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Isolation and Characterization of Zinc Solubilizing Bacteria from Rhizosphere Soils of Soybean

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Abstract

Zinc is one of the essential micronutrients required for the normal healthy growth and reproduction of crop plants. It plays a vital role in plant metabolism. Application of zinc in the form of synthetic fertilizers is proved to be inappropriate due to its unavailable to plants. This problem can be prevented by using zinc solubilizing microorganism which has highest potential to convert unavailable form to available form. In the present investigation rhizosphere soil samples from soybean crop were collected from the different location of Pune with the objective to isolate zinc solubilizing bacteria, their screening and characterization. Zinc sources were used. All the zinc solubilizing isolates were maintained for further screening for mineral solubilization of zinc. Among these ZSB 1, ZSB 2, ZSB 3 which were identified as *Bacillus* spp. and ZSB 4 identified as *Pseudomonas* spp. ZSB1 showed maximum solubilization index (S.I) and solubilisation efficiency (S.E) with 1.14 and 214.28.

Key words : Ssolation, characterization, zinc solubilizing bacteria, solubilization.

Zinc is one of the essential micronutrients required for the normal healthy growth and reproduction of crop plants. It plays a vital role in plant metabolism (Hughes and Poole, 1989). It occurs in soil as sphalerite, olivine, homblende, augite and biotite. However, availability of zinc from these sources is guided by many factors among which biochemical action of rhizomicroorganisms plays an important role in converting such unavailable sources into available ones (Singh *et al*., 2005). Zinc is required in relatively small concentrations in plant tissues (5-100 mg kg-1). Role of zinc in nutrition and physiology of both eukaryotic and prokaryotic organisms has been widely studied, especially its importance for activity of many enzymes. Zinc is present in the enzyme system as co-factor and it is metal activator of many enzymes (Parisi *et al*., 1969).

Zinc deficiency is well reported in soils of much of the world and it is one of the prevalent nutritional constraints in crop plants especially cereals and pulses. Among cereals, wheat and rice in are particularly suffer from Zn deficiency. Its deficiency is displayed as a remarkable reduction in plant height and plants develop whitish brown patches that turn necrotic subsequently (Parker *et al*., 1992).

In India, up to 50 per cent of the agricultural land, particularly the whole of the Indo- Gangetic belt, is reeling under zinc deficiency. This has serious consequences as plants grown on zinc-deficient soils have reduced grain yield (80%). The major reason for widespread occurrence of zinc deficiency problem in crop plants is especially its low solubility rather than a total low amount of zinc (Cakmak, 2008). The solubility of zinc is highly dependent on soil pH and moisture and hence arid and semi-arid areas of Indian agro-ecosystems are often zinc deficient. It can be corrected through exogenous application of soluble zinc sources but only 20 per cent of applied zinc is available for plant uptake and rest of the zinc is converted to

various unavailable forms. Zinc thus made unavailable is converted back to available form by inoculating bacterial strains which can solubilize it by release of organic acids and decrease in pH products containing living microorganisms which have the ability to mobilize nutritive.

Zinc availability in soil & in plant tissues are affected by the composition and abundance of microbial communities in the rhizosphere (Dotaniya and Meena, 2015). Hence, the inoculation of specific bacterial strains may be helpful to enhance both Zn availability and plant uptake in plant and soil. (Aketi *et al*., 2014). Among microbes, both bacteria and fungi have shown prolific ability to improve plant Zn availability in the rhizosphere and also enhance zinc in plant parts. (Subramanian *et al*., 2009). The present study deals on isolation and characterization of zinc solubilizing bacteria which has great importance in growth of the plant as well as increasing the yield capacity.

Material and Methodology

Present investigations on Laboratory experiments were carried out during 2022-2023 in the Biological Nitrogen Fixation Scheme and Department of Plant Pathology, College of Agriculture Pune-05.

Collection of sample : The healthy and vigorously growing soybean plants were uprooted from the field and soil adhering to the roots and around the root zone of the plant was collected in the clean disinfected polythene bags. The bags were labelled with the required information. The samples were brought to the laboratory and were stored in the refrigerator at a temperature of 40C. The soil samples were collected from different locations of College of agriculture, Pune and ZARS Ganeshkhind Pune.

Isolation of zinc solubilizing bacteria from soil : Isolation of zinc solubilizing bacteria was done using the serial dilution and pour plate technique. (Nivaas *et al*., 2019).1 gm of soil was serially diluted in distilled water up to 10-8 dilutions and 1ml of sample from conc. 10-4, 10-5, 10-6 and 10-7 were placed on the Bunt and Rovira medium and incubated at 30° C for 24 hr.

Purification and Maintenance of culture : After appearance of growth of the colonies on petri plates, colonies showing distinct colony characters were selected and sub-cultured several times to obtain pure cultures of the bacterial isolates.The well isolated colonies showing distinct colony characters were selected and these were picked using sterile needle and the inoculum was streaked on the Burnt and Rovira media slants and kept for incubation for 5-7 days in bacteriological incubator at 280C temperature. Later, the slants stored in refrigerator at 40C for further study.

Characterization of isolated zinc solubilizing bacteria : Preliminary characterization of outstanding zinc solubilizers was performed on the basis of morphological characteristics, colony features and biochemical tests like catalase test, urease test, methyl red test, voges-proskauer test, starch hydrolysis test, casein hydrolysis test, H_2S production test, gelatin liquefaction test, citrate utilization, indole production test. The morphological and biochemical characterization of the selected isolates were identified according to the Bergey's Manual of Systematic bacteriology.

Qualitative estimation of zinc solubilizing potential : In this the isolates were tested to find out the solubilization efficiency and solubilization index by using plate assay (Sunithakumari *et al*., 2016). Isolated test organisms were inoculated on Bunt and Rovira media containing zinc source. The plates were incubated at 30°C for 48 hrs. After incubation halo zones were observed around colonies which

were measured after 48 hrs. of incubation and zinc solubilization efficiency and zinc solubilization index were calculated by using the following formula:

$$\text{Soloubilization efficiency} = \frac{\text{Halo zone diameter}}{\text{colony diameter}} \times 100$$

$$\text{Soloubilization efficiency} = \frac{\text{Halo zone diameter - colony diameter}}{\text{colony diameter}} \times 100$$

Results and Discussion

Present study was taken to isolates potential zinc solubilizing bacteria (ZSB) from soybean rhizosphere. Screening was done to identify efficient ZSB and the isolates were subjected to biochemical and cultural characteristics. The results obtained on these aspects are being presented in following paragraphs.

Isolation of zinc solubilizing bacteria from rhizosphere soils of soybean : The soil samples were collected from different locations such as ZARS, Ganeshkhind, Pune and College of agriculture, Pune farms and the number of isolates obtained from the samples showed in (Table 1). These isolates were purified and maintained as pure culture on Bunt and Rovira medium slants and were preserved at 4°C in the refrigerator for further studies.

Characterization of zinc solubilizing bacterial isolates : Preliminary characterization of the isolated zinc solubilizing bacteria was performed on the basis of cultural characteristics, morphological characteristics and biochemical tests on the Petri plates after 2 days of incubation.

Morphological characterization : In the present investigation, some morphological characteristics *viz*., Gram staining reaction, cell shape and pigmentation of the zinc solubilizing bacterial isolates were studied. Results of the present investigation were presented in Table 2.

Results of the present investigation presented in Table 2 explicitly indicated that, three bacterial isolates were Gram positive and one bacterial isolate was Gram positive in reaction. Additionally, the isolates being investigated for pigment production. Results regarding pigmentation clearly indicated that, the isolates produced the pigments from creamy white to creamy bluish white. Regarding the shape of the cells of zinc solubilizing bacterial isolates, it was noticed that all the bacterial isolates were rod shaped.

Colony characterization : In the present investigation, the colony characteristics viz., shape, size, elevation, form, color were recorded on the Petri plates after 2 days of incubation. Result of the present investigation were presented in Table 3.

Result of the present investigation presented in table 3 explicitly indicated that, three bacterial isolates (ZSB1, ZSB2 and ZSB3) similar colony characteristics. Shape of all the zinc solubilizing

Table 1. Sources of soil samples collected from soybean rhizosphere used for isolation of zinc solubilizing bacterial isolates

District	Soil type	Crop	Location	No. of isolates
Pune	Black	Soybean	Ganeshkhind	1
Pune	Black	Soybean	College farm	1
Pune	Black	Soybean	College farm	1
Pune	Black	Soybean	Ganeshkhind	1

Table 2. Morphological characteristics of the zinc solubilizing bacterial isolates

Isolates	Cell Shape	Pigment	Gram staining
ZSB1	Rods	White	+ve
ZSB2	Rods	White	+ve
ZSB3	Rods	White	+ve
ZSB4	Short rods	Creamy bluish white	-ve

Table 3. Colony characteristics of the zinc solubilizers

Isolates	Colony shape	Colony size	Colony color	Elevation
ZSB1	Circular	Large	White	Flat
ZSB2	Circular	Small	White	Flat
ZSB3	Circular	Large	White	Flat
ZSB4	Circular	Small	Bluish white	Raised

bacterial colonies were circular. In regard to size of colony, ZSB1 and ZSB 3 showed large colonies, while ZSB2 and ZSB4 showed the small colonies. Elevation of the colonies in ZSB1, ZSB2 and ZSB3 were flat, while in ZSB4 showed raised elevation. Pertaining to colony form, colonies of the all the isolates were circular form. In regard to color of the colonies, three colonies (ZSB1, ZSB2 and ZSB3) showed white colour while ZSB4 showed bluish white in color.

Biochemical characterization : In the present investigation, all the bacterial isolates were subjected to different biochemical tests *viz*., catalase test, urease test, methyl red test, voges-proskauer test, starch hydrolysis test, casein hydrolysis, H_2S production test, indole formation test, citrate utilization test and gelatin liquefaction test. Result of the investigation were depicted in Table 4.

Table 4. Different biochemical tests of the zinc solubilizing bacterial strains

Isolates	ZSB1	ZSB2	ZSB3	ZSB4
Catalase test	+	+	+	+
Urease test	-	-	-	-
Methyl red test	+	+	+	-
Voges- Proskauer test	+	+	+	+
Starch hydrolysis test	-	+	-	+
Casein hydrolysis test	+	+	+	+
H2S production test	+	+	+	+
Gelatin liquefaction test	+	+	+	+
Citrate utilization	+	-	-	+
Indole production test	+	+	+	+
Probable Genus	B	B	B	P

B = *Bacillus* spp., P = *Pseudomonas* spp.

Results of the investigation depicted in Table 4 clearly indicated that, all the isolates were catalase positive, voges- proskauer test positive, casein hydrolysis positive, H_2S production positive and showed positive reaction for gelatin liquefaction and IAA production. In case of starch hydrolysis test and citrate utilization test some isolates showed positive and some showed negative reaction. In case of urease test all the isolates showed the negative reaction. In case of methyl red test ZSB1, ZSB2 and ZSB3 showed positive reaction while ZSB4 showed the negative reaction. All the bacterial isolates catalase positive, voges- proskauer test positive, casein hydrolysis positive, H_2S production positive and showed positive reaction for gelatin liquefaction and IAA production, which was in agreement with the results of earlier workers (Jagana *et al*., (2019), Desai *et al*., (2012) and Chibuogwu and Ezeobi , (2011).

From the results of morphological, cultural and biochemical tests, it was evident that ZSB1, ZSB2 and ZSB3 isolates obtained were *Bacillus* spp. and ZSB4 was *Pseudomonas* spp. (Bergey's Manual of Systematic Bacteriology, 2001-2012.)

Zinc solubilization activity of the isolates under different insoluble zinc sources in solid media (Bunt and Rovira-media; Plate assay) : In this study, the bacterial strains were tested by using plate assay. The bacterial strains were inoculated in the Bunt and Rovira agar medium amended with zinc source of zinc at 0.1 per cent. The solubilization efficiency of the isolates were calculated by measuring the diameter of the colony growth and the halo zone diametre. Zinc solubilizing potential varied with different zinc solubilizing isolates [Table - 4.1.3 and plate 3]. The isolated zinc solubilizing bacterial isolates were screened for solubilization of insoluble zinc oxide (ZnO) as a source of zinc. The halo zone is ranged from 9 mm to 15 mm, where higher solubilization

was found in strain ZSB 1 of 15 mm and least solubilization was 9 mm for ZSB 3.

Among all the bacterial isolates, strain ZSB 1 was recorded higher solubilization efficiency (214.28 %) followed by ZSB 4 of 200 per cent. The results indicated that all bacterial strains are capable of solubilizing ZnO. However, ZSB 1 recorded significantly higher solubilization. Among all the bacterial isolates, strain ZSB 1 was recorded higher solubilization Index (1.14) followed by ZSB 4 of 1. The results indicated that ZSB1 is highly efficient isolate for zinc solubilization. Among all the isolates obtained, the bacterial strain ZSB 1 solubilized more zinc oxide in plate assay. Hence, this isolate was chosen to evaluate its efficiency in evaluating solubilization and yield of soybean under field condition.

The results were in agreement with the findings by Sharma *et al*., (2012), who reported that Bacillus spp. showed highest solubilization on Tris-minimal agar medium containing zinc oxide, zinc phosphate, zinc carbonate. Aketi *et al*., (2014) reported Bacillus aryabhattai MDSR7 showed highest zinc solubilization in Tris-minimal broth. Also these findings were in agreement with Sentil kumar *et al*., (2004), who reported the zinc solubilizing organism (*Bacillus* spp.) identified proved to have favourable effect on the availability of P, K, Zn.

Conclusion

The present study concluded that, there is plenty of zinc after the application of synthetic fertlizers remains unavailable to the crop due to its insoluble form. The alternative tool for such problem is identifying efficient zinc solubilizing bacterial strain and its application.

Based on morphological, cultural, and biochemical traits, the current investigation's findings showed that, of the four zinc-solubilizing bacterial isolates recovered from soil samples, three belonged to Bacillus spp. and the fourth isolate to Pseudomonas spp. Compared to the other isolates, the ZSB1 exhibited the greatest zinc solubilization efficiency (214.28%) and zinc solubilization index (1.14).

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