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Screening of potassium and zinc solubilizing bacteria for plant growth promoting properties (PGPR) from different Rhizospheric soil

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Abstract
All the potassium and zinc solubilizing bacterial isolates were subjected to further studies to understand their Plant Growth Promoting Properties (PGPR) under *in vitro* conditions. Pure isolates were screened for mineral phosphate solubilization, siderophore production, ammonia production, IAA production and biocontrol activity. Among four KSB isolates 3 isolates were able to solubilize phosphate on Pikovskaya's media containing Tri calcium phosphate in the range of 7 mm to 9 mm. Among eight ZnSB isolates 2 isolates were able to solubilize phosphate on Pikovskaya's media containing Tri calcium phosphate in the range of 8 mm to 10 mm. Among four KSB isolates, KSB - 3 were positive for ammonia production in peptone water tubes. Among eight ZnSB isolates 2 isolates ZnSB-5 and ZnSB-6 were positive for ammonia production. IAA production varied with supplementation of L- tryptophan and without supplementation of L- tryptophan. Out of four isolates KSB-1 showed maximum IAA production (2.2 µg ml^{-1}) without supplementation of tryptophan, Enhancement in production of IAA was observed with the supplementation of L-Tryptophan @ 10 mg per liter by KSB-1 (6.60 µg ml^{-1}) and IAA production for ZnSB isolates were maximum for ZnSB-5 (3.516 µg ml^{-1}) without supplementation of L-tryptophan and 7.51 µg ml^{-1} with tryptophan. Out of four KSB isolates, KSB - 1 bacterial isolate produced siderophores moderately (++). Among eight ZnSB isolates 2 isolates ZnSB-1 and ZnSB-2 were strong (+++) for siderophore production Out of four KSB isolates, KSB-1 bacterial isolates produced HCN moderately (++) and among eight ZnSB isolates, 2 isolates ZnSB-4 and ZnSB-8 produced HCN. Antifungal activity of four potassium solubilizing bacterial isolates (KSB-1 to KSB-4) eight zinc solubilizing bacteria isolates (ZnSB-1 to ZnSB-8) were checked against *Rhizoctonia solani* under *in vitro* conditions using PDA media. Based on both characters inhibition and inhibition zone. The antifungal activity of strains tested varied with percent inhibition for KSB from 51.50 to 3.70 and for ZnSB 25.90 to 3.70.

Keywords: Potassium and zinc solubilizing bacteria, plant growth promoting properties, tryptophan, siderophore, antifungal activity

Introduction
Plant nutrition is the study of the mineral elements that are necessary for good plant growth and reproduction. Interaction between soil microbes and minerals play a major role in environmental cycling processes, which leads to the mobilization of nutrients from soil components into available forms for biological uptake. Plant nutrition is closely associated with the activity of plant growth promoting rhizobacteria (PGPR), including potassium and zinc solubilizing bacteria (KSB and ZnSB) which are used as bio fertilizer in many countries that where in soil are deficient in available potassium and zinc Plant growth promoting rhizobacteria (PGPR) are soil borne bacteria that colonize the rhizosphere, multiply and compete with other bacteria to promote plant growth (Kloepper and Okon, 1994) [6]. PGPR promote plant growth either by solubilizing and assisting nutrient acquisition or by releasing phytohormones or biocontrol agents to protect plant from various pathogens (Glick, 2012) [4]. Various PGPR have found to be effective zinc solubilizers. These bacteria improve the plant growth and development by colonizing the rhizosphere and by solubilizing complex mineral compounds into simpler ones, thus making available to the plants. Other mechanisms possibly involved in zinc solubilization include production of siderophores (Saravanan *et al*., 2011) [10] and proton, oxido-reductive systems on cell membranes and chelated ligands (Wakatsuki, 1995; Chang *et al*., 2005) [14, 2]. Various PGPR have shown enhanced growth and mineral content when inoculated in plants. These include Pseudomonas, Rhizobium strains

(Deepak *et al*., 2013; Naz *et al*., 2016) [3, 7], Bacillus aryabhattai (Ramesh *et al*., 2014) [8, 9], Bacillus sp. (Hussain *et al*., 2015), and Azospirillum. Keeping in view the above facts, this study was designed to identify and characterize pre-isolated bacteria from wheat and sugarcane for plant growth promoting (PGP) abilities, zinc solubilizing ability using plate assays and to evaluate the contribution (if any), of zinc solubilizing strains on growth and zinc content of wheat plants, through pot experiments.

Materials and Methods

Screening of isolates for plant growth promoting properties

Pure isolates were isolated by streaking isolates on respective media plates and screened for following Plant growth promoting properties.

Phosphate Solubilization

For this test sterilized Pikovskaya's agar was poured as a thin layer on to the sterilized petri plates and incubated for 24 h. After incubation the Pikovskaya's plates were spot inoculated with isolates and incubated at 28±1°C for 4-5 days. Formation of a clear zone around the colonies were considered as positive result for phosphate solubilization.

PSE (Phosphate Solubilization Efficiency) = Z / C x 100

Z- Clearance zone including bacterial growth

C- Colony diameter

Indole Acetic Acid Production

Indole acetic acid production was tested according to Gorden and Weber (1951). The active culture of each test isolate was raised in 5ml respective broth tubes and incubated at determined temperature and time. After incubation these cultures were centrifuged at recommended rpm and time. Two drops of O - phosphoric acid was added to 2 ml of supernatant and incubated for 30 min to develop the colour. Development of pink colour considered as positive for IAA production.

Siderophore Production

Siderophore production was estimated qualitatively. Chrome Azurol S (CAS) Agar medium (Schwyn and Neilands, 1987) [11]. For the detection of siderophores, each isolate was grown in synthetic medium, containing 0.5 µM of iron and incubated for 24 h on arotary shaker at room temperature. Chrome Azurol S (CAS) assay was used to detect the siderophores. The CAS plates were used to check the culture supernatant for the presence of siderophores. Culture supernatant was added to the wells made on the CAS agar plates and incubated at room temperature for 24 h. Formation of yellow to orange coloured zone around the well indicates siderophore production.

Hydrogen Cyanide Production (HCN)

The HCN production was tested by the method of Castric and Castric (1983) [1]. First respective media plates were prepared separately and incubated for 24h. After that, 1ml of culture of each test isolate was inoculated on respective media plates separately. A disc of whatman filter paper No.1 of the diameter equal to the petri plate size, impregnated with alkaline picric acid solution (0.5% picric acid (w/v) in 1% sodium carbonate) was placed in the upper lid of the inoculated petri plates under aseptic condition. The control plate did not receive the inoculum. The plates were incubated-upside up at 28±2°C for 48-72h. Change in colour from yellow to light brown, moderate or strong reddish brown was taken as indication of HCN production.

Antagonistic Activity

Pure isolates of common disease causing soil phytopathogens *viz*., *Rhizoctonia solani* were obtained from the Dept. of Plant Pathology, College of Agriculture, Rajendranagar Antagonistic activity was verified by following dual culture technique (Skidmore and Dickinson, 1976). First, the bacterial isolates were streaked on respective media plates and incubated at respective temperature and time. Loopful of each bacterial isolate was streaked on the potato dextrose agar plate at one end, which was pre-inoculated with 5 days old, 5mm mycelial disc of test fungal pathogen at the other end. Control plate was maintained by placing only pathogen mycelial disc in the centre without bacteria.

The assay plates were incubated at 28±1°C for 5 days and observations were made on inhibition of mycelial growth of the test pathogens. For each bacterial isolate three replications were maintained with suitable controls.

The per cent growth inhibition over control was calculated by using the formula:

$$\text{Percent Inhibition} = \frac{\text{Growth of pathogen in control (mm)} - \text{growth of pathogen in treatment (mm)}}{\text{Growth of pathogen in control (mm)}} \text{x100}$$

Note: In this the percent inhibition in control is taken as zero percent.

Result and Discussion

All the potassium and zinc solubilizing bacterial isolates were subjected to further studies to understand and their Plant Growth Promoting Properties (PGPR) under in vitro conditions. Pure isolates were screened for mineral phosphate solubilization, siderophore production, ammonia production, IAA production and biocontrol activity. Among four KSB isolates 3 isolates were able to solubilize phosphate on Pikovskaya's media containing Tri calcium phosphate in the range of 7 mm to 9 mm. Among 3 isolates KSB-3 recorded the highest solubilization zone (9 mm) followed by KSB-1 and KSB-4 (both 7 mm), KSB-2 do not solubilize phosphate (Table No. 1). Among eight ZnSB isolates 2 isolates were able to solubilize phosphate on Pikovskaya's media containing Tri calcium phosphate in the range of 8 mm to 10 mm (Table. 4.10 and Plate 4.6). Among 2 isolates ZnSB-6 recorded the highest solubilization zone (10 mm) followed by ZnSB-1 (8 mm) (Table No. 2). Among four KSB isolates, KSB - 3 were positive for ammonia production in peptone water tubes. The isolate KSB-3 produced ammonia moderately (++) (Table No. 1). Among eight ZnSB isolates 2 isolates ZnSB-5 and ZnSB-6 were positive for ammonia production. The isolate ZnSB-5 produced ammonia moderately (++) and ZnSB-6 was weak (+) ammonia producer and Plate 4.6) (Table No. 2). IAA production varied with supplementation of L- tryptophan and without supplementation of L- tryptophan. Out of four isolates KSB-1 showed maximum IAA production (2.2 µg ml -1) without supplementation of tryptophan, Enhancement in production of

IAA was observed with the supplementation of L-Tryptophan @ 10 mg per liter by KSB-1 (6.60 µg ml -1) (Table No. 1) and IAA production for ZnSB isolates were maximum for ZnSB-5 (3.516 µg ml -1) without supplementation of L-tryptophan and 7.51 µg ml -1 with tryptophan (Table 3 & 4 and Plate 5 & 6) (Table No. 2). Out of four KSB isolates, KSB - 1 bacterial isolate produced siderophores moderately (++). Among eight ZnSB isolates 2 isolates ZnSB-1 and ZnSB-2 were strong (+++) for siderophore production (Table No.4). Out of four KSB isolates, KSB-1 bacterial isolates produced HCN moderately (++) (Table No. 3) and among eight ZnSB isolates, 2 isolates ZnSB-4 and ZnSB-8 produced HCN. The ZnSB-4 was strong (+++) and ZnSB - 8 was moderately (++) HCN producer (Table No. 4). Antifungal activity of four potassium solubilizing bacterial isolates (KSB-1 to KSB-4) eight zinc solubilizing bacteria isolates (ZnSB-1 to ZnSB-8) were checked against Rhizoctonia solani under in vitro conditions using PDA media. Based on both characters inhibition and inhibition zone. The antifungal activity of strains tested varied with percent inhibition for KSB from 51.50 to 3.70 (Fig. 1) and for ZnSB 25.90 to 3.70 (Fig. 2). Similarly, Sharma *et al*. (2014) [12] assessed some selected Zn solubilizers for functional PGP traits *viz*. Indole acetic acid (IAA), P- solubilization and ammonium production in vitro condition using standard methods (Spaepen *et al*. 2007) [13]. In present study, 2 endophytic bacterial isolates *viz*. 1J (stem) and 19D (root) were identified as potent endophytic bacterial isolates with PGP traits *viz*. IAA, P- solubilization and NH 3 producers along with Zn solubilization. The results are in close agreement with the findings of Ramesh *et al*. (2014) [8, 9] who have also reported that three Bacillus aryabhattai strains MDRS7, MDRS11 and MDRS14 possessed IAA, siderophores and ammonia producing traits.

Table 1: Screening of KSB isolates for Plant Growth Promoting Activities *in vitro* Conditions

Isolate	Phosphate solubilization				Indole Acetic Acid production (IAA)	
	Zone diameter (mm)		Solubilization efficiency (%)	NH_3 Production	Without Tryptophan (µg ml^{-1})	With Tryptophan (µg ml^{-1})
	Solubilization zone	Culture diameter				
KSB-1	7	4	**175**	-	2.200	6.41
KSB-2	0	6	-	-	0.000	3.19
KSB-3	**9**	6	150	++	0.000	0.00
KSB-4	7	13	140	-	0.000	3.80

+ Weak production, ++ Moderate production +++ Strong production − No production IAA - Indole Acetic Acid KSB – Potassium Solubilizing Bacteria

Table 2: Screening of Zinc solubilizing bacterial isolates for plant growth promoting activities *in vitro*

Isolate	Phosphate solubilization				Indole Acetic Acid production (IAA)	
	Zone diameter (mm)		Solubilization efficiency (%)	Ammonia production	Without Tryptophan (µg ml^{-1})	With Tryptophan (µg ml^{-1})
	Solubilization zone	Culture diameter				
ZnSB-1	08.00	04.00	200.00	-	2.400	7.41
ZnSB-2	0.00	0.00	-	-	0.000	3.39
ZnSB-3	0.00	0.00	-	-	0.000	0.00
ZnSB-4	0.00	0.00	-	-	0.000	4.80
ZnSB-5	0.00	0.00	-	++	3.516	7.51
ZnSB-6	10.00	08.00	125.00	+	1.373	5.29
ZnSB-7	0.00	0.00	-	-	0.000	2.86
ZnSB-8	0.00	0.00	-	-	0.000	6.86
CD 5%	1.101				0.129	0.216

+ Weak production, ++ Moderate production +++ Strong production − No production IAA - Indole Acetic Acid KSB – Potassium Solubilizing Bacteria

Table 3: Study the biocontrol activity of KSB isolates

Isolate	Siderophore production	HCN production	Percent inhibition (%) of *Rhizoctonia solani*
KSB-1	++	-	03.70
KSB -2	-	++	03.70
KSB -3	-	-	51.50
KSB -4	-	-	22.20
Control			00
CD @ 0.05 probability			0.747
SE(m)			0.226
SE(d)			0.319
C.V.			1.92

+ Weak production, ++ Moderate production +++ Strong production − No production IAA - Indole Acetic Acid KSB – Potassium Solubilizing Bacteria

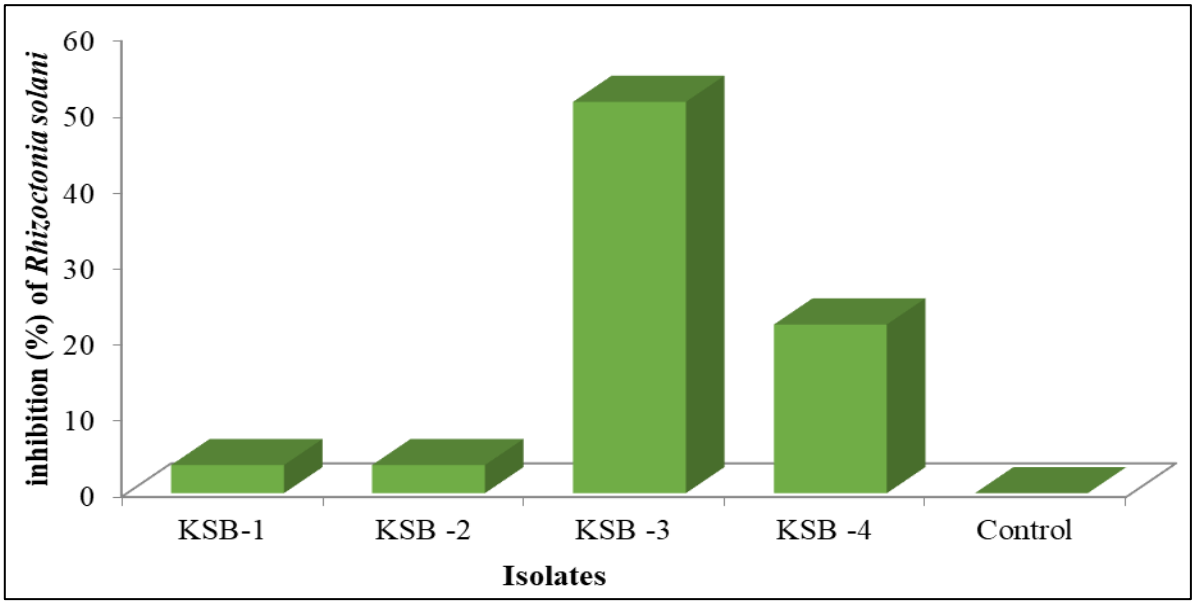

Fig 1: Study the antagonistic activity of KSB isolates

Table 4: Study the biocontrol activity of ZnSB isolates

Isolate	Siderophore production	HCN production	Percent inhibition (%) of *Rhizoctoniasolani*
ZnSB-1	+++	-	25.90
ZnSB -2	+++	-	18.50
ZnSB -3	-	-	07.40
ZnSB -4	-	+++	11.10
ZnSB -5	-	-	07.40
ZnSB -6	-	-	03.70
ZnSB -7	-	-	00.00
ZnSB -8	-	++	14.80
Control			0.00
C.D.			0.764
SE(m)			0.253
SE(d)			0.357
C.V.			3.95

+ Weak production, ++ Moderate production +++ Strong production − No production IAA - Indole Acetic Acid KSB – Potassium Solubilizing Bacteria

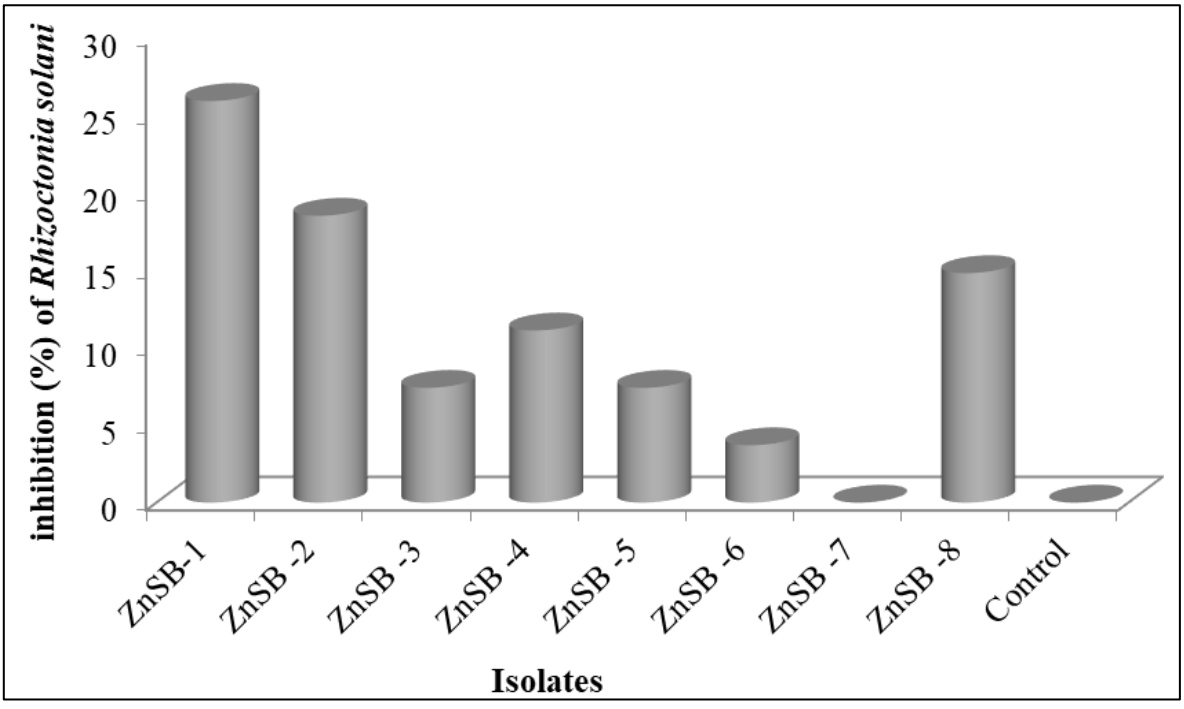

Fig 2: Study the antagonistic activity of ZnSB isolates

Conclusion

Among four KSB isolates 3 isolates were able to solubilize phosphate on Pikovskaya's media containing Tri calcium phosphate in the range of 7 mm to 9 mm. Among 3 isolates KSB-3 recorded the highest solubilization zone (9 mm) followed by KSB-1 and KSB-4 (both 7 mm), KSB-2 did not solubilize phosphate. Among eight ZnSB isolates 2 isolates were able to solubilize phosphate on Pikovskaya's media containing Tri calcium phosphate in the range of 8 mm to 10 mm. Among 2 isolates, ZnSB-6 recorded the highest solubilization zone (10 mm) followed by ZnSB-1 (8 mm). Among four KSB isolates, KSB - 3 was positive for ammonia production in peptone water tubes. The isolate KSB-3 produced ammonia moderately (++). Among eight ZnSB isolates 2 isolates ZnSB-5 and ZnSB-6 were positive for ammonia production. The isolate ZnSB-5 produced ammonia moderately (++) and ZnSB-6 was weak (+) ammonia producer. IAA production varied with supplementation of L-tryptophan and without supplementation of L-tryptophan. Out of four isolates KSB-1 showed maximum IAA production (2.2 µg ml -1) without supplementation of tryptophan, Enhancement in production of IAA was observed with the supplementation of L-Tryptophan @ 10 mg per liter by KSB-1 (6.41 µg ml -1). IAA production for ZnSB isolates were maximum for ZnSB-5 (3.516 µg ml -1) without supplementation of L-tryptophan and 7.51 µg ml -1 with tryptophan. Out of four KSB isolates, KSB - 1 isolate produced moderate (++) siderophore production. Among eight ZnSB isolates 2 isolates ZnSB-1 and ZnSB-2 were strong (+++) producers of siderophore. Out of four KSB isolates, KSB-2 produced (++) HCN moderately. Among eight ZnSB isolates 2 isolates, ZnSB-4 and ZnSB-8 performed good with HCN production. The ZnSB-4 was stronger (+++) one and ZnSB - 8 were moderate (++) HCN producer. Antifungal activity was tested with potassium solubilizing bacteria and eight zinc solubilizing bacteria isolates against Rhizoctonia solani under in vitro conditions using PDA media. The results varied with percent inhibition for KSB from 51.50 to 3.70 and for ZnSB 25.90 to 3.70.

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