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Short communication: Zinc solubilizing bacteria for plant growth isolated from SBACRF, Karaikudi

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Abstract
Zinc is an important micronutrient for plant since it involves in many cellular functions but their availability is less because of various environmental factors. Few microorganisms have the ability to solubilize zinc and make it available to the plant system. The present study aimed to isolate the ZSB from Bhendi, Brinjal, Chilli, and Marigold rhizosphere soils of Sethu Bhaskara Agricultural College and Research Foundation (SBACRF) field, Karaikudi, Tamil Nadu. A total of twenty three isolates were obtained based on high clear zone on Bunt and Rovira medium. From those, eleven isolates (ZSBBh6, ZSBBr9, ZSBC11, ZSBC12, ZSMC13, ZSBC14, ZSBC15, ZSBC16, ZSBC17, ZSBM22, and ZSBM23) were selected based on high solubilization index and titratable acidity. Morphological characterization revealed that most of them were belonged Gram negative except ZSBC 15. These isolates were screened for plant growth promoting (PGP) characters showed that most of the isolates solubilize P ions and only one isolate (ZSBC15) solubilize K. Most of the strains have produced HCN in qualitatively assay whereas IAA production was observed in two strains, ZSBC13 and ZSBC14 and siderophore production was observed in ZSBC13. Almost all strains have inhibited all the three tested pathogens (*Colletotrichum* sp., *Macrophomina* sp. and *Aspergillus* sp.). Hence, this study indicated that inoculation of ZSB have potential to promote plant growth.

Keywords: Zn solubilization, PGP activities, plant growth, and SBACRF

Introduction

Zinc is an essential trace element necessary for all crops. It is a key metal component vital for almost all metabolic and biological enzyme activities (Andreini *et al.*, 2009 and Jagana *et al.*, 2019) [2, 6]. Zn reservoir is not easily available to plants because of low solubility nature hence; the exogenous application of chemical fertilizers like Zinc sulphate may improve the bioavailability (Alloway, 2009) [1]. However, Zn deficiency in crop shows the symptoms like stunted growth, chlorosis of leaves, and spikelet sterility which ultimately reduce the crop yields thereby affects the one-third of the world's human population by causing critical nutritional and health issues (Sharma *et al.*, 2013 and Myers *et al.*, 2015) [12, 9]. Zn deficiency in soil is influenced by environmental factors (drying of upper horizons and sub-soil constraints) and their conditions (temperature, pH, organic matter, phosphorus, and iron availability) mostly in sandy, saline, and calcareous (Noulas *et al.*, 2018 and Dinesh *et al.*, 2018) [10, 4].

Microorganisms can play an important role in the solubilization, transport and deposition of metals and minerals in the soil and the common genera include *Acinetobacter*, *Bacillus*, *Gluconacetobacter*, *Pseudomonas*, and *Thiobacillus* (Sukhwal *et al.*, 2022) [13]. Saravanan and his colleagues (2003) reported that *Bacillus* sp. can solubilize ZnO, $ZnCO_3$ and ZnS which was isolated from zinc ore sphalerite. In the present study, forty zinc solubilizing bacteria were isolated based on clear zone formation from the rhizosphere soils of Bhendi, Brinjal, Chilli, and Marigold using Bunt and Rovira's agar medium supplemented with ZnO and the isolates were named as ZSBBh for Bhendi, ZSBBr for Brinjal, ZSBC for Chilli and ZSBM for Marigold. From that, 23 isolates (ZSBBh1, ZSBBh2, ZSBBh3, ZSBBh4, ZSBBh5, ZSBBh6, ZSBBr7, ZSBBr8, ZSBBr9, ZSBC10, ZSBC11, ZSBC12, ZSMC13, ZSBC14, ZSBC15, ZSBC16, ZSBC17, ZSBC18, ZSBC19, ZSBC20, ZSBM21, ZSBM22, and ZSBM23) showed strong solubilization index ranged from 1.00 to 6.00 (Fig. 1).

These findings are reliable with earlier studies that significant reduction in pH of the medium inversely correlates with the amount of soluble zinc released. The mechanism of zinc solubilization is mainly occurred by the production of organic acids followed by proton expulsion, and the production of chelating agents resulting in acidification of surrounding soil (Kamran *et al*., 2017 and Mumtaz *et al*., 2019) [7, 8]. Di Simine and his co-workers (1998) [3] found that gluconic acid and 2-ketogluconic acids produced in the culture broth aided in the solubilization of zinc phosphate by *Pseudomonas fluorescens* with a drop off pH of the medium.

Hence, we did titratable acidity by inoculating the culture in liquid medium with phenol red indicator. After incubation, the change in colour from red to yellow indicated the acid production by the isolates and also titratable acidity ranged from 0.38 to 2.33 g L^{-1}.

ZSB also have the ability to improve crop quality via producing various phytohormones and soluble nutrients (P and K), synthesizing exopolysaccharides and siderophores, and reducing environmental stress (Gupta *et al*., 2022) [5]. Most of the scientists reported that zinc solubilizing bacteria also isolates exhibit other plant growth-promoting traits, such as indole-3-acetic acid (IAA) and NH_3 production and potassium solubilization therefore, it could support plant growth. Hence, 11 best isolates (ZnSBBh6, ZnSBBr9, ZnSBC11, ZnSBC12, ZnSBC13, ZnSBC14, ZnSBC15, ZnSBC16, ZnSBC17, ZnSBM22 and ZnSBM23) were chosen based on zinc solubilization index and titratable acidity was taken for PGP activities (Table 1). The phosphorous and potassium solubilization ability was tested by standard plate assay showed that most of the isolates (SBBh6, ZSBBr9, ZSBC11, ZSBC12, ZSBC17, ZSBM22, and ZSBM23) solubilize P ions and only one isolate (ZSBC15) solubilize K.

The IAA production was qualitatively estimated for these strains and the results showed that only two strains such as ZSBC13 and ZSBC14 formed pink colonies on trypton soya agar medium when added with salkawski reagent. Only one strain (ZSBC13) has the ability to produce siderophore. Most of the strains have HCN production except three (ZSBC17, ZSBM22 and ZSBM23) was observed qualitatively on glycine nutrient agar medium. The isolates were tested for antagonistic activity towards three fungal pathogens (*Colletotrichum* sp., *Macrophomina* sp., and *Aspergillus* sp.) and the result showed all the strains have inhibited *Macrophomina* sp. Among them, only three isolates (ZSBBr9, ZSBC16 and ZSBC17) did not inhibit *Aspergillus* sp. and three isolates (ZSBC11, ZSBC16 and ZSBM22) did not inhibit *Macrophomina* sp. The present study extends the importance of zinc biofertilizers since the deficiency is occurred in most of the plants especially in vegetables. Even though, the applied zinc fertilizers are sometimes unavailable to the plant system due to their poor solubility.

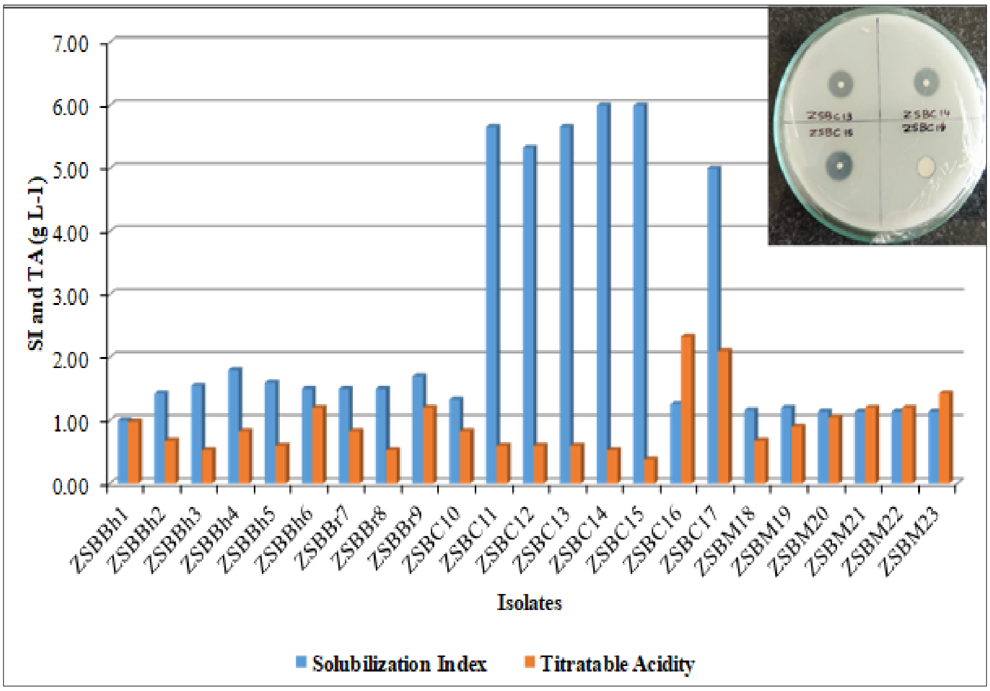

Fig 1: Solubilization index and titratable acidity of ZSB. Inset shows Clear zone formation of the isolates.

Table 1: Screening of the ZSB for PGP activities

S. No.	Bacterial isolates	Mineral solubilization		HCN	Siderophore	IAA	Antagonistic activity		
		P	K				*Macrophomina* sp.	*Colletotrichum* sp.	*Aspergillus* sp.
1	ZnSBBh6	+	-	+	-	-	+	+	+
2	ZnSBBr9	+	-	+	-	-	+	+	-
3	ZnSBC11	+	-	+	-	-	+	-	+
4	ZnSBC12	+	-	+	-	-	+	+	+
5	ZnSBC13	-	-	+	+	+	+	+	+
6	ZnSBC14	-	-	+	-	+	+	+	+
7	ZnSBC15	-	+	+	-	-	+	+	+
8	ZnSBC16	-	-	+	-	-	+	-	-
9	ZnSBC17	+	-	-	-	-	+	+	-
10	ZnSBM22	+	-	-	-	-	+	-	+
11	ZnSBM23	+	-	-	-	-	+	+	+

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