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SOLUBILIZATION OF POTASSIUM FROM TWO DIFFERENT INSOLUBLE POTASSIUM SOURCE BY POTASSIUM SOLUBILIZING BACTERIA ISOLATED FROM BANANA RHIZOSPHERE SOIL.

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ABSTRACT Potassium, the vital and important macro nutrient required by plants, is associated with major physiological processes and required in large quantities for a crop to achieve its growth and yield. Plants absorb potassium in its ionic form K^+. Feldspars and micas are minerals that contain most of the K. Potassium solubilizing bacteria solubilizes these insoluble form of potassium to soluble forms for plant uptake. Isolation of potassium solubilizer was carried out from banana rhizosphere soil amended with orthoclase feldspar and muscovite mica. Among the six isolates all the isolates were characterized and screened by biochemical tests and three isolates exhibited highest solubilization zone, produced highest amount of organic acids along with significant amount of potassium released. The present study suggest that these three isolates can be used as efficient potassium solubilizer in sustainable agriculture.

KEYWORDS : Potassium Solubilizing Bacteria, Biofertilizer, Rhizosphere, Mica, Feldspar

INTRODUCTION:

Potassium the third essential macronutrient that influences most of the biochemical and physiological processes and plays a vital role in plant growth and metabolism. Physiological processes of plants including enzyme activation and stomatal activity, photosynthesis, transportation of sugars and building of proteins are associated with potassium nutrition. Inadequate supply of potassium leads to stunted growth, increased susceptibility to diseases and reduced crop yield [6,19]. Total number of potassium are abundant in Indian soil, however, major portion of potassium containing minerals viz., muscovite, orthoclase, biotite, feldspar, illite, mica are present in the soil as a fixed form which are not directly taken up by the plant and thus very small amount of potassium are available to the plants. Depending on soil type, approximately 90-98 % of total soil K is found in non-exchangeable form. In nature, soil has limited potassium and expensive synthetic fertilizers along with its adverse effects leads to the substitution of potassium. Therefore, emerging importance of Potassium solubilizing bacteria are increasing in sustainable agriculture. Potassium solubilizing bacteria are rhizospheric microorganisms which are able to release potassium from insoluble potash minerals [16,19,1]. Potassium - solubilizing bacteria (KSB) undergoes few number of biological processes to make potassium available from unavailable forms. Total pool of soil potassium is extremely complex and this can be solubilized by KSB through production of acids and it will be available for plant [17,2,15]. Potassium solubilizers are diverse group of microorganisms was reported to involved in potassium solubilization of insoluble and fixed forms of potassium into available form of potassium which are easily absorbed by plants [5,20,4]. Potassium solubilizers that are able to solubilize potassium from rocks and minerals can enhance the yield and growth of plants, are also ecofriendly and economically viable. Therefore, the present study has taken up to isolate KSB from banana rhizosphere amended with two different insoluble potash minerals.

MATERIALS AND METHODS:

The experiment was conducted at Department of Agricultural Microbiology, UAS, GKVK Campus, Bengaluru. Banana rhizosphere soil were collected in a polythene cover from UAS, GKVK Campus, Bengaluru. Muscovite mica and Orthoclase feldspar were procured from Indian Bureau of Mines, Bengaluru which were powdered into 200 meshes. Muscovite mica and Orthoclase feldspar were mixed separately with banana rhizosphere soil which were used for isolation.

Enrichment of the sample:

Collected soil sample were mixed with insoluble potassium minerals (Orthoclase feldspar and muscovite mica) and incubated for one week at room temperature. After adaption 1gm of soil was inoculated in 100ml liquid medium containing 1 % glucose, 0.05 % yeast extract and 0.5% feldspar and mica and incubated at 37^0C on 120 rpm for 1 week.

Isolation and screening of KSB :

Enriched samples were inoculated by serial dilution technique followed by pour plate method on Aleksandrov medium and incubated at 37^0C for one week. Colonies exhibiting clear zone of potassium solubilization were selected as potassium solubilizers. Secondary screening was carried out on the basis of study of zone activity of the different isolates by using Khandeparkar's selection ratio.

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

Solubilization efficiency was calculated by using the following formula

% Solubilization efficiency = Solubilization diameter /growth diameter X100

Solubilization index was calculated by using the following formula

$$\text{Solibilization Index (SI)} = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter}}$$

Identification of KSB:

All the bacterial isolates were identified by different cultural, microscopically and based on biochemical characteristics.

Release of potassium from KSB isolates:

Potassium released in the broth cultures of the bacterial isolates were determined by flame photometer.

Detection of organic acids produced by KSB isolates:

Organic acids viz., oxalic acid, citric acid, pyruvic acid, tartaric acid, malic acid and succinic acid were detected by HPLC.

RESULTS:

KSB isolates exhibiting clear zone indicates the solubilization of potassium. Total six isolates were isolated and out of the six isolates two isolates showed the highest potassium solubilization (Table 1).

Solubilization of Potassium from insoluble potassium source:

All the KSB isolates showed solubilization of potassium from insoluble potassium sources in Aleksandrov medium. Among the six isolates, three isolates solubilized muscovite mica and other three had solubilized orthoclase feldspar. Isolate SAF and SBF showed highest solubilization zone and the solubilization was observed in Aleksandrov medium amended with feldspar. Maximum solubilization of muscovite mica was observed with the isolates SAM (Table 1).

Table1: Screening of Potassium Solubilization by KSB isolates

Isolates	Diameter of Zone of Clearance (D) mm	Diameter of Growth (d) mm	D/d (Ratio)	Solubilization Efficiency (%)	Solubilization Index (SI)	Slime Production	Shape & Nature
SAF	6.5	6.0	1.08	92.30 %	2.08	High	Rod
SBF	3.7	4.2	0.88	88.09 %	1.88	High	Rod
SCF	3.0	4.0	0.75	75.00 %	1.75	Low	Rod
SAM	3.9	4.7	0.82	82.97 %	1.82	High	Rod
SCM	3.1	4.0	0.77	77.50 %	1.77	Low	Rod
SDM	4.5	5.5	0.81	81.81 %	1.81	Medium	Rod
CD@5%	0.08	2.11	0.02	1.02	0.04		
SEM ±	0.03	0.68	0.60	0.33	0.01		

Slime production was observed in all the six isolates and it varied from high to low. Isolates SAF, SBF and SAM showed highest slime production whereas isolate SCM and SCF showed very low slime production. This result is in conformity with the findings of [9,16]. All the six isolates were found as gram positive rods.

Diameter of zone of solubilization exhibited by the six isolates was observed in varied range from 3.1 to 6.5. The result revealed that highest potassium solubilization was found with orthoclase feldspar compared to muscovite mica. Potassium solubilization was observed with the increase in incubation period. All the six isolates showed significantly good solubilization efficiency.

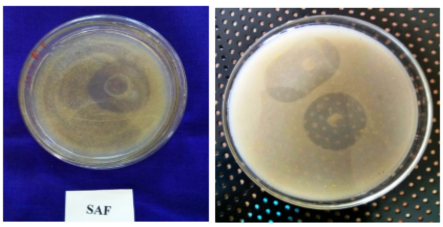

Plate 1: Solubilization of Potassium

Release of Potassium and effect of pH in broth culture

The data produced in Table 2 reveals the release of potassium and effect of pH in broth culture of KSB isolates.

Table2 :Effect of pH and release of potassium in broth culture by KSB isolates

KSB Isolates	Release of Potassium (ppm/ml)		Effect of pH	
	7 days	14days	7days	14days
SAF	18.53	**35.64**	6.7	5.3
SBF	3.78	8.12	6.8	6.2
SCF	2.33	6.53	6.9	6.5
SAM	5.09	8.85	6.8	5.7
SCM	1.33	5.43	6.8	6.5
SDM	2.45	7.07	6.7	5.8
CD@ 5%	17.51	34.76	14.42	12.91
SEM±	5.68	11.28	4.68	4.19

Significant decrease in pH was noticed in two KSB isolates in different incubation periods. Maximum decrease in pH was observed with SAF at 14 days incubation period followed by SAM and SDM. The decrease in pH may be due to production of organic acids in the broth culture by KSB isolates.

The release of potassium in broth culture was found significantly maximum in SAF compare to other KSB isolates. SAM, SBF and SDM also showed good amount of potassium released in both incubation period. The least amount of potassium release was noticed in SCM isolate.

Production of different organic acids by KSB isolates:

The data pertaining to the production of organic acids are given in Table 3.

Table3:Production of Organic acids by KSB isolates in broth culture medium

KSB Isolate	Production of Organic acid(ppm/ml)											
	Tartaric Acid		Citric Acid		Pyruvic Acid		Oxalic Acid		Maleic Acid		Succinic Acid	
	7days	14days	7 days	14days	7 days	14days	7days	14days	7days	14days	7days	14days
SAF	5.5	13.3	203.66	**592.7**	29.71	**69.80**	13.89	33.92	0.002	0.192	0.007	0.192
SBF	1.25	3.43	0.025	0.603	0.0001	0.01235	0.75	2.322	0.0001	**0.344**	**0.004**	**0.344**
SCF	2.1	3.51	46.12	113.6	0.053	1.17	9.25	15.8	0	0	0	0
SAM	6.62	**160.2**	82.56	121.8	0.087	3.02	72.15	**119.6**	0.0001	0.0599	0.006	0.0599
SCM	0.05	0.11	0.11	0.76	0.0001	0.02123	1.10	3.47	0.052	0.213	0.005	0.213
SDM	4.85	12.42	32.35	72.07	0.0001	0.0231	31.45	49.33	0.0003	0.125	0.003	0.125
CD@5%	0.16	0.53	0.82	550.11	26.16	61.83	70.71	119.08	0.00	0.00	0.00	0.00
SEM±	0.05	0.17	0.27	178.52	8.49	20.07	22.95	38.64	0.00	0.00	0.00	0.00

The results revealed that all the six KSB isolates had produced good amount of organic acids in broth culture medium. Highest organic acid production was observed in SAF and SAM isolates. Tartaric, citric, pyruvic, oxalic, maleic and succinic acid were determined in all the KSB isolates. Citric and pyruvic acid production was highest in SAF, whereas, Tartaric and oxalic acid was found highest in SAM. Production of maleic acid and succinic acid was very in low all KSB isolates, absolutely no production of maleic and succinic acid was observed in isolate SCF.

DISCUSSION:

The lowering of pH suggests the release of organic acids and protons by K solubilising organisms [3,20]. Rhizospheric microorganisms produced organic acids which leads to the dissolution of mineral K resulted in the slow release of exchangeable K, readily available exchangeable K or can chelate Si and Al ions associated with K minerals [12].The release of organic acids are related with microorganisms which solubilize insoluble form of potassium. The key processes of K solubilization includes acidolysis, chelation, complexolysis exchange reaction for the conversion of soluble forms [18] .The organic and inorganic acid production results in the solubilization of Potassium and the production of organic acids by potasssium solubilizing bacteria differs from other rhizospheric microorganisms [14]. Solubilization of Potassium from illite and feldspar by KSB isolates is due to the production of organic acid like oxalic acid, tartaric acid, gluconic acid, 2-ketogluconic acid, succinic acid, malic acid and citric acid [13]. Tartaric acid plays a vital role in mineral potassium solubilization [20,10,11]. Succinic acid, oxalic acid, citric acid, gluconic and ketogluconic acid are the key organic acids which are involved in the solubilization of insoluble K.

CONCLUSION:

Potassium availability in the soil is very low and very lesser amount of potassium is available to the crop plants as potassium are found in soil in a complex mineral form. Being the third and important macronutrient potassium is required for the plants to carry out different physiological processes. As potassium fertilization is very costly in India and the application of chemical fertilizer leads to decrease in soil health, there is a need to find alternative solution to this problem. KSB plays a great role in potassium fertilization. KSB solubilizes the insoluble form of potassium and made readily available to the plant in its ionic form. The results of the present study reveals that, KSB isolates which were isolated from banana rhizosphere soil amended with two different insoluble potassium source viz., muscovite mica and orthoclase feldspar. Among the six isolates, maximum solubilization, release of potassium, decrease in pH and production of organic acids was found significantly higher with the feldspar solubilization. Isolates associated with mica solubilization also showed good results. From this present study it can be concluded that, KSB isolated from rhizospheric soil has efficiency to solubilize orthoclase feldspar and muscovite mica and can be used as potassium solubilizers as a biofertilizer to the crop and can be replaced with chemical fertilizer.

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