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## Isolation and screening of potassium solubilizing soil bacteria from Rhizosphere of paddy plant

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**Abstract-** Potassium solubilizing bacteria were isolated from the rhizosphere of the Paddy plant. Altogether seven isolates were collected from the 15 villages of Madhepura district. All isolates were cultured in Aleksandrow medium and colonies were further sub-cultured in nutrient medium. Morphological and biochemical test were performed for colony morphology, gram-staining, Citrate-utilization test, Voges-Proskauer test, Catalase test, Oxidase test and Methyl test were performed. For the determination of solubilizing efficiency and solubilisation index bacteria were cultured in Aleksandrow medium, Halozone and colony diameter were measured and Solubilizing Efficiency (SE) and Solubilization Index (SI) were calculated. The highest value was observed in isolate no. K<sub>1</sub> (SE=11.33, SI=4.581) and the minimum for isolate no. K<sub>4</sub> (SE=5.66, SI=2.698).

**Key words:** Solubilization Index, Solubilizing Efficiency, Citrate test, Catalase test, Oxidase test

### INTRODUCTION

Potassium is an essential macronutrient for plants. Among the three essential nutrients required by the plant one of them is Potassium. It plays a vital role in enzyme activation, osmotic regulation, opening and closing of stomata, starch synthesis, Protein synthesis and water nutrient transport. Soil contain large amount of potassium, however most of the Potassium is unavailable for plant uptake. Application of chemical fertiliser is one of the sources to fulfil potassium requirement of plants but chemical fertilizers have a considerable negative effect on soil. The eco-friendly agriculture system has emerged as an important thrust area globally for long-term soil environmental sustainability and to minimise the environmental pollution associated with extensive use of

chemical fertiliser to conserve our existing resources and to minimise environmental pollution hazards.<sup>1-5</sup> Hence, the production and management of bio fertiliser containing KSB can be an effective alternative to chemical fertiliser. It is well-known that potassium solubilizing bacteria *Bacillus subtilis*, *Bacillus mucilaginous*, *Bacillus megaterium*, *Paenibacillus macerans*, *Paenibacillus agglomerans*, *Pseudomonas aureofaciens*, *Pseudomonas fluorescens*, *Agrobacterium radiobacter*, *Azospirillum brasilense*, *Azospirillum lipoferum*, *Azotobacter chroococcum* etc. can solubilize Potassium which become available to Plants.

These soil bacteria are commonly known as KSB (Potassium Solubilizing Bacteria). The diversity and ability of KBS depends upon soil and climatic condition. KSB are able to dissolve silicate minerals and release K through the production of organic and inorganic acids,

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Acidolyses, Polysaccharides, Complexolysis, Chelation and exchange reactions.

**MATERIAL & METHOD**

The soil samples were collected from rhizosphere of Paddy plants from different fields. Each sample consisted of 200 g of soil. Samples were collected in septic bags and transported to lab for further processing. Details of soil sample and culture media used are mentioned in Table no. 01.

**Isolation of Potassium solubilizing bacteria:**

1 gm of soil sample was mixed thoroughly in 10 ML autoclaved distilled water. Serial dilution was made up to 10<sup>-6</sup>. Diluted soil samples were placed on Aleksandrow medium. The plates were incubated at 30°C for three days. Colonies exhibiting clear zones were selected as potassium solubilizer. Selected colonies were placed on nutrient agar medium for obtaining pure culture. The pure culture was again inoculated in Aleksandrow medium to confirm their K solubilizing efficiency.

**Estimation of Solubilisation index and Solubilizing efficiency:**

Qualitative analysis of K solubilizing activity of various isolates was calculated by measuring halozone and colony diameter on Aleksandrow medium. Halozone and colony diameter was measured using Calipers.

Calculation of Solubilization Efficiency (SE) and Solubilization Index (SI) were calculated by following formula:

$$SE = \frac{\text{Halozone} - \text{Colony diameter}}{2}$$

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

**RESULT**

A total of 15 soil samples were collected from different Paddy fields of following villages of Madhepura district: Rampatti, Chousa, Budhma, Murliganj, Barbana. Details of soil sample, Date of collection, Culture medium and Isolate code are described in Table no. 01. All together seven isolates were selected as K solubilizing bacteria for morphological and biochemical characterisation. All isolates were examined for colony morphology, gram-staining, Methyl red test, Voges-Proskaurtest, Nitrate reaction test, Catalase test and Citrate test. The morphological characters are tabulated in Table no. 2. Biochemical characters are tabulated in Table no. 3. The value of Potassium solubilizing efficiency and Potassium solubilization index are mentioned in Table no. 4.

**Table 1- Details of K-solubilizing Bacteria isolated from different soil samples**

S. No.	Soil Sample	Place	Date	Culture medium	Isolate code
1	S <sub>1</sub>	Rampatti	03-08-20	NA-AM	K <sub>6</sub>
2	S <sub>2</sub>	Barbana	05-08-20	NA-AM	K <sub>2</sub>
3	S <sub>3</sub>	Chousa	09-08-20	NA-AM	K <sub>4</sub>
4	S <sub>4</sub>	Rampatti	12-08-20	NA-AM	K <sub>1</sub>
5	S <sub>5</sub>	Rampatti	17-08-20	NA-AM	K <sub>6</sub>
6	S <sub>6</sub>	Budhma	22-08-20	NA-AM	K <sub>5</sub>
7	S <sub>7</sub>	Chousa	28-08-20	NA-AM	K <sub>3</sub>
8	S <sub>8</sub>	Barbana	05-09-20	NA-AM	K <sub>1</sub>
9	S <sub>9</sub>	Murliganj	08-09-20	NA-AM	K <sub>4</sub>
10	S <sub>10</sub>	Chousa	12-09-20	NA-AM	K <sub>7</sub>
11	S <sub>11</sub>	Barbana	18-09-20	NA-AM	K <sub>4</sub>
12	S <sub>12</sub>	Budhma	22-09-20	NA-AM	K <sub>6</sub>
13	S <sub>13</sub>	Murliganj	24-09-20	NA-AM	K <sub>7</sub>
14	S <sub>14</sub>	Budhma	26-09-20	NA-AM	K <sub>1</sub>
15	S <sub>15</sub>	Murliganj	29-09-20	NA-AM	K <sub>2</sub>

NA: Nutrient-Agar medium

AM: Aleksandrow medium

**Table 2- Morphological Character**

Sl. No.	Isolate	Form	Elevation	Colony size	Colony colour	Cell shape	Gram staining	Remark
1	K <sub>1</sub>	Regular	Flat	Small	White	Oval	-ve	Cocci
2	K <sub>2</sub>	Regular	Flat	Small	White	Oval	-ve	Cocci
3	K <sub>3</sub>	Irregular	Flat	Small	White	Rod	+ve	Bacillus
4	K <sub>4</sub>	Irregular	Flat	Small	White	Oval	-ve	Cocci
5	K <sub>5</sub>	Regular	Flat	Small	White	Oval	-ve	Cocci
6	K <sub>6</sub>	Regular	Flat	Small	White	Oval	-ve	Cocci
7	K <sub>7</sub>	Regular	Flat	Small	White	Oval	-ve	Cocci

**Table 3- Biochemical Character**

Sl. No.	Biochemical Test	KBS Isolates						
		K <sub>1</sub>	K <sub>2</sub>	K <sub>3</sub>	K <sub>4</sub>	K <sub>5</sub>	K <sub>6</sub>	K <sub>7</sub>
1	Citrate Utilization	+	+	+	+	+	+	+
2	Voges-Proskauer	+	+	+	+	+	+	+
3	Catalase	+	+	+	+	+	+	+
4	Oxidase	+	+	+	+	+	+	+
5	Methyl red	+	-	+	-	+	+	+

**Table 4- K-Solubilization efficiency and K-solubilization index of Bacterial Isolates**

S. No.	Isolate	Colony Size (mm)	Solubilization Zone (mm)	Efficiency (mm)	Solubilization Index
1	K <sub>1</sub>	6.33	29.00	11.33	4.581
2	K <sub>2</sub>	7.2	21.00	6.9	2.916
3	K <sub>3</sub>	6.45	25.00	9.27	3.875
4	K <sub>4</sub>	6.67	18.00	5.66	2.698
5	K <sub>5</sub>	6.57	22.00	7.71	3.348
6	K <sub>6</sub>	7.3	26.00	9.35	3.561
7	K <sub>7</sub>	6.75	23.00	8.12	3.407

**CONCLUSION**

Potassium is one of the most essential nutrients for plant which is absorbed by plant from the soil. Potassium solubilizing bacteria fulfil the requirement of Potassium by the plants. In the present investigation, altogether 7 isolates of Potassium solubilizing bacteria were isolated from rhizosphere of Paddy plant. All isolates were tested for their morphology, biochemical test and Potassium solubilizing capacity. One isolate (K<sub>3</sub>) was gram positive while all other were gram negative. The biochemical test for Citrate-utilization, Voges-proskauer, Catalase and Oxidase were positive for all isolates. Methyl test for isolates no. K<sub>2</sub> and K<sub>4</sub> were negative and for all other was positive. Solubilizing efficiency (SE) and Solubilization index (SI) was highest for isolate no. K1 (SE=11.33, SI=4.581). SE and SI of isolate no. K<sub>4</sub> was minimum (SE=5.66, SI=2.698).

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