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Characterization of Potassium Solubilizing Bacteria from Rhizospheric soils of walnut (Juglans regia L.)

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ABSTRACT

Potassium (K) is one of the essential nutrient for plant growth and development. It is available in various forms in soil like K ions in the soil solution, as an exchangeable cation, tightly held on the surfaces of clay minerals, organic matter and tightly held by weathered micaceous minerals. Soil contains large amount of K than other nutrients. The concentrations of soluble potassium in the soil are usually very low and more than 90 per cent of potassium in the soil exists in the insoluble form. The rhizospheric bacteria have the only ability to dissolve potassium from insoluble or locked minerals. Rhizosphere bacteria of walnut (Juglans regia L.) have been found to dissolve potassium from insoluble K- bearing minerals. In this study bacterial isolates were obtained on modified Aleksandrov medium containing mica powder as potassium source. From the 32 isolates 8 bacterial strains (KSB1, KSB2, KSB3, KSB9, KSB10, KSB11, KSB12 and KSB13) were selected which showed highest Zone of Clearance on Aleksandrov medium and were Characterized on the basis of morphological and biochemical Characteristics.

Keywords: Aleksandrov medium, Mica powder, Organic matter, Potassium Solubilizing Bacteria (KSB), Walnut.

I.INTRODUCTION

Global crop production has Intensive cultivation practices like the use of chemical fertilizers have improved crop yield, but contaminated both food and the environment, thus leading to a global food crisis[8][21] .After Phosphorus (P), and Nitrogen (N), potassium (K) is the essential nutrient which plays important role in growth and development of plants .In addition to disease resistance ,pests, and abiotic stresses, K is required to activate over 80 different enzymes responsible for plant and animal processes .E.g. such as energy metabolism , nitrate reduction, photosynthesis, and sugar degradation [6][1][13][23][10].Although K is present as an abundant element in soil, only 1 to 2 percent of this element is available to plants [5] rest is in the locked condition. Since cost of K-fertilizers (the price of potash $470 per ton since 2011) is increasing every year [18]and these chemical fertilizers have harmful effect on environment ,so it is necessary to find the way for maintaining availability of potassium in soil for sustainable plant growth. Potassium solubilizing microorganisms (KSM) can help in enhancing the availability of nutrients playing an essential role in dynamic soil environment by contributing release of key nutrients from primary minerals and ores. These key macronutrients are central for nutrition of microbial population present in the soil and in turn also benefit to plant nutritional status[19][3][16]

.In addition to release of plant growth regulating substances ,antibiotics, biodegradation of organic matter and nutrients cycling in the soil by KSM can also be benefited for crop productivity and ecological sustainability [4].Among the microorganisms, potassium solubilizing bacteria have attracted the attention of agriculturalist as inoculum to promote the plant growth and yield. The KSB are effective in releasing potassium from inorganic sources of K by solubilization [15][17][9] .It has been reported that the inoculation with these KSB strains produced beneficial effect on growth of various different plants [12][22]. Mechanism of K-solubilization could be mainly attributed to excrete organic acids which either directly dissolves rock K or chelate silicon ions to bring K in to solution [7]. Therefore the objective of this research was to isolate, characterize KSB from rhizospheric soils of walnut (Juglans regia L.).

II.MATERIAL METHODS : SOIL SAMPLING

The soil samples were taken from the root attached soils of walnut (Juglans regia L.). Some top surface soil was removed before the collection of soil samples from walnut tree fields. The surface soil was digged to 10 cm soil layer, were roots of walnut trees are concentrated .From about 0 to 2.5 mm away from the root surface, a zone of soil is located that is significantly influenced by living roots and is referred to as the rhizosphere .Rhizosphere soil and roots were separated from the bulk of the soil by hand. The 20 samples from 10 locations were taken randomly in every block. All the samples were sealed in a zip lock bags stored in fridges and were used in 10-20 hours .All the samples were collected from May to July 2016.

2.1ISOLATION OF K SOLUBILIZING RHIZOBACTERIA

Modified Aleksandrov medium, with waste mica powder as a sole source of K, was used to screen K - solubilizing bacteria. The serially diluted soil samples were plated on modified Aleksandrov medium which contain, 5g glucose, 0.005g $MgSO_4.7H_2O$, 0.1g $FeCl_3$, 2.0g $CaCO_3$, 3g mica powder as a sole source of K, 2g Ca_3PO_4 and 20 g agar [20].The plates were then incubated at 28 ± 2^0C.After seven days the colonies showing formation of Clear Zones around them were considered to be KSM and selected for further studies [11] .Screened isolates were gram stained for presumptive identification and pure colonies were transferred to sterile slants on nutrient agar medium. Further in another set of experiments, Zone of solubilization of purified KSB isolates were measured, using a scale after 7 days of plating on Modified Aleksandrov medium .The diameter of the solubilization zone was measured in centimeter.

2.2 CHARACTERIZATION OF THE POTASSIUM SOLUBILIZING BACTERIA

The identification of the different bacteria, isolated from rhizospheric soils of walnut (Juglans regia L.) was made based on its Morphological and Biochemical Characteristic studies.

2.3 SCREENING OF POTASSIUM SOLUBILIZERS ON THE BASIS OF ZONE RATIO

Screening of potassium solubilizing bacteria were done on Aleksandrov medium on the basis of zone ratio (Zone diameter /colony diameter) and solubilization index [20]

2.4 MORPHOLOGICAL CHARACTERIZATION

All the selected isolates were examined for the colony morphology, cell shape, gram reaction and ability to form spores as per the Standard procedures given by [2].

2.5 BIOCHEMICAL CHARACTERIZATION

The Characterization of the isolates were carried out as per the procedures outlined by Bergey's manual of systematic Bacteriology 9th Edition (1993).MR-VP test, Urea hydrolysis, Gelatine hydrolysis, Starch hydrolysis, Casein Hydrolysis, Hydrogen sulphide production, Acid production ,Gas production, Utilization of sucrose, maltose, citrate were performed**.**

2.6 EFFECT OF pH ON GROWTH OF K SOLUBILIZER

The effect of pH was studied by using nutrient broth and nutrient agar medium and pH adjusted to the required level by 0.1M HCl and NaOH. Medium was inoculated with 1 loopful of previously activated bacterial culture in 10 ml N. broth medium and nutrient gar plates with different pH were streaked and then incubated at 37 ^{o}C for 48 hours. Then the growth was checked visually.

2.7 EFFECT OF TEMPERATURE ON GROWTH OF K SOLUBILIZERS

The effect of temperature was studied by using nutrient broth and nutrient agar medium. Medium was inoculated with 1 loopful of previously activated bacterial culture in 10 ml N. broth medium and nutrient agar plates were streaked and incubated at 37 ^{o}C for 48 hours. Then the growth was checked visually.

III.RESULT AND DISCUSSION

3.1 ISOLATION AND SCREENING

Colonies exhibiting zone of clearance indicating potassium solubilization were selected, colonies were selected which are morphologically different. A total of 32 different type colonies were isolated on different media like Nutrient agar, kings B, YEMA. The isolated colonies were streaked on media and then pure isolated colonies were tested for K solubilization on modified Aleksandrov medium which contains Mica powder as K source. Out of 32 colonies 8 showed the highest zone of Clearance**.**

TABLE.1. ZONE OF CLEARANCE SHOWN BY EIGHT KSB ISOLATES.

KSB Isolates	Diameter of zone of clearance (cm)	Diameter of colony (cm)	Solubilization Index (SI)
KSB1	3.50	1.00	4.50
KSB2	2.30	0.80	3.88
KSB3	1.50	0.50	4.00
KSB9	1.50	0.60	3.50
KSB10	1.20	0.60	3.00

KSB11	1.00	0.60	2.67
KSB12	1.50	0.60	3.50
KSB13	1.90	0.70	3.71

Fig 1: Potassium Solubilization on Aleksandrov Medium.

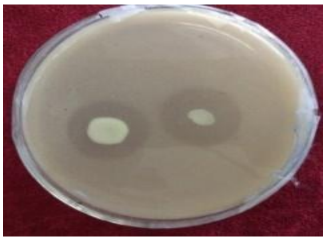

TABLE.2: COLONICAL AND MORPHOLOGICAL CHARACTERISTICS OF ISOLATES:

isolates	Pigment- -ation	Margin	Spore formation	Gram reaction	Slightly Raised	Highly raised	Transparent	Opaque
KSB1	Creamy	Entire	+	G +IVE	-	+	-	+
KSB2	White	Entire	+	G +IVE	+	-	-	+
KSB3	Whitish	Entire	+	G +IVE	+	-	+	-
KSB9	White	circular	+	G +IVE	+	-	-	+
KSB10	whitish	irregular	+	G +IVE	+	-	-	+
KSB11	creamy	circular	+	G +IVE	+	-	-	+
KSB12	White	circular	+	G+IVE	+	-	-	+
KSB13	creamy	Entire	-	G –IVE	-	+	-	+

TABLE 3: BIOCHEMICAL CHARACTERIZATION

Isolates	VP	MR	UH	GH	SH	CH	H2S	A.P.	G.P	70% NaCl	S	M	C
KSB1	-	+	+	+	+	+	-	+	-	-	+	+	+
KSB2	-	+	+	-	+	+	-	+	-	-	+	+	+

KSB3	-	+	+	+	+	+	-	+	-	+	+	+	+
KSB9	-	+	+	-	+	+	-	+	-	+	-	+	-
KSB10	-	+	-	-	+	+	-	+	-	-	-	+	-
KSB11	-	+	-	-	+	+	-	+	-	-	+	+	-
KSB12	-	+	+	+	+	+	-	+	-	-	+	+	-
KSB13	+	+	+	+	-	+	-	+	-	-	+	+	-

VP = V.P test, MR = Methyl red test, UH = urea hydrolysis, GH = gelatin hydrolysis, SH = starch hydrolysis, CH = Casein Hydrolysis, H_2S = Hydrogen sulphide production, 70% NaCl = growth at 70% NaCl, S = utilization of Sucrose, M = Maltose, C = Citrate. S = Starch hydrolysis, A.P = Acid production, G. P = Gas production.

TABLE 4: GROWTH OF ISOLATES ON DIFFERENT pH.

S. NO	pH	KSB1	KSB2	KSB3	KSB9	KSB10	KSB11	KSB12	KSB13
1	2	-	-	-	-	-	-	-	-
2	4	-	-	-	-	-	-	-	-
3	5	+	++	++	++	++	++	++	++
4	6	+	++	++	++	++	++	++	++
5	7	++	++	++	++	++	++	++	++
6	8	+	++	-	++	++	++	-	++
7	10	-	-	-	+	-	-	-	-
8	12	-	-	-	-	-	-	-	-

TABLE.5: GROWTH OF ISOLATES ON DIFFERENT TEMPERATURES

S.NO	Temp.	KSB1	KSB2	KSB3	KSB9	KSB10	KSB11	KSB12	KSB14
1	5 ^{0}C	-	-	-	-	-	-	-	-
2	10 ^{0}C	-	-	-	-	-	-	-	-
3	15 ^{0}C	-	-	-	-	-	-	-	-
4	20 ^{0}C	+	+	+	+	+	+	+	+
5	25 ^{0}C	++	++	+++	++	++	+++	++	++
6	30 ^{0}C	+++	+++	+++	+++	+++	+++	+++	+++
7	35 ^{0}C	++	++	++	++	++	++	++	++
8	40 ^{0}C	-	-	-	-	-	-	-	-

IV. CONCLUSION

Because of fixation 90 to 98 percent of potassium becomes unavailable to plants. Rhizospheric microorganisms contribute significantly in solubilization of locked form of soil minerals in the soil.in this study 32 bacterial strains were isolated from Rhizospheric soil of walnut (Juglans regia L.) out of them only 8 isolates (KSB1,KSB2,KSB3,KSB9,KSB10,KSB11,KSB12,KSB13) were found to Solubilize Potassium on Aleksandrov medium supplemented with mica. All the 8 isolates were Characterized morphologically, and Biochemically. These eight strains can be used as best Biofertilizers.

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