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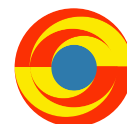

Zinc Solubilizing Bacteria from Rhizospheric Soil of Mangroves

Beulah Jerlin[1], S. Sharmila[2], K. Kathiresan[3], K. Kayalvizhi[3]

[1]Department of Botany, St. Mary's College (Autonomous), Thoothukudi, India

[2]Department of Botany, Vellalar College for Women (Autonomous), Erode, India

[3]Faculty of Marine Sciences, Annamalai University, Chidambaram, India

Email address:
beulah_jerlin@yahoo.co.in (B. Jerlin)

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Abstract: Zinc (Zn) is among the essential micronutrients required for optimum plant growth. Inorganic zinc in soil is generally in unavailable form for plant assimilation. However, Zinc Solubilizing Bacteria (ZSB) makes the inorganic zinc in to biologically available form. Such studies in mangroves habitats are almost non-existing. Hence, the present study explored the presence of ZSB from mangrove soil. The ZSB were in a range of 9.53% to 13.9% in non-mangrove soil and *Rhizophora* mangrove root soil respectively. Out of 24 morphologically distinct strains of ZSB, three strains (ZSB-4, ZSB-13, ZSB-14) displayed high Zn solubilization efficiency on solid medium amended with ZnO (382%), $ZnCO_3$ (365%) and $ZnSO_4$ (336%). These strains exhibited significant release of Zn at the concentrations of 2.3 2.12 and 2.09 ppm by ZSB-14, ZSB-4 and ZSB-13 respectively on 10th day of incubation in broth medium amended with ZnO. The strains released acids as evident by decline in pH of the broth medium. They also secreted IAA with the maximum of 14.5 ppm by ZSB-4 with ZnO as source of Zn. The potential strains for Zn solubilization were identified using 16S rRNA as *Pseudomonas aeroginosa* for further application as bioinoculants to mangrove soil.

Keywords: Mangroves, Rhizospheric Soil, Zinc Solubilizing Bacteria, Indole 3 Acetic Acid

1. Introduction

Zinc is an essential micro-nutrient for plant growth and development. The zinc occurs as a free ion that drives and boosts the rate of many metabolic reactions of the plants [1] Zn plays an essential role in the biosynthesis of IAA through the formation of tryptophan, the precursor of IAA. Usually in the absence of IAA, plant growth is stunted [2]. This is because that IAA regulates many biological functions of plants such as cell division, elongation, fruit development and senescence. Zinc is also involved in the control of gene expression; and it appears important in stabilizing RNA and DNA structure, and maintaining the activity of RNA degrading enzymes [3]. Zn deficiency will result in inhibition of physiological and biochemical activities of the plants leading to abnormal growth. The zinc deficiency also increases membrane leakiness as zinc containing enzymes are involved in the detoxification of membrane damaging oxygen radicals [4]

Exogenous application of Zn to counter its deficiency in plants in the form of zinc sulphate also gets transformed into unavailable forms like Zn (OH) and $Zn(OH_2)$ depending on pH of soil [5]. The application of Zn is converted to $ZnCO_3$ in calcium-rich alkali soils, and to $Zn\ (PO_3)_4$ in near neutral to alkali soils of high P application [6]. Though there is plenty of zinc in the soil, the crop plants exhibit Zn deficiency due to the presence of the unavailable forms of Zn. However, bacteria, especially those associated with the rhizosphere have the ability to transform unavailable form of a metal including Zn into available form through solubilization mechanism [7, 8 & 9]. Only a few studies are available for Zinc Solubilizing Bacteria (ZSB) and such studies for mangrove habitats are non-existing in India. The mangroves are among the world's most productive ecosystems, enriched with microbial diversity [10] The mangroves may efficiently trap trace metals in non-bioavailable forms by rapid precipitation of stable metal sulfides under anaerobic condition [11] and by strong binding

with organic complexes [12]. However, the reports of zinc solubilizing bacteria in mangroves are largely missing, and hence the present study was made to isolate and enumerate the zinc solubilizing bacteria associated with rhizospheric soil of mangroves in relation to physic-chemical characteristics of the soil.

2. Materials and Methods

2.1. Collection of Soil Samples

The sediment soil samples adhering to the root system of two mangroves species (*Rhizophora mucronata* and *Avicennia marina*) and non-mangrove soil samples were collected carefully along the Vellar estuary (Lat. 11° 29' N; Long. 79° 46' E), situated in southeast coast of India [Figure-1 & 2]. Samples were randomly collected three times from the rhizosphere of plants at a depth of 10 – 20 cm. Collected samples were air dried, crushed and passed through 2 mm sieve before being mixed in to a single composite sample. Then the samples were stored at 5°C for further studies.

Figure 1. *View of the study area – Vellar estuary.*

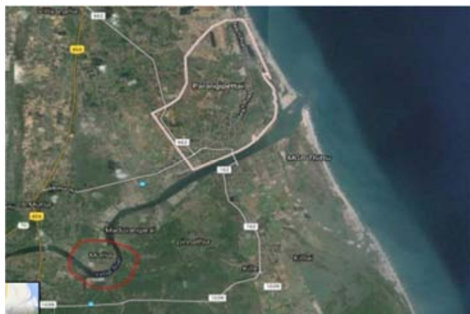

Figure 2. *Cuddalore District Satellite map showing the location of the study area.*

2.2. Physico-Chemical Characters of Rhizospheric Soil Samples

The texture of soil samples was calculated by Pipette method [13]. Temperature was measured in sediment soil using a mercury centigrade thermometer with 0.5°C accuracy. The pH of sediment was measured in the samples diluted at 1: 2.5 sediment: water ratio. The pH in the solution was measured using a pH meter, calibrated with standard buffer solution prior to use. Electrical conductivity (EC) was measured in the sediment samples prepared for analyzing total amount of soluble salts present in the soil. The electrical conductivity (EC) is expressed as dsm^{-1}. The EC of the soil extract was determined by using Systronic EC meter (digital) in a soil water suspension of 1: 5 ratio [13]. Pour water salinity of soil samples was measured by using hand refractometer, after crushing a small amount of soil through a syringe. For this a known amount of sediment samples was moisturized with double distilled water up to the moisture saturation level of the sediment. Oven-dried (60°C) soil samples were taken for bulk density analysis. Total organic carbon in sediment soil was estimated by adopting the method of [14].

2.3. Nutrient Analysis of Rhizospheric Soil

Besides bacterial studies, rhizospheric soil samples were also analysed for soil nutrients. Nitrogen was estimated by using Kjeldahl method [15]. Phosphorus was estimated by using colorimetric method [16] and potassium was estimated by using flame photometer [17]. The micronutrients viz., iron, manganese, zinc and copper were estimated by using Atomic Absorption Spectrometer (AAS) [18].

2.4. Isolation of Bacteria

Rhizosphere soil samples were collected during the low tide from three different zones (*Avicennia, Rhizophora* and non-mangrove zones) and brought to the laboratory immediately for analyses within 3 hrs. Soil samples were collected in sterile polythene bags using a sterile spatula and shade-dried to a constant weight. Before that the plant roots and other debris were removed from the soil samples. Then the sediments were ground and sieved (mesh size of 2 mm) with filter. Then the soil samples were stored at 5°C for further study. A known weight of soil (1 g) was aseptically weighed and transferred to a stoppered (150 ml) sterile conical flask containing 99 ml of sterile diluents. The sediment-diluents mixture was agitated by means of mechanical shaking for about 10 min. and later subjected to bacteriological examination. For isolation of zinc solubilizing bacteria, the Bunt Rovira Agar Medium was prepared for 1 liter with 50% seawater [19]. Samples were serially diluted up to 10^{-5} with sterilized 50% seawater and were plated with Bunt Rovira Agar Medium for zinc solubilizing bacterial counts. For plating, one milliliter of the serially diluted samples of soil was pipetted out into sterile Petri-dish. Sterile media were then poured into dishes aseptically and swirled for thorough mixing. After solidification, the plates were incubated at 28±2°C. All the determinations were carried out in duplicates. After the incubation period of 3 to 4 days for zinc solubilizing bacteria, colonies were counted. The counts are expressed as colony forming units (cfu) per gram of the soil. Inorganic zinc solubilizing bacteria were identified by clear solubilizing zones that were formed around their colonies at the end of the third day of incubation. After 7-10 days of incubation, zinc solubilizing bacteria were formed as mucus-like colonies [Figure 2].

Figure 2. *Bacterial isolates – Sub-culture & Broth culture.*

2.5. Enumeration and Isolation of Zinc Solubilizing Bacteria

Enumeration of zinc solubilizing bacteria (ZSB) present in the soil samples was done by plate count method. The Bunt and Rovira agar medium supplemented with different insoluble sources of zinc (ZnO, $ZnCO_3$ and $ZnSO_4$) at 0.1% was used in the enumeration of the bacteria. The plates were incubated for three days at 30°C in an incubator. The colonies that displayed the clearing zone were considered as zinc solubilizers. The clear-zone forming organisms were counted, isolated and purified. The 24 morphologically distinct bacterial colonies were selected for qualitative assay after designating them as ZSB-1, ZSB-2 ZSB-24.

2.6. Qualitative Estimation of Zinc Solubilizing Potential

In the qualitative study, all the 24 bacterial isolates were tested for solubilization efficiency by using plate assay using the Bunt and Rovira agar medium containing 0.1% of ZnO, $ZnCO_3$ and $ZnSO_4$ as insoluble Zn source. The plates were incubated at 30°C for 48 h. The diameter of the clear zone and colony growth was measured for calculating Zn solubilization efficiency [20].

$$\text{(Formula -1) Solubilization efficiency} = \frac{\text{Solubilization diameter}}{\text{Diameter of colony growth}} \times 100$$

Based on the results of the plate assay, three isolates (ZSB-4, ZSB-13 and ZSB-14) which showed high solubilization of zinc were identified. They were subjected to further experimental studies such as quantitative estimation (broth assay), influence of the isolates on pH of the medium and production of IAA.

2.7. Identification of Zn Solubilizing Microorganisms Using Molecular Markers

Bacterial genomic DNA was isolated using Nucleo Spin® Tissue Kit (Macherey – Nagel) following manufacturer's instructions. Using 16S-RS-F as forward and 16S-RS-R as reverse primers.The PCR amplification was carried out in a PCR Thermal cycler (Gene Amp PCR system 9700. Applied Biosystem).The PCR product was mixed with 2 µl of ExoSAP – IT for the removal of unwanted primers and dNTPs. Sequencing reactions were performed using Big Dye Terminator v 3.1 cycle sequencing kit (Applied Biosystems, USA). Following manufactures protocol. The sequence quality was checked using sequence scanner software v1 (Applied Biosystems).The sequence alignment and required editing of the obtained sequence were carried out using Geneious Pro v 5.6 [21]. A comparison of the gene sequences was done with those available in GenBank (National Center for Biotechnology Information; NCBI) to obtain the best homologous sequences.

2.8. Quantitative Estimation of Zinc Solubilizing Potential of the Isolates (Broth Assay)

The three bacterial isolates were tested to find out the amount of zinc solubilized in the broth by growing them in 100 ml Erlenmeyer flasks containing 50 ml of Bunt and Rovira broth supplemented with 0.1% ZnO, $ZnCO_3$ and $ZnSO_4$. Uninoculated controls were maintained. All the treatments were replicated. The bacterial cultures were withdrawn after the sixth, eighth and tenth day of incubation at 30°C for the estimation of soluble Zn. The bacterial cultures were centrifuged at 15,000 rpm for 20 min and the

supernatant was passed through 0.2 µm membrane filter so as to obtain the culture filtrate containing only the soluble forms of Zn [22]. Then the sample was analysed for Zn in an Inductively Coupled Plasma / Optical emission Spectrometer (ICP – OES).

2.9. Influence of Zinc Solubilizing Organisms on pH

The three strains were inoculated in the flasks containing 50 ml of Bunt and Rovira medium containing 0.1% of ZnO, $ZnCO_3$ and $ZnSO_4$ as insoluble sources. An uninoculated control was also maintained. After incubation the samples were drawn on the sixth, eighth and tenth day for analysis. The bacterial cultures were centrifuged at 15,000 rpm for 10 min and filtered using Whatman No. 42 filter paper. pH of the ZSB culture filtrates was measured using a pH meter (Elico).

2.10. Quantitative Estimation of Indole 3 Acetic Acid by ZSB

The three bacterial strains were tested for Indole 3 acetic acid production by inoculating in the flasks containing 50 ml of Bunt and Rovira medium supplemented with 0.1% ZnO. Another set without Zn source was also inoculated. All the treatments were supplemented with 0.1% tryptophan and incubated for seven days. The quantity of Indole 3 acetic acid produced by the organisms was estimated by the method of [23]. Salkowski's reagent was added on the cellulose membrane after 48 hrs of incubation. Pink coloration indicated the production of Indole 3 acetic acid. Production of Indole 3 acetic acid was quantitatively analyzed at 520 nm by using UV- Spectrophotometer and quantified by using a tryptophan standard curve.

Statistical analysis was done by Analysis of Variance (ANOVA) followed by Duncan's

Multiple Range Tests [24].

3. Results and Discussion

3.1. Physico-chemical Characteristics of Soil Samples

The physico-chemical parameters and distribution of elements in soil, sampled around the root system of two different mangrove zones and non-mangrove zones of the Vellar estuary are shown in Table 1.

Table 1. *Physico-chemical characteristics of different rhizosphere soil samples (NMZ – Non Mangrove Zone; AZ – Avicennia Zone; RZ – Rhizophora Zone).*

Source	Soil Temperature	pH	EC(dSm^{-1})	Pour water salinity (ppt)	Bulk density (g/cm^{-3})	Total organic carbon (%)
NMZ	25.98±0.45	7.98±0.12	4.68±0.78	43.98±2.56	0.64±0.35	0.53±0.12
AZ	26.93±0.52	8.08±0.25	2.88±0.95	25.98±1.45	0.60±0.12	0.36±0.36
RZ	24.98±0.45	8.28±0.36	4.88±1.23	47.98±3.65	0.67±0.36	0.44±0.12
Zones	**	**	**	**	**	**

Values are expressed as the mean ± SD; **Statistical significance (p<0.05) calculated by one way ANOVA using SPSS.16.0.

Table 1. *Continue.*

Source	N (%)	P (%)	K (%)	Fe (ppm)	Mn (ppm)	Zn (ppm)	Cu (ppm)
NMZ	56.98±5.63	8.98±1.25	194.98±20.12	18.4±1.25	9.25±1.25	0.84±0.25	1.22±0.15
AZ	26.98±2.56	15.98±2.12	162.98±14.25	20.2±1.24	11.3±2.54	0.70±0.14	1.2±0.30
RZ	62.98±4.25	9.78±1.45	209.98±25.26	17.3±2.48	8.3±1.20	0.90±0.26	1.26±0.21
Zones	**	**	**	**	**	**	**

Values are expressed as the mean ± SD; **Statistical significance (p<0.05) calculated by one way ANOVA using SPSS.16.0.

Soil characteristics are one of the important environmental factors that directly affect mangrove growth. The soil type was sandy clay with alkaline pH in all the three sites. The soil samples from mangrove sites displayed the electrical conductivity of above 4 ds/m and hence the samples were saline, as per [25]. However, the soil sample from non-mangrove site was non-saline as the electrical conductivity value was only 2.9 ds/m. This trend was similar to pour water salinity which was 26, 44 and 48 ppt in non-mangroves, *Avicennia* and *Rhizophora* zones respectively (Table 1). Generally conductivity values that fall in the range of 4-8 dsm/m are not inhibitory to plant growth in most semi-arid to arid region unless other factors compound the salinity problem [26]. Accordingly, the salinity of mangrove soil samples, which was less than 5 ds/m in the present study, was not adverse to the growth of mangroves. State exhibit Zn deficiency due to the factors such as tropical climate, low total Zn content, neutral or alkaline pH, high salt concentration, high calcium carbonate content and calcareous soil [9].

The total organic carbon was found to be low with a range of 0.26% to 0.37%, due to high biological activity as suggested by [27]. The levels of nitrogen, and potassium were significantly higher in mangrove sites than the sites without mangroves. The levels of N and K were higher in *Rhizophora* zone (63%and 210% respectively) than those in *Avicennia* zone (57% and 195% respectively). In contrast, the mangrove soil was found to have lower level of phosphorus in mangrove soil than the non-mangrove soil (Table 1).

Micronutrients are required in very small quantities. Also, they are harmful when the available forms are present in the soil in larger amount than the level that could be tolerated by plants. In the present study, the levels of Fe and Mn were found to be lower in mangrove soil than those in non-mangrove soil. However, a reverse trend was observed for the levels of Zn and Cu (Table 1). Alkaline pH is known to

precipitate and accumulate the heavy metals [28]. Adequate Zn nutrition is important in controlling the uptake of P by roots [29]. Increasing the availability of P in the growth medium can induce Zn deficiency in plants by altering soil and plant factors [30].

3.2. Total Zinc Solubilizing Bacteria (ZSB) in Soil Samples

The present study isolated 24 morphologically distinct ZSB strains from the soil samples. Based on the plate assay, three strains which displayed high Zn solubilization were detected. The viable counts of ZSB from soil samples using ZnO, $ZnCO_3$ and $ZnSO_4$ as Zn source are given in the Table-2. The colony forming units of ZSB was limited in a range of 8.23-15.87% of total heterotrophic bacterial counts in all the three soil samples. The counts of ZSB varied with the soil type and zinc source. The maximum ZSB were found higher in mangrove soils than non-mangrove soil sample. *Rhizophora* soil exhibited the maximum counts of ZSB in all the three sources of zinc: ZnO (15.87% of total heterotrophic bacteria), $ZnCO_3$ (12.90%) and $ZnSO_4$ (12.96%).

Table 2. *Total zinc solubilizing bacteria (ZSB) in the different rhizosphere soil samples and sources of Zn.*

Source	Microbial counts (CFU x 10-6/g)		Zinc Solubilizing Bacteria (% of THB)
	Total heterotrophic bacteria (THB)	Zinc Solubilizing Bacteria	
Mangroves Zones:			
NMZ	33.88±0.35	3.22±0.25	9.53±3.85
Avicennia Zone	39.78±0.58	5.00±0.45	12.45±1.25
Rhizophora Zone	44.44±0.26	6.22±0.65	13.91±2.14
Zinc source in the medium:			
$ZnCO_3$	36.55±0.25	4.22±0.25	11.44±1.89
ZnO	43.77±1.25	5.67±0.48	12.55±2.47
$ZnSO_4$	37.78±2.45	4.56±0.65	11.90±1.47
Mangroves Zones	**	**	**
Zinc source	**	*	*

Values are expressed as the mean ± SD; Statistical significance (p) calculated by one way ANOVA by SPSS.16.0. ** is significant at the 0.01 level (2-tailed). * is significant at the 0.05 level (2-tailed).

3.3. Qualitative Estimation of Zinc Solubilizing Potential

In the qualitative study, the bacterial strains were tested using plate assay. The 24 bacterial isolates were inoculated in the Bunt and Rovira agar medium amended with different insoluble sources (ZnO, $ZnCO_3$ and $ZnSO_4$) of Zn at 0.1%. The solubilization efficiency of the isolates was calculated by measuring the diameter of the colony growth and the solubilization zone. Zinc solubilizing potential varied with ZSB isolate and it ranged between 112% and 382% depending on the zinc sources used [Figure–3]. Among the isolates, ZSB- 4, ZSB-13 and ZSB-14 exhibited the highest solubilizing efficiency of ZnO (382%), $ZnCO_3$ (365%) and $ZnSO_4$ (336%) [Figure-3]. The formation of halo zone is due to the movement of organic acids, secreted from ZSB for Zn solubilization [31]. The maximum solubilization here noted was with ZnO followed by $ZnCO_3$ and $ZnSO_{4.}$ A similar study has found that *Bacillus* species exhibits higher clearing zone with zinc sulphide as Zn source, whereas *Pseudomonas* species produces higher halo zone with ZnO and $ZnCO_3$ [32].

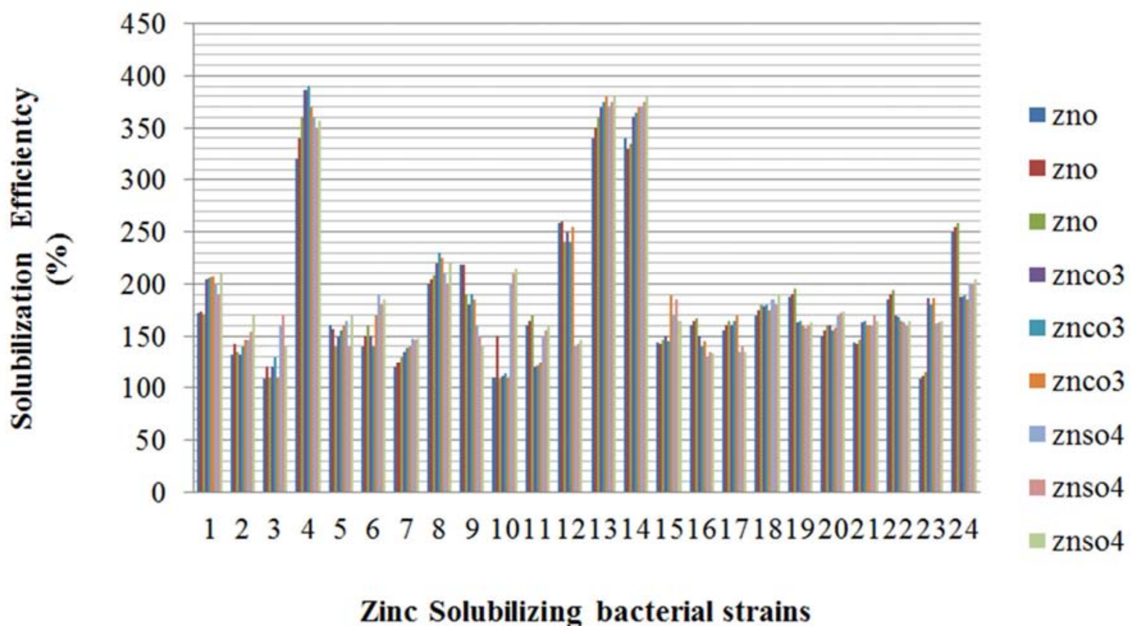

Figure 3. *In vitro zinc solubilising potential of the 24 bacterial strains with different Zn sources (Plate assay).*

3.4. Molecular Identification of Potential Strains

The three strains (ZSB-4, ZSB-13, ZSB-14) which showed highest Zn solubilization efficiency were identified using molecular marker 16S rRNA. The data obtained from the partial 16S rRNA sequencing of the 1400 bp long PCR-amplified product were subjected to BLAST. The query sequence revealed that all the strains were *Pseudomonas aeruginosa* [Figure-4]. This species with high Zn solubilization efficiency has also been isolated from three different zones (*Avicennia, Rhizophora* and non-mangrove zones) [9].

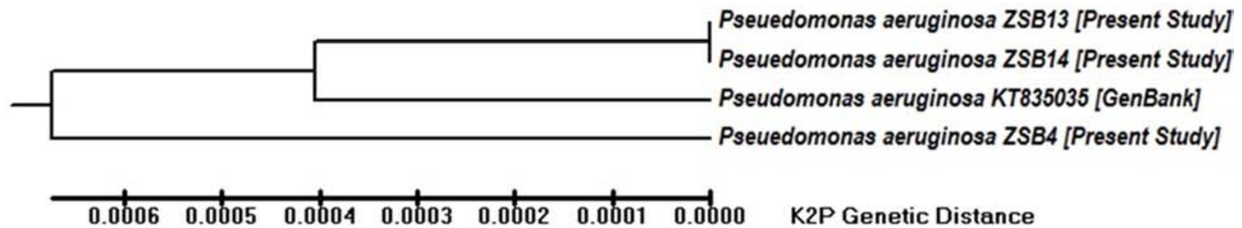

Figure 4. *Phylogenetic tree based on 16S rRNA gene sequence comparison showing the position of ZSB.*

3.5. Quantitative Estimation of Zinc Solubilizing Potential

In the quantitative assay, the three bacterial strains were tested by growing them in Bunt and Rovira liquid medium supplemented with 0.1% of ZnO, $ZnCO_3$ and $ZnSO_4$. The bacterial cultures were drawn-out after the sixth, eighth and tenth day of incubation at 30°C for determination of soluble Zn in the broth medium by using ICP-OES (5100 Agilant Technologies) [Table 3]. The solubilization efficiency of the strains varied with strains and increased with days of incubation. After 10 days of incubation, maximum solubilization potential of 2.12 mg/l by ZSB-4, 2.09 mg/l by ZSB-13, and 2.30 mg/l by ZSB-14 was recorded with ZnO as substrate (Table 3) have confirmed the solubilization of insoluble zinc compounds such as $ZnCO_3$ and ZnO by *G. diazotrophicus* by using radiotracers. The zinc compounds ($ZnCO_3$ and ZnO) tagged with 65Zn. 65ZnCO3 and 65ZnO have been found to be effectively solubilized and the uptake of Zn by the plants is also more in *G. diazotrophicus* inoculated treatments than the uninoculated treatments.

Table 3. *Quantitative zinc solubilizing potential of the bacterial strains in terms of concentration of Zn released in broth culture.*

Isolates	Zinc source in the medium	6th Day	8th Day	10th Day
ZSB-4	ZnO	$1.86{\pm}0.02^{b}$	$1.96{\pm}0.02^{b}$	$2.12{\pm}0.08^{a,b}$
	$ZnCO_3$	1.63±0.49a	$1.73{\pm}0.02^{b}$	$1.85{\pm}0.03^{b}$
	$ZnSO_4$	$1.82{\pm}0.02^{b}$	$1.81{\pm}0.06^{a}$	$1.97{\pm}0.02^{a}$
ZSB-13	ZnO	$1.81{\pm}0.20^{a}$	$1.83{\pm}0.03^{a}$	$2.09{\pm}0.11^{a}$
	$ZnCO_3$	$0.66{\pm}0.01^{a}$	$0.71{\pm}0.01^{a}$	$1.81{\pm}0.08^{a,b}$
	$ZnSO_4$	$0.72{\pm}0.01^{a}$	$0.78{\pm}0.02^{a}$	$2.04{\pm}0.14^{a}$
ZSB-14	ZnO	$1.92{\pm}0.03^{c}$	$2.02{\pm}0.08^{b}$	$2.30{\pm}0.10^{b}$
	$ZnCO_3$	$1.08{\pm}0.09^{c}$	$1.22{\pm}0.04^{c}$	$1.24{\pm}0.06^{b}$
	$ZnSO_4$	$1.18{\pm}0.01^{c}$	$1.14{\pm}0.20^{b}$	$2.03{\pm}0.06^{a}$

Values are expressed as the mean ± SD; Statistical significance (p) calculated by one way ANOVA followed by Duncan's Multiple Range Test.

3.6. Influence of ZSB on Ph of Growth Medium

The growth of ZSB strains on pH of the medium was assessed at different intervals (sixth, eighth and tenth day after incubation) using a pH meter (Elico) [Table 4]. All the culture filtrates showed a drop in pH with increasing the incubation period. After 10 days of incubation the insoluble source of ZnO showed maximum decline in pH: 5.07-4.13 for ZSB-4, 5.87-3.83 for ZSB-13 and 5.12-3.8 for ZSB-14. The decline of pH value in $ZnCO_3$ was 5.73-3.90 by ZSB-4, 5.77-3.55 for ZSB-13 and 4.83-3.37 for ZSB-14, whereas in $ZnSO_4$ the decline in pH value was 6.13-4.27 for ZSB-4, 5.10-3.27 for ZSB-13 and 5.23-3.23 for ZSB-14. A drop in the pH of the broth amended with insoluble Zn compounds has been argued by various authors to result from organic acid production and subsequent acidification of the medium [31].

Table 4. *Influence of zinc solubilizing bacteria on pH of the growth medium.*

Bacterial strains	Zinc source in the medium	pH of the growth medium		
		6th Day	8th Day	10th Day
ZSB – 4	ZnO	$5.07{\pm}0.06^{a}$	$4.67{\pm}0.06^{b}$	$4.13{\pm}0.06^{b}$
	$ZnCO_3$	$5.73{\pm}0.06^{a}$	$5.33{\pm}0.15^{c}$	$3.90{\pm}0.02^{b}$
	$ZnSO_4$	$6.13{\pm}0.06^{a}$	$5.47{\pm}0.06^{c}$	$4.27{\pm}0.06^{b}$
ZSB – 13	ZnO	$5.87{\pm}0.06^{a}$	$4.27{\pm}0.06^{a}$	$3.83{\pm}0.06^{a}$
	$ZnCO_3$	$5.77{\pm}0.06^{a}$	$4.03{\pm}0.06^{a}$	$3.53{\pm}0.06^{b}$
	$ZnSO_4$	$5.10{\pm}0.10^{a}$	$4.37{\pm}0.06^{b}$	$3.27{\pm}0.06^{a}$
ZSB - 14	ZnO	$5.1{\pm}0.10^{b}$	$4.37{\pm}0.06^{a}$	$3.80{\pm}0.01^{a}$
	$ZnCO_3$	$4.83{\pm}0.16^{a}$	$3.93{\pm}0.06^{a}$	$3.37{\pm}0.06^{a}$
	$ZnSO_4$	$5.23{\pm}0.06^{b}$	$4.17{\pm}0.06^{a}$	$3.23{\pm}0.012^{a}$

Values are expressed as the mean ± SD; Statistical significance (p) calculated by one way ANOVA followed by Duncan's Multiple Range Test.

3.7. Production of Indole 3 Acetic Acid by ZSB

Zinc is an important metalloprotein which is responsible for the synthesis of tryptophan, which in turn acts as a precursor for the production of Indole 3 acetic acid. The production of Indole 3 acetic acid by ZSB with or without ZnO in the Bunt and Rovira liquid medium supplemented with 0.1% of tryptophan was estimated [Table 5]. The results revealed that all the strains produced Indole 3 acetic acid in the medium supplemented with tryptophan, and that there was further enhancement in Indole 3 acetic acid production by the strains due to the addition of Zn source (ZnO). This may be due to the induction of high Zn solubilizing efficiency of the isolates, which results in the stimulation of

IAA synthesis. Among the three strains, ZSB-4 was found to produce more Indole 3 acetic acid (14.5 mg/l) followed by ZSB-13 (13.73 mg/l) and ZSB-14 (10.17 mg/l) in the presence of zinc (ZnO) than in its absence [Table 5]. Similar observations were also made by several other workers [34, 35 & 36]. The limited counts of ZSB [Table 2] and less production of Indole 3 acetic acid [Table 5] may be attributed as among the reasons for the stunted growth of mangroves in the present study area, and this deserves further investigation by using ZSB strains as bioinoculants to overcome the stunted growth.

Table 5. *Production of indole-3-acetic acid by zinc solubilizing bacteria.*

Treatment	IAA (mg/l)
Control	0
ZSB – 4 + ZnO	14.50 ± 0.50^a
ZSB – 4 alone	6.66 ± 0.29^a
ZSB – 13 + ZnO	13.73 ± 0.06^b
ZSB – 13 alone	9.16 ± 0.29^b
ZSB – 14 + ZnO	10.17 ± 0.15^c
ZSB – 14 alone	4.63 ± 0.15^c

Values are expressed as the mean ± SD; Statistical significance (p) calculated by one way ANOVA followed by Duncan's Multiple Range Test.

4. Conclusion

Zinc is a key micro nutrient at small concentration but lethal at elevated concentration. The solubilization of zinc might limit the growth of the bacteria at higher level. At higher level of zinc concentration the cultures tolerate its solubilization may not prolong. Continuous application of fertilizers principally in zinc also becomes eccentric because of the transformation into unavailable fractions soon after application and accumulation in the soil. Thus identification of an elite strain capable of transforming unavailable forms of Zn into available forms will be an alternative tool to alleviate zinc deficiency in plants. The above experimental study revealed that 3 strains that formed clearing zone in plate assay and available zinc in broth assay were found to be potent to solubilize the zinc and to produce plant growth regulator IAA under saline and alkaline conditions. Selection and inoculation of zinc solubilizing bacteria either alone in soils inherently rich in native zinc or along with cheaper insoluble zinc compounds, like ZnO, $ZnCO_3$ and $ZnSO_4$ will lead to lot of saving in crop husbandry, besides curtailing the expenditure on agro input. The plant growth promoting zinc solubilizing bacterial strains are proved to be good alternative of chemicals for increasing the plant growth and yield and help reduction in the use of hazardous agro-chemicals and used for bioinoculant. Further studies are at progress to use the bacterial strains as bioinoculants for zinc availability and growth stimulation to overcome the problem of stunted growth of mangroves.

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References

[1] Parisi, B. and Vallee, B. L. 1969. Metal enzyme complexes activated by zinc. J. Biol. Chem. 179: 803-807.

[2] Normanly, J., Cohen, J. D. and Fink, G. R. 1993. Arabidopsis thaliana auxotrophs reveal a tryptophan-independent biosynthetic pathway for indole-3-acetic acid. Proc. Natl. Acad. Sci. USA, 90(21), 10355–10359.

[3] Brown, P. H., Cakmak, I. and Zhang, Q. 1993. Form and function of Zinc in soils and plants. Chapter 7 in Robson A. D. (ed) Zinc in soils and plants, Kluwerb Acadamic Publishers, Dordrecht pp. 90 – 106.

[4] Cakmak, I. 2009. Enrichment of fertilizers with zinc: an excellent investment for humanity and crop production in India. J. Trace Elem. Med. Biol., 23, 281–289.

[5] Srivastava, P. C. and Gupta, U. C. 1996. Trace Elements in Crop Production, Oxford and IBH Publishers, New Delhi, 356pp.

[6] Krishnaswamy, R. 1993. Effect of phosphatic fertilization on zinc adsorption in some vertisol and inceptisol. J. Indian. Soc. oil Sci., 41, 251–255.

[7] Cunninghan, J. E. and Kuiack, C. 1992. Production of citric acid and oxalic acid and solubilization of calcium phosphate by Penicillium billai. Appl. Environ. Microbio l.58, 1451–1458.

[8] Irum Naz., Habib Ahmad., Shahida Nasreen Khokhar., Khalid Khan. and Azhar Hussain Shah. 2016. Impact of Zinc Solubilizing Bacteria on Zinc Contents of Wheat. American-Eurasian J. Agric. & Environ. Sci., 16 (3): 449-454.

[9] Sunithakumari, K., Padma Devi, S. N. and Vasandha, S. 2016. Zinc solubilizing bacterial isolates from the agricultural fields of Coimbatore, Tamil Nadu, India, Current Science, 110, 196 2, 25.

[10] Kathiresan, K. and Bingham, B. L. 2001. Biology of mangroves and mangrove ecosystems. Advances in Marine Biology. 40: 81-251.

[11] Mackey, A. P. and Mackay, S. 1996. Environ. Pollut. 93: 205.

[12] Lacerda, L. D., Carvalho, C. E. V., Tanizaki, K. F., Ovalle, A. R. C. and Rezende, C. E. 1993. The biogeochemistry and trace metals distribution of mangrove rhizospheres. Biotropica 25: 252-257.

[13] Piper, C. S., 1966. Soil plant analysis. Hans publications, Bombay.

[14] EI Wakeel S. K and Riley, J. P. 1956. The determination of organic carbon in marine muds, J. Cons., 22: 180 -183.

[15] Subbaih, B. V. and Asija, G. L. 1956. A rapid procedure for the determination of available nitrogen in soils. Curr. Sci. 31: 196.

[16] Olsen, S. R., Cole, C. V., Watanabe, F. S. and Dean. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. Circ. U. S. Dep. Agric., 939.

[17] Guzman, H. and Jimenez, C. E. 1992. Contamination of coral reefs by heavy metals along the Carribbean coast of Central America. (Costal Rica and Panama). Mar. Poll. Bull. 24(11): 554-561.

[18] Lindsay, W. L. and Norvell, W. A. 1978. Development of DTPA Soil Test for Zinc, Iron, Manganese and Copper. Soil Sci. Soc.Am. J., 42: 421 – 428.

[19] Buchanan R. E. and Gibbons, N. E., eds. 1974. Bergey's Manual of Determinative Bacteriology. 8th ed. Williams & Wilkins Co., Baltimore, Md. 21202. Xxvi + 1246 pp. $45.00.

[20] Nyugen, C., Yan, W., Tacon, F. L. and Lapyire, F. 1992. Genetic variability of phosphate solubilizing activity by monocaryotic and dicaryotic mycelia of the ectomycorrhizal fungus Laccaria bicolor (Maire). Plant Soil, 143, 193–199.

[21] Drummond A. J. Ashton B. Buxton S. Swidan. Cheun M. Duran C. Heled J. Kearse M. Markowitz S. Moir R. Stones-Havas S. Sturrock S. Swidan F. Thierer T. Wilson A. (2012) Geneious v 5.6, Available from http://www.geneious.com

[22] Francis, A. J., Dodge, C., Chendrayan, K. and Quinby, H. 1988. An-aerobic microbial dissolution of lead oxide and production of organicacids. US Patent No. 4758345.

[23] Brick, J. M., Bostock, R. M. and Silverstone, S. E. 1991. Rapid in situ assay for indole acetic acid production by bacteria immobilized on a nitrocellulose membrane. Appl. Environ. Microbiol. 57(2), 535–538.

[24] Duncan, B. D. 1957. Multiple range test for correlated and heteroscedatic means. Biometrics, 13: 359-364.

[25] Rickard, W. H. 1965. The influence of greasewool on soil mixture penetration and soil chemistry. Northwest Sci. 39: 36–42.

[26] Munshower, F. F. 1993. Practical hand book of disturbed land revegetation. Lewis Publishers, London. Tokyo.

[27] Lowe, L. E. (1975) Fractionation of acid-soluble components of soil organic matter using polyvinyl pyrrolidone. Can. J. Soil. Sci. 60: 219 -229.

[28] Ajmal, M and Khan, A. V. (1987). Heavy metals in water sediments, fish and plants of river Hindson, U. P., India. Hydrobiologia. 148: 151-157.

[29] Marschner, H. 1995. Mineral nutrition of higher plants. Second Edition, Academic press, London.

[30] Robson, A. D. and Pitman, M. G. 1983. Interactions between nutrients in higher plants. In: Lauchli, A., Bieleski, R. L. eds. Encyclopedia of plant physiology. Vol 15A. New series, Berlin and New York: Springer-Verlag, pp. 287-312.

[31] Fasim, F., Ahmed, N., Parsons, R. and Gadd, G. M. 2002. Solubilzation of zinc salts by bacterium isolated from the air environment of a tannery. FEMS Microbiol. Lett., 213, 1–6.

[32] Saravanan, V. S., Subramanian, S. and Anthoni Raj, S. 2003. Assessing *in vitro* solubilization potential of different zinc solubilizing bacterial isolates. Braz. J. Microbiol., 34: 121–125.

[33] Sarathambal, C., Thangaraju, M., Paulraj, C. and Gomathy, M. 2010. Assessing the Zinc solubilization ability of *Gluconacetobacter diazotrophicus* in maize rhizosphere using labelled 65Zn compounds. Indian J Microbiol. 50 (1): 103–109.

[34] Henri, F., Laurette, N., Annette, D., John, Q., Wolfgang, M. and Xavier, F., 2008, Solubilization of inorganic phosphates and plant growth promotionby strains of *Pseudomonas fluorescens* isolated from acidic soils of Cameroon. Afr. J. Microbiol. Res., 2, 171–178.

[35] Lin, T. F., Huang, H. I., Shen, F. T. and Young, C. C. 2006,, The protons of gluconic acid are the major factors responsible for the dissolu-tion of tricalcium phosphate by Burkholderia cepacia CC-A174. Bioresour. Technol., 97, 957–960.

[36] Song, O. R., Lee, S. J., Kim, S. H., Chung, S. Y., Cha, I. H. and Choi, Y. L., 2008, Isolation and cultural characteristics of a phosphate-solubilizing bacterium, *Aeromonas hydrophila* DA 57. J. Korean Soc. Agric. Chem. Biotechnol., 44, 257–261.