iJRASET
International Journal For Research in Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 7 **Issue:** II **Month of publication:** February

DOI: http://doi.org/10.22214/ijraset.2019.2153

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 6.887
Volume 7 Issue II, Feb 2019- Available at www.ijraset.com

Isolation of Potassium Solubilizing Bacteria from Rhizospheric Soil

Jenifer Udhaya P[1], Auxzilia Preethi K[2]
[1, 2]*Department of Plant Biology and Biotechnology, Loyola College, Nungambakkam, Chennai-34*

Abstract: ***Potassium solubilizing bacteria helps in converting the insoluble K^+ into the soluble form. The objective of the study was to isolate, characterize and to assess for K^+ solubilization in soil bacteria. Rhizospheric bacteria with K^+ solubilizing ability were isolated from soil of Maize and Sorghum fields using Aleksandrov medium. Four bacterial isolates showing K^+ solubilization were characterized on the basis of colony morphology, biochemical and molecular studies. K^+ solubility index for the different isolates was calculated using Flame photometry.***
Keywords: Potassium solubilizing bacteria (KSB), Rhizospheric soils, 16Sr RNA

I. INTRODUCTION

Soil is the natural body, which contains a mixture of components like organic matter, minerals, liquids etc. The important minerals that are present in the earth crust are Nitrogen (N), Phosphorous (P) and Potassium (K). As we know that, Potassium is the third most important essential macronutrient in the earth's crust. Potassium is the fourth most abundant nutrient constituting about 2.5 percent of the lithosphere. However, the actual potassium concentration in the soil range from 0.04 – 3% (Sparks and Huang, 1985). The form of the silicate minerals (microcline, muscovite, orthoclase biotite and feldspars etc.) in soil exists with 98% of potassium. (Buchholz and Brown,1993). Potassium plays an important role in the development and metabolism of the plants. The major activities of the Potassium in soil to plant are: helps in the activation of the enzymes, helps in enhancement of photosynthesis, improves the yield of the crops, helps in the transfer of sugars and starch to the plant, helps in maintaining the cell turgor, helps in water holding capacity and resistant to pests. Without sufficient supply of Potassium, the plants will have poor development of roots, slow growth, produce small seeds and lower yield (White and Karley, 2010). Plants require Potassium ions for protein synthesis and for opening and closing of stomata.

Microorganisms play a major role in the potassium cycle naturally. Some species of the rhizobacteria have the capacity to converts the insoluble form of potassium to the soluble form in the soil. The plants can utilize the soluble form. Microorganisms like bacteria, fungi have a role in potassium solubilization.

II. MATERIALSAND METHODS:

1) *Sample Collection:* The rhizospheric soil samples were collected from Maize and Sorghum fields from Chittoor District- Andhra Pradesh. The samples were transferred to sterile polythene bags and stored for further use.
2) *Isolation Of Ksb From Rhizospheric Soils:* Potassium solubilizing bacteria were isolated from collected soil samples by serial dilution and plated onto Aleksandrov medium(HIMEDIA), which is a selective medium for isolation of KSB. The plates were incubated at room temperature (30±1°C) for 3 days. The selected isolates were tested for Potassium solubilizing activity.
3) *Characterization Of The Bacterial Isolates:* Bacterial isolates were characterized using colony, morphology, biochemical and molecular characteristics. The staining procedures and motility tests were performed for the bacterial isolates. Colony characteristics such as size, margin, opacity, elevation, consistency and color were examined. Biochemical characteristics such as catalase, oxidase, citrate utilization, urease, methyl red, VP test, indole and nitrate production, TSI and various carbohydrate fermentations were studied.
4) *Extraction Of Genomic DNA Of Bacterial Isolates:* Overnight bacterial culture was inoculated in Luria Broth (LB) and the cultures were kept in the shaker at 30°C and 120 rpm for 18h. The cells were harvested by centrifugation at 13000 rpm for 4 min. The supernatant was removed and 600µl of the Lysis buffer (TE buffer, 10% SDS, 20mg/ml Protienase K; pH 8) was added to the pellets and vortexed. The tubes were incubated for 30 minutes at room temperature and centrifuged after at 10000 rpm for 5 minutes. Precipitation was achieved using ethanol. The eppendorff tubes were incubated for 10 minutes at 20º and centrifuged at 13000 rpm for 5 min at 4ºC. The DNA pellets obtained were dissolved in 100µl of TE buffer and stored.
5) *Amplification Of Bacterial Isolates Using 16s R RNA Gene:* The universal primers used for the amplification of 16S r RNA gene are 27f and 1492r, 8f and 1490r. The PCR was performed for 30 cycles with initial denaturation at 94°C for 4 min,

denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec,elongation at 72°C for 1 min, post elongation at 72°C for 7 min The PCR product was electrophoresed and visualized using a Alpha Image Gel documentation unit. The sequences obtained were then compared using rdp (ribosomal Database Project) sequence database.

6) *K Solubilization Efficiency Of Bacterial Isolates:* K solubilization efficiency of bacterial isolates was assessed by spot test method. Aleksandrov medium was prepared of varying pH of 5, 7 and 9. A loop of bacterial isolate was spotted on the plates with different pH. The plates were incubated in the incubator for 7 days at 30°C. The clear zone indicated the solubilization of K in the media. The clear zone obtained can be used to calculated using Solubility Index.

$$SI = \frac{\text{Halozone diameter- colony diameter}}{\text{Colony diameter}}$$

7) *Flame Photometry:* K^+ release by bacterial isolates were studied in Alexandrove broth. The inoculated tubes were kept in the shaker for 72 hours at28±2°C.The amount of K^+ released in solution the bacterial isolate was analysed using Flame photometry.

III. RESULTS

In this study, the potassium solubilizing bacteria were isolated from the rhizospheres' soil of Maize and Sorghum. The bacterial cultures obtained were characterized and sequenced using 16S r RNA. The K solubilizing ability of the isolates were assessed by analyzing the Solubility Index (SI). The potential K solubilizer (potassium aluminosilicate) was studied at different pH and time duration.

A. Isolation of KSB from Rhizospheric Soils

Totally four bacterial isolates were obtained from rhizospheric soil of maize and sorghum collected from the fields of Chittoor district Andhra Pradesh. Potassium solubilizing bacterial isolates were obtained using Aleksandrov medium. Four isolates were selected based on colony morphology and named as SRSM11, SRSM21 SRSM41and SRSS21

B. Identification of KSB

The Gram staining results showed that three isolates were gram- negative rods and one isolate showed gram-positive rods. In accordance to the Bergey's manual and the biochemical results, the isolates SRSM11, SRSM21, SRSM41 and SRSS21 were identified to belong to *Enterobacter sp, Pseudomonas sp, Bacillus sp* and *Burkholderia sp* respectively. The colony, morphological and biochemical characteristics of bacterial isolates were shown in the table 1-2.

Table 1. Colony morphology of isolates

Colony Morphology	Bacterial Isolates			
	SRSM11	SRSM21	SRSM41	SRSS21
Size	Large	Small	Small	Small
Margin	Round	Round	Round	Wavy
Opacity	Translucent	Opaque	Opaque	Opaque
Elevation	Raised	Convex	Flat	Convex
Consistency	Moist	Moist	Moist	Moist
Color	Colorless	Yellow	Cream	White

Table 2.Biochemical characterization of bacterial isolates:

Biochemical Characterization		Bacterial Isolate			
		SRSM11	SRSM21	SRSM41	SRSS21
Catalase		+	-	+	+
Oxidase		-	-	-	+
Citrate Utilization		+	+	-	+
Urease		+	-	-	-
Indole Production		-	-	-	+
Methyl Red		+	+	+	+
Voges Proskauer		-	-	-	-
Nitrate Production		+	-	+	+
Carbohydrate fermentation	Lactose	-	-	-	+
	Sucrose	+	-	+	+
	Glucose	+	+	+	+
TSI	H_2S	-	-	-	-

All the isolates had the ability to oxidase glucose with the production of high concentration of the acid in methyl red test. In addition, all the isolates had the ability to degrade and ferment glucose with the production of acid and gas. Expect SRSM21, all the other isolates had the ability to degrade hydrogen peroxide by producing the enzyme catalase and to ferment citrate as a sole carbon source. Only SRSM11 had the ability to degrade urea by the enzyme urease.

C. Molecular Identification Of The Bacterial Isolates

The bacterial genomic DNA of the isolates was successfully extracted by physical method. The purity of DNA of three isolates showed A_{260}/ A_{280} values of slightly higher than 2.0 and one isolate showed A_{260}/ A_{280} value of 1.8. 2 % of agarose was used for gel electrophoresis to run the PCR products. The DNA samples extracted were used as template for PCR amplification for 16S r RNA gene. 27f and 1492r, 8f and 1490r primers are used for the amplification of 16S r RNA gene sequencing. In which, SRSM11, SRSM21 and SRSM41 are amplified using 27f and 1492r, SRSS21 was amplified using 8f and 1490r. The electrophoresis of PCR product for 16Sr RNA gene was shown in figure 1. The 16srDNA was sequence and BLAST was performed which correspond to Table.4

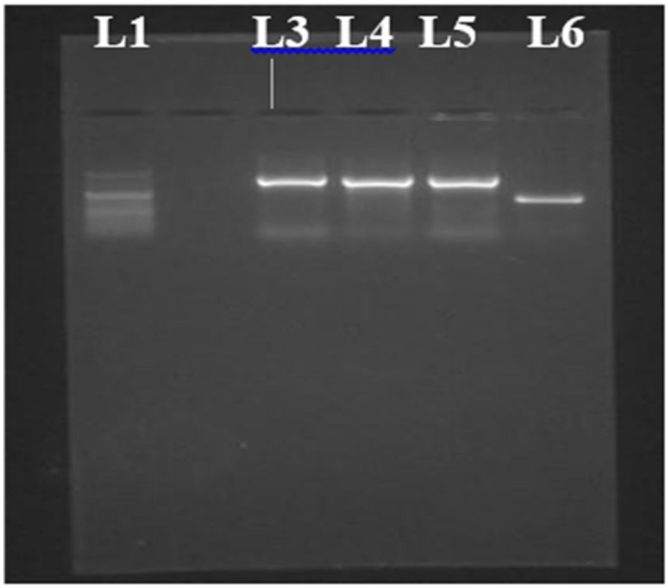


Figure 1: Electrophoresis of 16S r RNA PCR product
Lane 1- DNA ladder,Lane 2- SRSM11, Lane 3-SRSM21, Lane 4- SRSM41 and Lane 5- SRSS21

Table. 4 Sequence results for the obtained bacterial isolates:

Bacterial Isolate	Primer used	Obtained organism
SRSM11	27f and 1492r	*Enterobacter cloacae*
SRSM41	27f and 1492r	*Bacillus citrulans*
SRSS21	8f and 1490r	*Burkholderia seminails*

D. Assessment Of Potassium Solubilization Efficiency

Bacterial isolates were assessed for potassium solubilization at varying pH and time duration. The potassium solubilizing ability was tested by spot test method, which was performed using Aleksandrov medium. Solubility Index (SI) calculated clear zone formed by bacterial isolates. It was found that out of four bacterial isolates, one isolate showed the significant zone to solubilize potassium aluminosilicate.

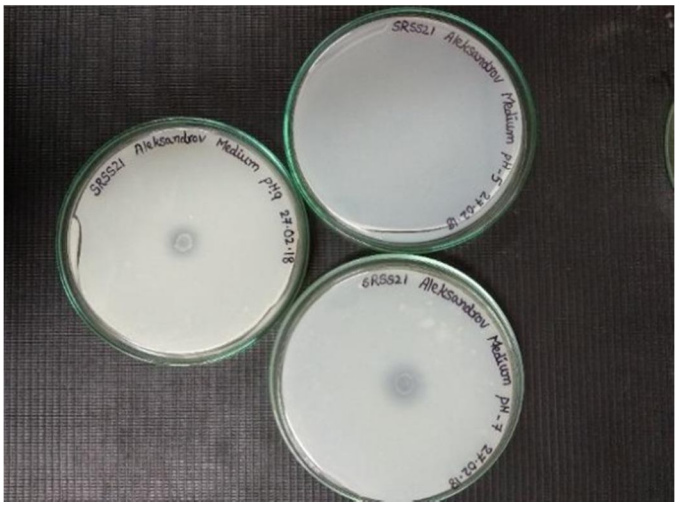
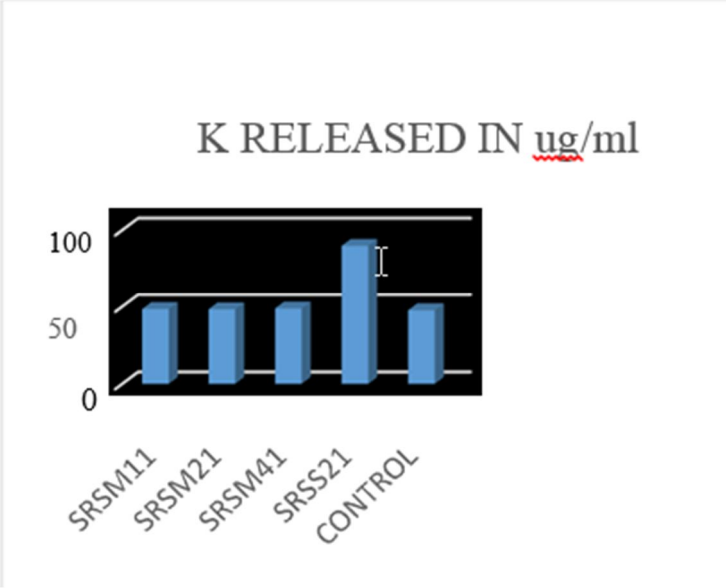


Figure 2: Solubilized zone of SRSS21 in Aleksandrov medium containing potassium aluminosilicate



Graph1. Amount of potassium released by the activity of the isolates.

ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 6.887
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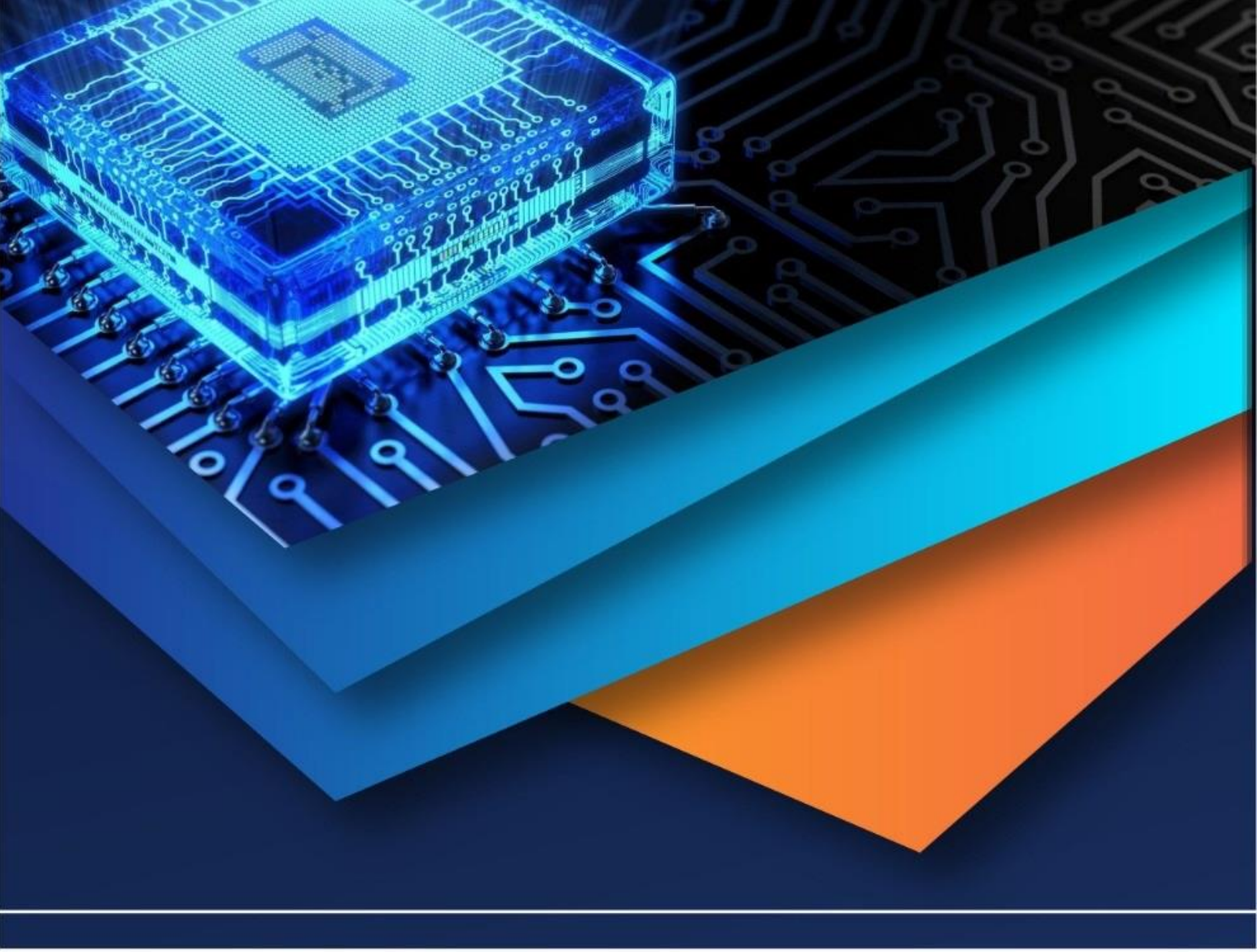
IV. DISCUSSION

Potassium solubilizing bacteria were isolated and characterized from rhizospheric soils of Maize and Sorghum. K^+ solublizers are natural rhizosphere inhabitants which can uptake and enhance plant growth in complex mineral deficient environmants.Four K^+ solublizer were assessed for their potassium solublization using spot test and flame photometry. Studies show that bacteria dissolves complex rocks by releasing acids and thereby enhance K^+ release in to soil. Out of the four isolates only SRS21 showed activity in the medium. Solublisation was also tested at pH 5, 7 & 9. SRS21 showed generous solublization at pH 5& 7. This has been already reported in Liu et al,2006 who recorded that bacteria produce large amount of acids which leads to dissolution of minerals. Results show the measured zone of clearance decreased as the pH increased. This is indicative that under acidic environment dissolution of minerals occur. The assessment of K solubilization of the bacterial isolates were done after 72 hours of incubation by flame photometry. The amount of K in the control was found to be 47.62 ug/ml. SRSS21 showed the highest K^+ solubilization after 72 hours, which was around 89.84 ug/ml. Bacteria possess differential ability to solublise minerals they encounter. From the current investigation it is evident that SRSS21 is a potential K^+ solublizer, from the amount of K^+ released into solution. K^+ solublizers can be used in agricultural formulations for plant growth enhancement.



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