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Potassium Solubilizing Bacteria Having Antagonistic Effect on Plant Pathogenic Fungi and Inducing Effective Brinjal Seed Germination

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Authors' contributions

This work was carried out in collaboration among all authors. Author KP and BM designed the study, performed the statistical analysis. Author DG managed the literature searches All authors read and approved the final manuscript.

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ABSTRACT

The aim of this research was to identify potassium-solubilizing bacteria (KSB) and evaluate their ability to release available potassium (K) from the soil for enhanced plant uptake. After assessing various strains for plant growth-promoting (PGP) traits and potassium-solubilizing effectiveness, five significant strains were selected. Using the BIOLOG system for further identification, it was determined that two of the most effective strains belong to the genus Bacillus. In laboratory conditions, potassium solubilization ranged from 1.89 to 46.52 μg/ml. The most effective strain, Bacillus licheniformis, achieved a potassium solubilization of 46.52 μg/ml, while Bacillus subtilis

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followed with 40.58 µg/ml. These KSB strains also demonstrated phosphate solubilization, IAA production, and potential for biocontrol against plant pathogenic fungi. In an *In vitro* experiment with brinjal seeds, three highly efficient KSB isolates (KSB-1, KSB-3, and KSB-20) with strong phosphate solubilizing and antagonistic abilities were tested. Bacillus licheniformis (KSB-1) showed the highest potassium solubilization, increased potassium content in plants, and improved growth parameters, with Bacillus subtilis (KSB-3) showing significant, though slightly lower, effects.

Keywords: Potassium; potassium-solubilizing bacteria; PGPR; antagonism.

1. INTRODUCTION

“Potassium (K) ranks as the seventh most abundant element in the earth’s crust, comprising approximately 2.5 percent of the lithosphere. However, actual soil concentrations of this vital nutrient vary widely, ranging from 0.04 to 3.00 percent” [1]. The highest concentrations of potassium in soils are found in insoluble rocks and minerals [2], such as micas, illite, feldspar, and orthoclases. Potassium plays a pivotal role in carbohydrate translocation, photosynthesis, water regulation, insect, and disease resistance, and maintaining the balance between monovalent and divalent cations [3]. “While potassium deficiency is not as widespread as that of nitrogen and phosphorus, the introduction of high-yielding varieties and hybrids during the green revolution, along with the progressive intensification of agriculture, has led to faster depletion of potassium reserves in soils. Extensive use of chemical fertilizers has been shown to degrade soil structure and exacerbate environmental pollution by contaminating groundwater. Various types of microorganisms inhabit soil, particularly the rhizosphere, and play a crucial role in plant growth and development. K-solubilizing bacteria could release potassium from insoluble minerals, contributing to nutrient availability for plants” [4].

“A wide range of KSMs *viz. Bacillus mucilaginosus*, *Bacillus edaphicus*, *Bacillus circulans*, *Paenibacillus spp*., *Acidothiobacillus ferrooxidans*, *Pseudomonas*, *Burkholderia”* [4-7] have been reported to release potassium in accessible form from K-bearing minerals in soils. “The release of various types of organic acids like oxalic acid, tartaric acid, gluconic acid, 2-ketogluconic acid, citric acid, malic acid, and succinic acid are responsible for solubilisation of illite and feldspar” [5].

“Identifying microbial strains adept at efficiently mobilizing potassium minerals while showcasing superior efficacy and plant growth-promoting traits offers a pathway to conserve resources and rejuvenate the productivity of degraded agricultural soils [8,9]. Excessive chemical fertilizer use often leads to soil degradation and reduced productivity. Thus, our investigation focuses on assessing the genetic diversity of proficient indigenous potassium-solubilizing bacteria from southern Gujarat” [10]. Furthermore, we aim to explore their effectiveness by employing these microbial strains as biocontrol agents to augment maize (*Zea mays* L.) productivity.

2. MATERIALS AND METHODS

“Samples of soil and root system from healthy rhizosphere of maize were collected in sterile plastic bags from ten plants each at random from the field. Each sample consisted of 100 g soil and 10 g roots. one gram of rhizosphere soil and root sample was mixed thoroughly in 100 ml sterile water and was processed following serial dilution agar plate technique” [11]. Suitable dilutions (10^{-5} and 10^{-6}) of both rhizosphere and rhizoplane solutions were plated on Aleksandrov medium [12].

“The potential of potent KSB isolates to solubilize insoluble potassium was assessed both by *In vitro* plate assay (qualitative) and in vivo broth assay (quantitative). To evaluate the potassium-solubilizing ability, the strains were placed on Aleksandrov agar media and the plates were incubated at 28 ± 2 °C for 5–7 days” [13]. The diameter of the bacterial colonies and halozones were measured and the values were used to calculate the solubilization index by following the formula:

SI = [Colony diameter + Halozone diameter]/ Colony diameter

“A quantitative estimation of potassium solubilization by the bacterial cultures was determined using flame photometry [14]. To the 100 mL of sterilized Aleksandrov broth bearing mica as a mineral potassium source in a 250 mL Erlenmeyer flask, 1% of bacterial suspension were inoculated and incubated at 28 ± 2 °C for 21 days. For each isolate, three flasks were inoculated on rotary shaker (150 rpm) at 30 ± 2 °C and after 7, 14, and 21 days, each flask was checked for potassium release by flame photometry" [4].

All the KSB strains were further assessed for multiple plant growth-promoting activities, viz., IAA production, GA_3 production, antagonistic activity and phosphate solubilization according to the standard methodologies [15,16].

"Brinjal seeds were surface sterilized using 1% sodium hypochlorite for 30 seconds, rinsed with sterilized distilled water, and dried overnight under a sterile airflow in a laminar flow hood. KSB isolates, grown in Nutrient Broth (NB), were transferred to screw-capped tubes with 100 mg of carboxymethylcellulose (CMC) as an adhesive. Five seeds were soaked in 10 ml of rhizobacterial suspension (10^8 cfu/ml) for 10 minutes [17]. Plant growth-promoting activity of the KSB isolates was evaluated using the seedling vigor index with the standard roll towel method. Bacterized seeds were placed on pre-soaked germination paper, covered with another pre-soaked paper strip, and gently pressed. The seeds, wrapped in a polythene sheet, were incubated in a growth chamber for 10 days, with three replicates per treatment. Seeds soaked in sterile distilled water served as control. Root length, shoot length, and germination percentage of the seedlings were measured" [13,18].

Biolog carbon substrates utilization patterns Biolog GP2 MicroPlates (Biolog, Inc., Hayward, CA, USA) were inoculated in duplicate using the standard procedures. Similarity index (SIM), correlated with genetic distance (DIS) was calculated among each strain and the 10 most closely related species present in the database. A correct identification was attained when the SIM and DIS values were 40.50 and 05.00, respectively [19].

3. RESULTS AND DISCUSSION

Twenty-five strains were isolated and purified from rhizospheres' soil samples using modified Aleksandrov agar media plates supplemented with mica as the sole source of potassium. These plates were then incubated at 28 ± 2°C to screen for potassium-solubilizing bacteria. After seven days, all strains exhibited distinct colonies with transparent halo zones, indicating their potassium-solubilizing ability. These strains, showing prominent halo zones, were chosen for further analysis and spot tested. Subsequently, the selected colonies underwent repeated subculturing to achieve single pure colonies and were purified using the streak plate method for subsequent studies.

The potassium-solubilizing potential of the identified potent KSB isolates was assessed by examining the formation of large clear or halo zones around bacterial colonies on Aleksandrov agar media plates. All isolates demonstrated efficient potassium solubilization. Among the isolates, KSB-1 recorded maximum solubilization index (5.20) followed by KSB-3 (5.00) and KSB-9 (4.60), KSB-17 (4.56), KSB-20 (3.78).

The isolates that displayed solubilization zones on Aleksandrov agar medium underwent further assessment for their capacity to release potassium (K) in broth media. Flame photometric analysis was employed to quantify the amount of potassium released from muscovite mica in the broth by the isolates at 7, 14, and 20 days after incubation under laboratory conditions. The documented potassium release ranged from 1.89 to 46.52µg/mL.

All the KSB strains were further assessed for multiple plant growth-promoting activities, viz., IAA production, GA_3 production, antagonistic activity, and phosphate solubilization and the results are summarized in the Table 2.

The results of phosphate solubilization indicated that the isolate KSB-1 showed highest solubilization index 2.63 when compared to other isolates KSB-3 (1.70) and KSB-12 (1.23). All KSB isolates were found to secrete IAA in the ranged between 13 to 26 µg/ml (Table 2). Highest concentration of IAA was observed from isolate KSB-1 (26 µg/ml) followed by KSB-3 (22 µg/ml). Lowest concentration of IAA was produced in KSB-18 (13µg/ml). Gibberellins are tetracyclic diterpenoid acids involved in several developmental and physiological processes in plants. Among all the tested 25 KSB isolates maximum GA_3 was found in KSB 3 and minimum in KSB 7. In order to study the antagonistic activity, inhibition tests with 25 KSB isolates against maize specific root rot fungi (*Macrophomina phaseolina*) were employed. A total of 7 KSB strains were found to exhibit antagonistic activity.

Efficacy of potent KSB on seed germination under *In vitro* conditions: In paper towel method, the KSB isolates KSB-1, KSB-3 and KSB-20 showed more than 85 per cent seed germination

Table 1. Solubilization index and *In vitro* solubilization assay of isolated KSB

Sr Number	KSB Isolates	Solubilization Index (SI)	Quantitative *In vitro* Solubilization Assay		
			7 DAI* (μg/ml)	15 DAI (μg/ml)	20 DAI (μg/ml)
1	KSB-1	5.20	21.32	43.29	46.52
2	KSB-2	1.20	0.56	2.36	3.69
3	KSB-3	5.00	25.76	38.56	40.58
4	KSB-4	1.30	3.24	4.98	5.55
5	KSB-5	0.50	3.58	6.70	7.19
6	KSB-6	1.00	3.16	5.55	5.90
7	KSB-7	2.60	2.90	6.80	7.89
8	KSB-8	3.24	7.36	18.39	21.46
9	KSB-9	4.60	6.48	19.65	30.21
10	KSB-10	1.35	0.96	1.98	5.22
11	KSB-11	2.64	2.98	7.41	11.63
12	KSB-12	3.25	4.26	14.67	21.12
13	KSB-13	3.68	8.71	18.22	26.87
14	KSB-14	2.70	6.06	13.58	20.21
15	KSB-15	2.80	3.15	5.21	6.38
16	KSB-16	3.17	6.78	10.68	22.21
17	KSB-17	4.56	18.66	26.32	39.76
18	KSB-18	1.20	0.98	2.58	3.62
19	KSB-19	2.30	11.69	19.65	22.86
20	KSB-20	3.78	12.58	22.78	34.75
21	KSB-21	1.26	8.25	16.47	20.97
22	KSB-22	1.90	5.31	8.22	10.26
23	KSB-23	2.40	4.98	11.67	21.26
24	KSB-24	2.72	2.68	4.26	4.76
25	KSB-25	3.28	3.97	13.68	22.62
26	control	--	0.06	0.09	0.09

**DAI- Days After Inoculation*

and produced higher shoot and root length as well as fresh and dry weight of brinjal seedlings with enhanced vigour index after 15 days [20]. The maximum vigour index of 20.00 was recorded in KSB1 treated seeds followed by 16.64 and 17.82 vigour index in KSB 20 and KSB 3 treated seeds respectively. Least germination (68.00%), less shoot and root length as well as less fresh and dry weight of seedlings with less vigour index of 8.55 was observed in untreated control.

Identification of potent isolates using Biolog system: The Biological system is one of the most versatile and authentic system for the identification of various microorganism on the basis of its metabolic profile [21,22]. The plate contains 26 sugars, two hexose phosphate, nine amino acids, nine hexose acids, 18 carboxilic acids, testers, and fatty acids, two acidic pH (5 and 6), three level of NaCl (1%, 4% and 8%), lactic acid, reducing agent (Tetrazolium blue and Tetrazolium violet) and antibiotics. "Potent bacterial isolates were analysed in the Biolog system and were scanned after 36-48h after incubation as per the instructions of system manual. Software assisted computation identified KSB-1 isolate as *Bacillus licheniformis*. The isolate KSB-3 was identified as *Bacillus subtilis"* [4].

Table 2. Studies of PGPR attributes of KSB isolates

Sr. Number	KSB Isolates	Solubilization index (SI) for Phosphate	IAA production (μg/ml)	GA3 (μg/mL)	PGI* %
1	KSB-1	2.63	26.00	0.832	12
2	KSB-2	-	14.80	0.420	-
3	KSB-3	1.70	22.17	0.947	10

Sr. Number	KSB Isolates	Solubilization index (SI) for Phosphate	IAA production (µg/ml)	GA3 (µg/mL)	PGI* %
4	KSB-4	-	20.67	0.552	-
5	KSB-5	-	13.67	-	-
6	KSB-6	0.62	14.35	-	8
7	KSB-7	-	15.00	0.253	-
8	KSB-8	-	19.65	0.621	-
9	KSB-9	0.80	19.00	0.880	-
10	KSB-10	0.74	15.73	0.598	-
11	KSB-11	0.10	17.85	0.927	-
12	KSB-12	1.23	14.32	0.878	-
13	KSB-13	-	19.27	0.741	10
14	KSB-14	-	17.38	0.563	-
15	KSB-15	1.00	13.89	0.589	-
16	KSB-16	-	17.56	0.747	-
17	KSB-17	0.22	21.00	0.868	21
18	KSB-18	-	13.00	0.625	-
19	KSB-19	-	18.47	0.870	-
20	KSB-20	1.20	17.00	0.562	-
21	KSB-21	-	14.00	0.429	8
22	KSB-22	-	14.00	0.398	6
23	KSB-23	0.80	17.00	0.456	-
24	KSB-24	-	16.00	0.780	-
25	KSB-25	1.12	20.00	0.529	-

***** *PGI%: Percent Growth Inhibition*

Table 3. Plant growth promoting activity of selected KSB isolates in brinjal seeds under *In vitro* conditions (Paper towel method)

PGPR isolates	Germination (%)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	SVI
KSB-1	85.00	5.3	4.44	0.13	0.02	20.00
KSB-3	84.00	5.1	4.16	0.12	0.04	17.82
KSB-20	80.00	5.0	4.16	0.12	0.02	16.64
Control	68.00	3.4	3.7	0.09	0.01	8.55
S.Em ±	0.70	0.13	0.13	0.004	0.003	
CD@1%	2.11	0.39	0.40	0.014	0.011	
CV%	2.50	6.25	7.81	8.80	31.62	

4. CONCLUSION

In current research potassium solubilising bacteria were isolated and characterized. Further the efficiency of the isolates to solubilize insoluble mineral potassium, production of plant growth promoting substances and other agronomically beneficial traits were studied under laboratory condition. From morphological and biochemical characterization all the 25 isolates were identified up to genus level. The Biological system identified the two most effective isolates screened from the laboratory and pot trials as Bacillus subtilis and Bacillus licheniformis, respectively. Isolate KSB-1 was identified as such. With a solubility of 46.52µg/ml, KSB-1 was determined to be the most superior isolate out of all of them. Their innate, specific processes can greatly boost plant development and antagonistic action. Synthetic K fertilizers may be replaced with a microbial consortium consisting of these effective native strains.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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