Solubilization of Potassium by *Klebsiella quasipneumoniae* from *Triticum aestivum L.*

[1]Richa Mishra, [2]Twinkal Shriwas, [3]V. Shanthi
[1]Assistant Professor Microbiology, [2]Research Scholar Microbiology, [3]Assistant Professor Microbiology
[1]Department of Microbiology
[1]A.P.S.G.M.N.S. Govt. P.G. College, Kawardha-491995, Chhattisgarh, India
[1]richa.micro10@gmail.com, [2]twinkalshriwas21@gmail.com, [3]vshanthistc@gmail.com

***ABSTRACT*- Plants need potassium (K) for their growth and development because K is the third major and vital nutrient; however, its presence in the soil is 98% insoluble and only 2% soluble. From 10 bacterial isolates, only one bacterial isolate was selected which exhibited the highest K-solubilization on Aleksandrow agar medium and was characterized based on morphology, biochemically and identified as *Klebsiella quasipneumoniae* by 16S rRNA gene sequencing. The contribution of microbes in the solubilization of K by bounded minerals and providing soluble or available forms of K for plant uptake is opening a new way of improving soil fertility and quality crop productivity.**

Key Words: Solubilization, Potassium, Nutrition, Klebsiella quasipneumoniae.

I. INTRODUCTION

K- Ion in rich in soil with high adsorption of minerals [1]. Apart from nitrogen and phosphorus, potassium is the third macronutrient in plants and plays the valuable role in multiple biological functions such as protein synthesis, photosynthesis, enzyme activation and reduction of disease, drought and cold tolerance [2]. Only 1-2% of K available for plant uptake and 98% K bound with other minerals including mica, orthoclase and lignite, which are unavailable for plant uptake [3]. Some rhizospheric microorganisms perform central contribution in the solubilize nutrients in the soil [4]. Although some bacteria are present in the soil like, *Burkholderia, Pseudomonas* and *Bacillus* have been identified as K-solubilizer, because of the capability to mineralization, these bacteria are called potassium solubilizing bacteria (KSB) [5]. The level of K in the soil is decreasing by soil erosion, water runoff, leaching out and nutrient depletion, and soil infertility by imbalanced application of chemical fertilizer, if K-level scanty in soil or not available in adequate amount than plant growth and effected by the reduced yield and poor crop development, therefore it’s mandatory to use alternative source which can minimize this loss [6]. The KSB are able to solubilize K-bearing minerals rapidly and can preserve our extant resources and decrease the environment hazards caused by chemical fertilizer [7]. These KSB produced some organic acid, polysaccharide, siderophore and complexolysis to solubilize K-bearing minerals and for its survival in soil [8]. The higher concentration of KSB in rhizospheric soil can promote the crop quality and productivity [9]. Application of these KSB as K-biofertilizer can improve the growth and development of crop [10]. Thus, in-depth knowledge of understanding the KSB as current challenges in sustainable agriculture is must important [11].

Bacillus cereus was used as K-biofertilizer and gave best effects on potato growth in the field trials by [12]. *Paenibacillus mucilaginosus* as KSB was isolated from apple orchard and showed effects on apple seedling growth by [13]. Significant effects of *Klebsiella oxytoca* on the maize plant growth was studied by [14]. The evaluation of growth effects on garlic by Bacillus mucilaginosus was conducted during two seasons [15].

II. MATERIALS AND METHODS

Sample collection: The rhizospheric soil from the wheat rhizosphere was collected in the area of Durg district. Around twenty wheat rhizospheric soil samples (0-10” depth) were collected during the rabi season and brought to the laboratory of St. Thomas college, Bhilai, Chhattisgarh.

Adaptation and Enrichment: One gram of soil sample was mixed with 10 ml Aleksandrow broth containing potassium aluminium silicate, which gives the insoluble K-source and kept for one week at room temperature for enhancing the quality of solubilization and more fit for existence and adaptation.

Isolation and Screening: After enrichment, 1ml of enriched soil sample was diluted up to 10^{-5} suspension; each dilution was inoculated on Aleksandrow agar medium which is selective medium, constituted 0.50g $MgSO_4$, 0.10g $CaCO_3$, 2.00g Potassium aluminium silicate, 5.00g glucose, 0.005g $FeCl_3$, 2.00g $Ca_3(PO_4)_2$, 20g Agar, pH= 7.2, suspended in 1L distilled water and incubated at 30°c for up to 6 days.

Characterization of potent KSB: The isolates were characterized using different morphological, physiological, biochemical and other activity tests.

Morphological characterization: The selected isolates of KSB were cultured on Aleksandrow medium. Colony characteristics such as shape, forms, elevation, margin, colour, density and consistency were examined, gram staining, endospore staining were carried out for the isolates.

Biochemical characterization: The potent KSB were characterized by some biochemical parameters such as: methyl red test, Voges-Proskauer test, indole test, citrate utilization test, catalase test, nitrate reduction, starch hydrolysis, urease test and gelatin hydrolysis test.

Molecular Identification: A Pure culture of the selected isolate (W6-5) was sent to the National Collection of Industrial Microorganisms (NCIM), Pune, Maharashtra, for sequencing. The data of 16S rRNA gene sequencing were compared with the database at Gene Bank using BLAST-N search program in National Center Biotechnology Information (NCBI). And the phylogenetic analysis was constructed by the neighbour-joining method based on 1000 bootstraps.

III. OBSERVATION AND RESULT:

Isolation and Screening: A total of three efficient bacterial isolates were selected out of ten isolates found as K-solubilizers on the basis of zone formation around the colony.

Figure 01: Solubilization by KSB strain (W6-5, W10-3 & W10-8) on Aleksandrow medium

Table 01: Solubilization by Three KSB

S.N.	Isolates	Diameter of zone of the clearance (D), average value in cm	Diameter of Growth of the organism (d), average value in cm	(D/d) Ratio
1.	**W6-5**	2.9±0.03	0.5±0	5.8
2.	**W10-3**	1.4±0.03	0.3±0.03	4.6
3.	**W10-8**	1.8±0.03	0.6±0	3

Description: Best solubilization given by W6-5 with 5.8 cm.

Morphological and Biochemical Characterization: Three efficient isolates of KSB are studied by morphological and biochemical tests are presented in Table 02. Most of these isolates are non-spore forming and W6-5 is gram negative, while all isolates are gram positive and biochemical result of the three KSB are presented in Table 2.

Table 2: Morphological and Biochemical characteristics of efficient isolates

Isolates	Colony character	Morphological characters		Biochemical tests								
		Gram reaction	**Spore formation**	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**8.**	**9.**
W6-5	Circular, raised, entire, white, opaque, mucoid	-ve Rods	Non-sporulating	+	+	-	+	-	+	-	-	+/-
W10-3	Circular, convex, entire, cream, opaque, mucoid	+ve Round	Non-sporulating	+	+	-	+/-	-	+	-	-	+/-
W10-8	Circular, convex, entire, cream, opaque, mucoid	-ve Round	Non-sporulating	+	+	-	+/-	-	+	-	-	-

Description: 1- Methyl red test, 2- Indole test, 3- Voges-proskauer test, 4- Nitrate reduction test, 5- Citrate utilization test, 6- Catalase test, 7- Gelatin hydrolysis test, 8-Starch hydrolysis test, 9-Urease test

Molecular Identification and Phylogenetic Analysis: The best isolate of KSB was selected and identified using the maximum aligned sequence through BLAST search. The result has shown that, W6-5 have highest homology (100%) with *Klebsiella quasipneumoniae.* And phylogenetic tree was generated by MEGA 11 software.

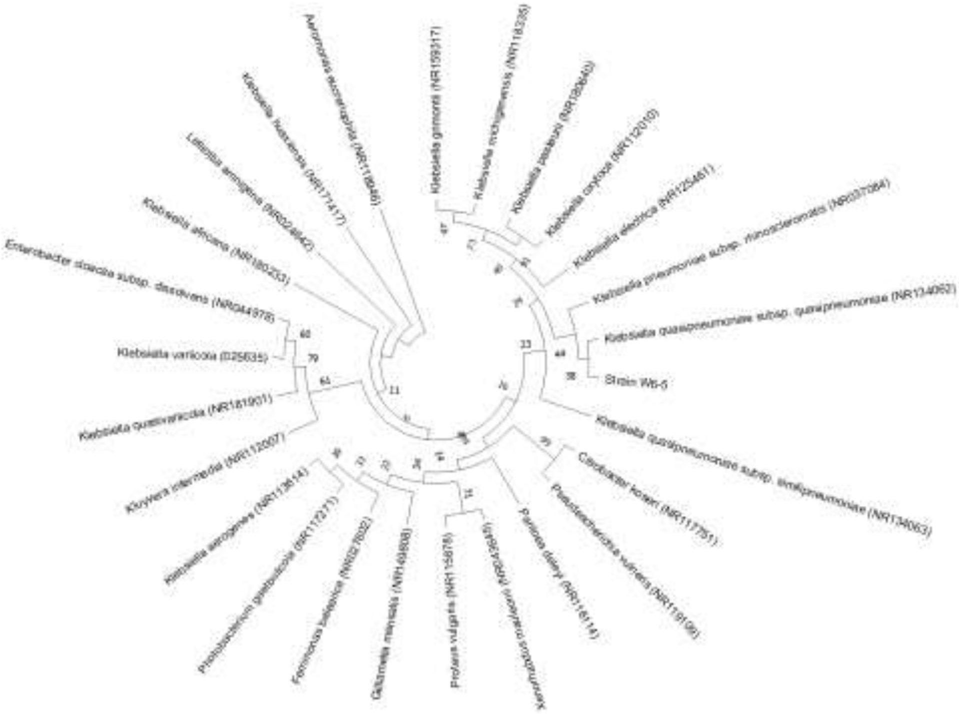

Figure 02: Comparative analysis of W6-5. The GenBank sequence accession numbers were indicated in brackets after the strain names.

IV. DISCUSSION:

For crop's better-quality growth and enhanced yield, K is the major nutrient. Due to imbalanced and excessive application of chemical fertilizer, agricultural land is becoming degraded therefore, it’s important to understand the beneficial application of soil microbes for improved soil health and yield [16]. Among the three rhizobacterial isolates, the best one showed the highest 5.8 cm in diameter zone of clearance around the colony on Aleksandrow agar plate, which was isolated from the wheat (*Triticum aestivum* L.) rhizosphere of the crop land area of Durg district (C.G.) 491001. The characterization and biochemical study of the efficient KSB strain was studied in this research and is shown in the Table No. 02. The zone of K-solubilization was found to be maximum an optimum pH of 7 and 30°c incubation temperature. The pure culture of the W6-5 strain of KSB was sent to NCIM for 16S rRNA gene sequencing with 1200bp and identified with 99.9% homology with *Klebsiella quasipneumoniae.* Phylogenetic tree based on comparative analysis of the 16S rRNA gene sequence, showing the relationship among strains W6-5 and other related species and genera. The tree was generated using the neighbour-joining method by MEGA-11 and includes the percentage bootstrap values generated. The KSB isolated and studied. In this research will be further used in bioformulation with pot and field trials in eco-friendly manner for better adaptation and substitute of K-chemical fertilizer. It can enhance the soil condition which is deficient of K-nutrition.

V. ACKNOWLEDGMENT:

The authors are thankful to the Department of Microbiology and Biotechnology, St. Thomas College Durg, Chhattisgarh, for providing all the facilities for the research. Authors are thankful to “Dr. Richa Mishra”, Assistant Professor, A.P.S.G.M.N.S. Govt. P.G. College, Kawardha, Chhattisgarh, for her valuable support and guidance. Also thankful to “Dr. V. Shanthi”, Head of the department of Microbiology and Biotechnology from St. Thomas College, Bhilai, for her cooperation and advice.

VI: REFERENCE:

[1] Prajapati and Modi (2012); Isolation and characterization of potassium solubilizing bacteria from ceramic industry soil. *CIBTech Journal of Microbiology*, 7-14.

[2] Das I and Pradhan M (2016); Potassium Solubilizing Microorganisms and their Role in Enhancing Soil Fertility and Health. *Potassium Solubilizing Microorganisms for Sustainable Agriculture*, 281-291.

[3] Meena VS, Bahadur I, Maurya BR, Kumar A, Meena RK, Meena SK, and Verma JP (2016); Potassium-solubilizing microorganism in evergreen agriculture: an overview. *Potassium solubilizing microorganisms for sustainable agriculture*, 1-20.

[4] Priyadharsini P and Muthukumar T (2016); Interactions between arbuscular mycorrhizal fungi and potassium-solubilizing microorganisms on agricultural productivity. *Potassium solubilizing microorganisms for sustainable agriculture*, 111-125.

[5] Sharma A, Shankhdhar D and Shankhdhar SC (2016); Potassium-solubilizing microorganisms: mechanism and their role in potassium solubilization and uptake. *Potassium solubilizing microorganisms for sustainable agriculture*, 203-219.

[6] Teotia P, Kumar V, Kumar M, Shrivastava N, and Varma A (2016); Rhizosphere microbes: potassium solubilization and crop productivity–present and future aspects. *Potassium solubilizing microorganisms for sustainable agriculture*, 315-325.

[7] Zahedi H (2016); Growth-Promoting Effect of Potassium-Solubilizing Microorganisms on Some Crop Species. *Potassium Solubilizing Microorganisms for Sustainable Agriculture*, 31-42.

[8] Etesami H, Emami S and Alikhani HA (2017); Potassium solubilizing bacteria (KSB): Mechanisms, promotion of plant growth, and future prospects A review. *Journal of Soil Science and Plant Nutrition*, 897-911.

[9] Rajavat MVS, Ansari WA, Singh D and Singh R (2019); Potassium Solubilizing Bacteria (KSB). *Microbial Interventions in Agriculture and Environment*, 189-209.

[10] Pandey D, Kehri HK, Zoomi I, Singh U, Chaudhri KL and Akhtar O (2020); Potassium Solubilizing Microbes: Diversity, Ecological Significances and Biotechnological applications. *Plant microbiomes for Sustainable Agriculture*, 263-286.

[11] Sun F, Ou Q, Wang N, Guo Zx, Ou Y, Li N, Peng C (2020); Isolation and identification of potassium-solubilizing bacteria from *Mikania micrantha* rhizospheric soil and their effect on *M. micrantha* plants, *Global Ecology and Conservation.*

[12] Ali AM, Awad MYM, Hegab SA, Gawad AMAE, Eissa MA (2020); Effect of potassium solubilizing bacteria (*Bacillus cereus*) on growth and yield of potato. *Journal of Plant Nutrition*, 411-420.

[13] Hui CY, Zhu YX, Zhang L, Hong AX, Peng MR, Qing LY and Gang CC (2020); Efficiency of potassium solubilizing *Paenibaciullus mucilaginosus* for the growth of apple seedling. *Journal of Integrative Agriculture*, 2458-2469.

[14] Imran M, Shahzad M, Arif MS, Yasmeen T, Ali B and Tanveer A (2020); Inoculation of potassium solubilizing bacteria with different potassium fertilization sources mediated maize growth and productivity. *Pakistan Journal of Agriculture Research*, 1045-1055.

[15] Mounir A, Osman YMM, Khalil O (2020); Impact of potassium solubilizing bacteria on growth and yield of garlic. *Plant Archives*, 8374-8388.

[16] Verma A, Patidar Y and Vaishampayan A (2016); Isolation and purification of potassium solubilizing bacteria from different regions of India and its effect on crop's yield. *Indian Journals of Microbiology Research*, 483-488.