	1.44
MPS4C4	14	9	1.56
MPS5C3	12	13	0.92
MPS5C4	15	8	1.88
MPS5C2	11	6	1.83
MPS5C1	29	9	3.22
UPS1C1	30	6	5.00
UPS2C1	33	8	4.13
UPS3C1	30	8	3.75

Cultural, Microscopical and Biochemical Characteristics

Modified Alekandrove media was used to study cultural behavior of purified bacterial isolates. From the 14 isolates only seven bacterial isolates were picked to identify and study their microscopical and biochemical characteristic which is reported in Table 3 and 4.

Table 3: Colonical and Morphological characteristic of KSB

	Colony characters	Morphological characters			
Isolates	**Appearance**	**Gram reaction & cell shape**	**Spore Formation**	**Capsule Formation**	**Motility**
MPSIC2	Medium, opaque, elevated, smooth, round	+ve cocci	Non-sporulating	Capsulated	Motile
MPS2C5	Medium, opaque, elevated, smooth, round	+ve small rods	Non-sporulating	Non capsulated	Motile
MPS2C4	small, opaque, slightly raised, smooth, round	+ve small rods	Non-sporulating	Non capsulated	Motile
MPS5C1	small, opaque, slightly raised, smooth, round	+ve small rods	Non-sporulating	Non capsulated	Motile
UPS1C1	small, transparent, slightly elevated, smooth, round	+ve small rods	Non-sporulating	Capsulated	Non motile
UPS2C1	small, translucent, slightly elevated, smooth, round	+ve small rods	Non-sporulating	Capsulated	Non motile
UPS3C1	small, translucent, slightly elevated, smooth, round	+ve small rods	Non-sporulating	Capsulated	Non motile

Table 4: Biochemical characteristics of KSB

Test /soil sample	MPS1C2	MPS2C5	MPS2C4	MPS5C1	UPS1C1	UPS2C1	UPS3C1
Indole test	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Starch hydrolysis test	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Methyl red test	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Simmon's citrate test	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Triple sugar iron's test	Positive	Positive	Positive	Positive	Positive	Positive	Positive
Casein hydrolysis test	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Vogesproskauer	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Catalase test	Positive	Positive	Positive	Positive	Positive	Positive	Positive
H_2S test	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Gas production	Negative	Negative	Negative	Negative	Negative	Negative	Negative

Optimum Conditions for Efficient growth and K Solubilization of bacteria

Seven bacterial strains i.e., MPS1C2, MPS2C5, MPS2C4, MPS5C1, UPS1C1, UPS2C1 and UP53C1 were selected on the basis of their zone size, to check their efficiency of potassium solubilization under optimum conditions of pH, temperature and potassium sources used.

In order to determine the effect of different pH on the growth of isolated bacteria and zone of potassium solubilization the isolates were grown at pH range from 5, 6, 7, 8, 9. It was found that maximum growth and potassium solubilization was achieved at pH 7.0 with sharp decline in growth and potassium solubilization from pH 8 onwards.(Fig. 1). Maximum K solubilization was observed with bacterial strain UPS1C1 at pH 7.0 followed by strain UPS2C1.

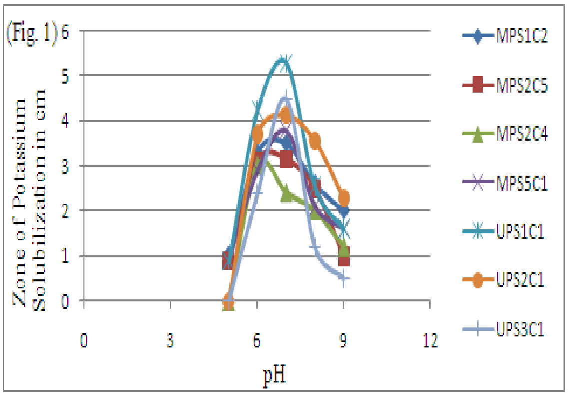


Fig. 1: Effect of different pH on Potassium Solubilizing Bacteria

In order to study the optimum temperature for the growth of bacteria and its ability to solubilize potassium the isolated bacteria were grown on modified Alekendrove broth at different temperature ranging from 25.30.35.40.45.50°C. It was found that bacterial strain UPS1C1 showed maximum solubilization (5.0cm) at 25°C and decreased with increase in temperature. Bacterial strain UPS2C1 showed maximum solubilization (4.13cm) at 28±2°C, whereas other bacterial strains showed significant solubilization in the temperature range of 25°C to 35°C. K solubilization decreased at higher temperature of incubation i.e., 45°C with all the bacterial strains. (Fig. 2)

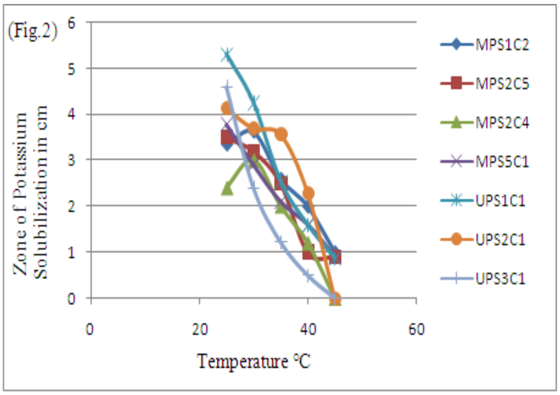
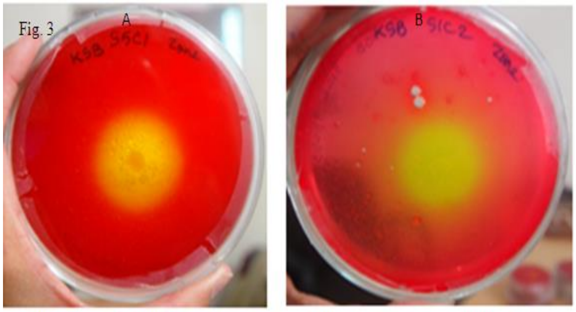


Fig. 2: Effect of different temperature on Potassium Solubilizing Bacteria

In order to show the effect of different forms of potassium on the growth and solubilization zone modified Alekandrove's agar media was supplemented with KCl, K_2SO_4 and $AlK(SO_4)_2 \cdot 12H_2O$ along with mica, it was found that K was solubilized maximum by all the bacterial strains in KCl and K_2SO_4 supplemented media than $AlK(SO_4)_2 \cdot 12H_2O$ and mica powder containing samples (Fig. 3).



A- Potassium Solubilizing Bacteria grown on KCl, B-Potassium Solubilizing Bacteria grown on K_2SO_4 media.

Fig. 3: Maximum zone of potassium solubilization was achieved in KCl and K_2SO_4

Identification of isolated bacteria: On the basis of different biochemical analysis performed (Catalase test, starch hydrolysis test, Gram staining, endospore and capsule staining and using Bergey's manule of determinative Bacteriology isolated bacterial strain are *Micrococcus varians* and *Corynebacterium kutscheri*

Effect of KSB on groundnut crop: Total 14 isolates were isolated from media. Seven isolates were selected according to their greatest K solubilization. They were numbered as MPS1C2, MPS2C5, MPS2C4, MPS5C1, UPS1C1, UPS2C1, UPS3C1. These isolates were tested for their solubilization capacity from alkaline leached soil. The maximum potassium solubilization potential was in UPS1C1. By incubating these bacteria with Groundnut the number of un-germinated seeds significantly decreased in the inoculated treatment (Table 5)

Table 5: Effect of KSB isolates on groundnut germination and growth

	Control	**Treated with KSB isolates**
Total ungerminated seeds(%)	30%	3%
Average root length (cm)	12.5	25
Average shoot length (cm)	20	45
Average root mass (g)	2	8
Average shoot mass(g)	10	30
Total no. of branches	6	18
Total no. of pods per plant	7	16
Total no. of seeds per plant	21	72

The average root and shoot length and mass is considerably higher in trial field treated with KSB and comparatively lower in control trial field. The study showed that there is 3.43 times more production in KSB treated field as compared to control one.

Discussions

For plant's better growth and development and to enhance crop's yields potassium is the major nutrient. Due to excess application of Urea and phosphatic fertilizers agricultural land is becoming eroded. Therefore we have made efforts to understand the beneficial effect of combination of both bacteria and rock material its combined application in soil have improved soil texture and increased crop yield. Among the 14 rhizobacterial isolates studied in this experiment, seven strains showed zone of clearance on modified Aleksandrov medium plates supplemented with mica powder (Table 1). Three bacterial isolates i.e., UPS1C1, UPS2C1 and UPS3C1 showed large zone of clearance around the colonies. Similarly, bacteria showing zone of clearance on mica plate have been isolated from the roots of cereal crops. (Mikhailouskaya, 2005) and soil (Zeng 2012).

The study of Optimum conditions for efficient solubilization of potassium by seven bacterial isolates shows that MPS1C2, MPS2C5, MPS2C4, MPS5C1, UPS1C1, UPS2C1, UPS3C1 showed highest D/d ratio 3.13, 3.50, 2.0, 3.22, 5.00, 4.13, 3.75. Zone of potassium solubilization was comparatively less on mica plate media with all the seven bacterial strains. Bacterial strain UPS1C1 caused maximum zone of potassium solubilization at 25°C to 28°C, whereas strain MPS2C5 caused maximum zone of potassium solubilization at 30°C. Zone of potassium solubilization decreased at 45°C with all the bacterial strains. The zone of potassium solubilization was found to be maximum when bacteria were grown in a media having optimum pH 7.0. With the increase in pH of the medium, growth of bacteria and zone of potassium solubilization decreased considerably.

Other workers also reported similar difference in potassium solubilization. Sheng and Huang (2002), reported that its pH and dissolve oxygen which releases potassium from minerals. When treated with KSB it was found that potassium increased by 84.8 to 127.9 per cent when compared with the control. Potassium solubilizing bacterial strains resulted in release of 35.2 mg L-1 potassium in 7 days at 28°Cat pH range from 6.5-8.0. Badr (2006) reported that there is release of 35.2mg/L of potassium and 490mg to 758mg/l in seven days by phosphorus silicate solubilizing bacteria at pH 6.5 to 8.0.

When mica was replaced with KCl and K2SO4 it was found that maximum K solubilization was obtained with KCl as compared to mica which in term, is another field of study to understand the behavior of bacteria with different substrate.(Liu, 2006). The above result implies that more efforts has to be made towards the behavior of bacteria in soil and its beneficial effect on plant growth. It is important to select those efficient bacterial strains from rhizospheric region which could adopt to the particular conditions of the soil. Thus, potassium solubilizing bacteria isolated and studied in this experiment will be further used in field trial for better adaptation and screening of more efficient bacteria. This may lead to improve the soil condition which is deficient of K nutrition.

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