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Isolation, Screening and Characterization of Zinc Solubilizing Microorganisms from Direct Sown Paddy (*Oryza sativa* L.) Rhizospheric Soils of Andhra Pradesh

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A B S T R A C T

Rice (*Oryza sativa* L.) is one of the vital staple food for more than 50 % of the world's population provided that major source of the food energy. Rice occupies a key role in Indian agriculture contributing to 20 - 25 % of agricultural income. It pays about 43 % of the whole food grain production and about 46 % of the overall cereal production in India (FAO, 2017).In the present study collected twenty (20) direct sown paddy rhizosphere soil samples from four districts of Andhra Pradesh and recorded their geographical position of collected soil samples. From that thirty two (32) Zinc Solubilizing Bacteria (ZnSB) isolated by using zinc phosphate [$Zn_3(PO_4)_2$] as a insoluble source of zinc in the tris-mineral salts media. Zinc solubilizing isolates coded based on their soil sample collected geographical position, recorded the morphological, cultural characteristics of ZnSB. ZnSB Isolates screened based on their zinc solubilization efficiency both qualitatively and quantitatively. Among all 32 ZnSB isolates Zn KJJ-4 has the high ability to solubilize zinc both qualitatively and quantitatively among the all and lowest was showed by ZnAUU-1. Zn KJJ-4 showed the positive for the starch hydrolysis test, hydrogen sulphide test, catalase test, oxidase test, gelatine liquefaction test, methyl red test, Vogues Proskauer test, citrate utilization test and ammonia production test. ZnKJJ-4 showed the negative for indole production test.

Introduction

The plants essential several macro and micro nutrients for their growth and development. Zinc (Zn) is one of the most important micronutrients is necessary for the normal healthy growth and reproduction of plants. In rice zinc has the crucial role for the growth and development. In the recent days importance of Zinc Solubilizing Bacteria (ZSB) has increased and these are probable candidate for improving bioavailable fraction of Zn to host plant for enlightening the crop growth, yield and quality without affecting the environment is being adopted in the agriculture. Alternatively, several

microorganisms, especially those associated with roots, have the ability to rise plant growth and productivity (Rodriguiz *et al.,* 2004) by enhancing the supply of mineral nutrients of low mobility in the soil like P, Zn and Cu (Thompson, 1996). Bacterial strains that have been reported to show zinc solubilisation on lab scale include *Pseudomonas aeruginosa* (Fasim *et al.,* 2002), *Gluconacetobacter diazotrophicus* (Saravanan *et al.,* 2007), *Bacillus* spp., *Pseudomonas striata, Pseudomonas fluorescence, Burkholderia cenocepacia* (Pawar *et al.,* 2015), *Serratia liquefaciens, Serratia marcescens*, and *Bacillus thuringiensis* (Abaid – Ullah *et al.,* 2015).

Materials and Methods

Collection of soil samples

Rhizospheric soils were collected from different places of Kurnool, Prakasam, Guntur and Anantapur districts such as soils of direct sown paddy growing areas by using quadratic method of soil sample collecting procedure. In Kurnool District (Atmakur, Kothapalle, Jupadu bungalow, Pamulapadu and Velugodu), Prakasam District (Tripuranthakam, Yerragondapalem, Dornala, Markapuram and Giddalur), Guntur District (Vinukonda, Narasaropet, Chilakaluripet, Sattenapalle and Piduguralla) and Anantapur District (Guntakal, Gooty, Pamidi, Tadipatri and Uravakonda) along with particular GPS coordinates for each sampling area was fixed (Table 1) soil samples were collected from each Mandal from different farmer's fields.

Isolation of different bacterial isolates following serial dilution method and plating techniques using suitable media and incubated conditions were followed. Pure cultures must be obtained by the streak plate method. These pure cultures of different bacterial isolates were preserved and used for further analysis.

Characterization of zinc solubilizing bacterial isolates by morphological, cultural and biochemical characters

Morphological characterization

All the zinc solubilizing bacterial isolates were checked for their purity and then studied for the colony morphology and pigmentation. The cell shape and gram reaction was also recorded as per the standard procedures given by Barthalomew and Mittewar (1950).

Colony morphology

Morphological characteristics of the colony of each isolate were examined on Nutrient agar and specialized medium and incubated for according to isolate. Cultural characterization of isolates was observed by different characteristics of colonies such as shape, size, elevation, surface, margin, colour, odour, pigmentation etc. were recorded.

Biochemical and physiological characterization of zinc solubilizing bacterial isolates

Starch hydrolysis (MacFaddin, 2000)

Sterile starch agar plates were spotted with 10 µl overnight broth cultures of the isolates and incubated at 28 ± 2^{o}C for 24-48 hours. After incubation, the plates were flooded with an iodine solution. The formation of a transparent zone around the colony was taken as a positive reaction to the test.

Hydrogen sulfide test (Beishir, 1991)

Sterilized Hydrogen Sulfide-Indole-Motility agar (SIM agar) stabs were inoculated along the wall of the tubes with overnight cultures of the isolates and incubated for 48 hours at 28 ± 2^{o}C. Visualization of black colour along the line of inoculation indicated a positive reaction to the test.

Indole production (Isenberg and Sundheim, 1958)

Sterilized hydrogen sulfide-indole-motility agar (SIM agar) slants were inoculated with the overnight cultures of the isolates and incubated for 48 hours at 28 ± 2°C. Following incubation, 10 drops of Kovac's indole reagent was added to each tube. The isolates showing the production of red colour were recorded as positive for indole production.

Catalase test (Rangaswami and Bagyaraj, 1993)

This test was performed to study the presence of catalase enzyme in bacterial colonies. Pure isolates (24 hours old) were taken on glass slides and one drop of H_2O_2 (30%) was added. The appearance of the gas bubble indicated the presence of catalase enzyme.

Oxidase test (Collins and Lyne, 1970)

The overnight cultures of the test isolate were spotted on plates poured with sterile Trypticase Soy Agar (TSA) and the plates were incubated for 24 hours at 28 ± 2°C. After incubation, 2-3 drops of N, N, N', N'-tetramethyl-p-phenylenediamine-dihydro chloride (Wurster's reagent) was added onto the surface of growth of each test organism. The isolates showing the change of colour to maroon were noted as oxidase positive.

Gelatin liquefaction (MacFaddin, 2000)

The overnight cultures of the test isolates were inoculated to sterilized nutrient gelatin deep tubes and incubated for 24 hours at 28 ± 2°C. Then the tubes were kept in the refrigerator for 30 minutes at 4°C. The isolates showing liquefied gelatin were taken as positive and those which resulted in the solidification of gelatin on refrigeration were recorded as negative for the test.

Methyl red test (Crown and Gen, 1998)

Sterilized glucose-phosphate broth tubes were inoculated with the test culture and incubated at 28 ± 2°C for 48 hours. After incubation five drops of methyl red indicator were added to each tube and gently shaken. Red colour production was taken as positive and yellow colour production was taken as negative for the test.

Vogesprausker's test (MacFaddin, 2000)

To the pre-sterilized glucose-phosphate broth tubes, test cultures were inoculated and incubated at 37°C for 48 hours. After incubation ten drops of Barritt's reagent-A was added and gently shaken followed by the addition of 10 drops of Barritt's reagent-B. The development of pink colour in the broth was taken as positive for the test.

Citrate utilization (MacFaddin, 2000)

Isolates were streaked on Simmon's citrate agar slants and incubated at 28 ± 2°C for 24 hours. Change in colour from green to blue indicates the positive reaction for citrate utilization.

Ammonia production (Juanda, 2005)

The isolates were tested for ammonia production by inoculating the isolates into 10 ml of pre-sterilized peptone water in test tubes. The tubes were incubated for 48-72 hours at 36 ± 2°C. After that Nessler's reagent (0.5 ml) was added in each tube. Change in colour of the medium from brown to yellow colour was taken as a positive test for ammonia production.

Isolation of zinc solubilizing bacteria

Serial dilution method was used for isolating the Zinc Solubilizing Bacteria (ZnSB) from the rhizosphere soil samples. 1.0 g of rice rhizosphere samples was suspended in 9.0 ml

of saline distilled water in test tubes. From the first dilution 1 ml was transferred to test tube containing 9 ml of sterile distilled water to get 10^{-2} dilution. The same method was followed for preparing up to 10^{-6} dilution. The tris-mineral salts medium was prepared containing dextrose - 10.0 g, $(NH_4)_2$ SO_4 - 1.0 g, KCl - 0.2 g, K_2HPO_4 - 0.1 g, $MgSO_4$ - 0.2 g in distilled water (1000 ml and pH was adjusted 7.0). The source of insoluble zinc compounds such as ZnO, $ZnCO_3$ and Zn_3 $(PO_4)_2$ was supplemented at 1% and agar was added into the medium individually and autoclaved at 121^0C for 30 min. The agar medium was poured in sterile petri plates under aseptic condition. After solidification 0.1 ml from 10^{-4}, 10^{-5} and 10^{-6} dilutions of rice rhizosphere soil samples were taken by sterile pipette, transferred and spread on to petri plates. The inoculated plates were incubated at 27 - 30^0C for 48 h.

Qualitative and quantitative assay for zinc solubilization

Zinc solubilizing ability of bacterial isolates were evaluated using zinc phosphate in both plate and broth media assays.

Plate assay (Qualitative study)

All the bacterial strains were screened for their ability to solubilize zinc in mineral salts agar medium was amended with 0.1% of zinc phosphate Zn_3 $(PO_4)_2$. The actively growing cultures (5 µl) were spot inoculated onto the medium, incubated at 28^oC and solubilization zone will be measured after 5 days of inoculation and cleared zone was expressed as solubilization efficiency in percent and area in square millimeter (mm).

Zinc solubilization in broth assay (Quantitative study)

Analysis of insoluble zinc solubilized in liquid medium by the bacterial culture was determined quantitatively by following the protocol of Fasim *et al.,* (2002) using Atomic Absorption Spectrophotometer (AAS). In the broth assay, the selected bacterial isolates were grown in 100 ml of mineral salts medium broth supplemented with insoluble source of zinc phosphate at 0.1% concentration and incubated for 48 hrs at 28^oC in an orbital shaker at 120 rpm. There were three replicates (n=3 for each treatment) maintained for each bacterial isolates. The cultured broth was filtered through Whatman No. 42 filter paper and centrifuge at 10,000 rpm for 10 minutes. The culture supernatant was fed directly to the Atomic Absorption Spectroscopy (Varian Model SPECTRAA 220) for the determination of soluble zinc content. The amount of zinc solubilized was obtained by subtracting the soluble Zn of the inoculated sample from the un-inoculated control and expressed as (mg Zn/100ml) culture.

Results and Discussion

Isolation of zinc solubilizing microorganisms from direct sown paddy rhizosphere soils

Rhizospheric soils were collected from different places of Kurnool, Prakasam, Guntur and Anantapur districts such as soils of direct sown paddy growing areas by using quadratic method of soil sample collecting procedure. In Kurnool District (Atmakur, Kothapalle, Jupadu bungalow, Pamulapadu and Velugodu), Prakasam District (Tripuranthakam, Yerragondapalem, Dornala, Markapuram and Giddalur), Guntur District (Vinukonda, Narasaropet, Chilakaluripet, Sattenapalle and Piduguralla) and Anantapur District (Guntakal, Gooty, Pamidi, Tadipatri and Uravakonda) along with particular GPS coordinates for each sampling area was fixed (Table 1) soil samples were collected from each Mandal from different farmer's fields. Coding of the isolates based on the GPS

location of the collected soil samples (Table 2).

Cultural and morphological characterization

More number of zinc solubilizing microorganisms was present in the rhizosphere and they are metabolically more active than others. Morphological and cultural characteristics of all the isolates were studied viz., shape, size, margins and colour etc., The morphological features on zinc solubilizing agar plate was studied and they showed small to medium size, dull white or off white, flat, smooth, irregular colonies and there was no pigment production (Table 3).

These isolates were found to be gram negative, short stumpy, rod shaped cells when observed under microscope. On the basis of biochemical reactions it was found that *Pseudomonas* spp. The thirty two isolates were named based on the geographical position of soil sample collected area. Based on microscopic examination and cultural characteristics, Preeti *et al.,* (2011) identified four isolates as *Pseudomonas* spp. and others as *Bacillus* spp.

Biochemical characterization

All the isolates were tested for biochemical characterization viz., Starch hydrolysis, Hydrogen sulphide test, Indole production, Catalase test, Oxidase test, Gelatine liquification, Methyl red test, Vogues Proskauer test, Citrate Utilization, Ammonia production results (Table 2) were revealed that all the zinc solubilizing bacterial isolates were positive for starch hydrolysis expect ZnKAA-4, ZnPYY-1 and ZnGPP-3.

For hydrogen sulphide test all the isolates were positive expect ZnGCC-1, ZnAPP-2.All the isolates were positive for indole production test expect ZnKAA-1, ZnKJJ-4, ZnPTT-3, ZnPYY-2 and ZnAPP-2.For catalase test all isolates are showed positive expect ZnKJJ-2, ZnPMM-3 and ZnGCC-3 are negative. For oxidase test ZnKAA-2, ZnPTT-1, ZnPMM-1 and ZnAUU-3 are showed negative remaining all are showed positive.

In the test of gelatine liquefaction all the isolates were positive expect ZnKJJ-1, ZnPYY-1, ZnGCC-2 and ZnGPP-2. For methyl red test ZnKAA-2, ZnKAA-3, ZnPTT-1, ZnPGG-3 and ZnAUU-1 were negative remaining were showed the positive. For the test of vogues proskauer all the isolates were showed the positive expect the ZnKAA-1, ZnKJJ-1, ZnPYY-1, ZnPGG-1 and ZnGPP-1 were showed the negative.

In the test of citrate utilization ZnKJJ-3, ZnPMM-2 and ZnAPP-2 isolates were showed the negative result remaining were all showed the positive result. All the isolates were showed the positive for ammonia production test expect ZnKAA-3, ZnPMM-3 and ZnGCC-3 isolates were showed the negative.

These results are agreed with the Anitha and Kumudini (2014) isolated *Pseudomonas* from fifteen rhizospheric samples from different regions of India. They characterized morphologically and biochemically and concluded as genus *Pseudomonas*.

Screening of isolates for their zinc solubilization efficiency

Qualitative method in plat assay

All the thirty two zinc solubilizing isolates were able to form clear zone of zinc solubilization on zinc solubilizing media plate ranged from 5.12 - 16.12 mm. Among them ZnKJJ-4 of *Pseudomonas* spp detected the highest solubilization zone (16.12 mm) followed by ZnPGG-1 (15.32 mm) and the lowest solubilization zone was observed with ZnAUU-1 (5.12 mm).

Table.1 Details of soil samples collected from different districts of Andhra Pradesh

S.No.	District	Mandal	Village	Geographical Location		Sample Code	Soil type	Cropping Pattern
				Latitude	Longitude			
1.	Kurnool	Atmakur	Atmakur	$15^0$91'53" N	$78^0$70'84" E	KAA	Black soil	Rice - Rice
2.	Kurnool	Pamulapadu	Pamulapadu	$15^0$81'87" N	$78^0$50'77" E	KPP	Black soil	Rice - Blackgram
3.	Kurnool	Jupadu Bungalow	Jupadu Bungalow	$15^0$85'42" N	$78^0$36'16" E	KJJ	Black soil	Rice - Rice
4.	Kurnool	Kothapalle	Kothapalle	$15^0$97'84" N	$78^0$48'63" E	KKK	Black soil	Rice - Rice
5.	Kurnool	Velugodu	Velugodu	$15^0$72'02" N	$78^0$57'28" E	KVV	Black soil	Rice - Rice
6.	Prakasham	Tripuranthakam	Tripuranthakam	$15^0$97'80"N	$79^0$44'61" E	PTT	Black soil	Rice - Blackgram
7.	Prakasham	Yerragondapalem	Yerragondapalem	$15^0$99'96"N	$79^0$31'54" E	PYY	Black soil	Rice - Rice
8.	Prakasham	Dornala	Dornala	$15^0$90'33"N	$79^0$11'81" E	PDD	Black soil	Rice - Rice
9.	Prakasham	Markapuram	Markapuram	$15^0$74'28"N	$79^0$26'77" E	PMM	Black soil	Rice - Rice
10.	Prakasham	Giddalur	Giddalur	$15^0$37'24"N	$78^0$95'92"E	PGG	Black soil	Rice - Rice
11.	Guntur	Vinukonda	Vinukonda	$16^0$04'85"N	$79^0$75'12"E	GVV	Black soil	Rice - Rice
12.	Guntur	Narasaropet	Narasaropet	$16^0$23'48"N	$80^0$07'04"E	GNN	Black soil	Rice - Blackgram
13.	Guntur	Chilakaluripet	Chilakaluripet	$16^0$09'93"N	$80^0$18'13"E	GCC	Black soil	Rice - Blackgram
14.	Guntur	Sattenapalle	Sattenapalle	$16^0$39'92"N	$80^0$13'70"E	GSS	Black soil	Rice - Blackgram
15.	Guntur	Piduguralla	Piduguralla	$16^0$47'29"N	$79^0$90'63"E	GPP	Black soil	Rice - Blackgram
16.	Anathapuram	Guntakal	Guntakal	$15^0$16'52"N	$77^0$37'54"E	AGG	Black soil	Rice - Rice
17.	Anathapuram	Gooty	Gooty	$15^0$11'23"N	77.63'19"E	AGoGo	Black soil	Rice - Rice
18.	Anathapuram	Pamidi	Pamidi	$14^0$94'40"N	$77^0$58'57"E	APP	Black soil	Rice - Rice
19.	Anathapuram	Tadipatri	Tadipatri	$14^0$91'21"N	$78^0$00'32"E	ATT	Black soil	Rice - Rice
20.	Anathapuramu	Uravakonda	Uravakonda	$14^0$94'74"N	$77^0$22'56"E	AUU	Black soil	Rice - Rice

Table.2 Coding of ZnSB isolates collected from different soil samples according to their geographical position of Andhra Pradesh

S.No.	Type of Organism	Number of isolates	Isolate code
1.	**ZnSB**	32	ZnKAA-1, ZnKAA-2, ZnKAA-3, ZnKAA-4, ZnKJJ-1, ZnKJJ-2, ZnKJJ-3, ZnKJJ-4, ZnPTT-1, ZnPTT-2, ZnPTT-3, ZnPTT-4, ZnPYY-1, ZnPYY-2, ZnPMM-1, ZnPMM-2, ZnPMM-3, ZnPGG-1, ZnPGG-2, ZnPGG-3, ZnGCC-1, ZnGCC-2, ZnGCC-3, ZnGPP-1, ZnGPP-2, ZnGPP-3, ZnAPP-1, ZnAPP-2, ZnAPP-3, ZnAUU-1, ZnAUU-2, ZnAUU-3.

Table.3 Morphological and cultural characterization of Zinc solubilizing bacterial (ZnSB) isolates of different soil samples

S. No.	Isolate code	Gram reaction	Cell shape	Colony morphology			
				Colour	Form	Elevation	Margin
1.	ZnKAA-1	-ve	Rod	White	Irregular	Raised	Undulate
2.	ZnKAA-2	-ve	Rod	Creamy white	Circular	Flat	Entire
3.	ZnKAA-3	-ve	Rod	Creamy white	Irregular	Umbonate	Lobate
4.	ZnKAA-4	-ve	Rod	Yellowish white	Irregular	Convex	Curled
5.	ZnKJJ-1	-ve	Rod	Creamy white	Irregular	Raised	Undulate
6.	ZnKJJ-2	-ve	Rod	White	Circular	Convex	Entire
7.	ZnKJJ-3	-ve	Rod	White	Circular	Flat	Curled
8.	ZnKJJ-4	-ve	Rod	White	Irregular	Flat	Entire
9.	ZnPTT-1	-ve	Rod	White	Irregular	Convex	Entire
10.	ZnPTT-2	-ve	Rod	Off White	Irregular	Umbonate	Curled
11.	ZnPTT-3	-ve	Rod	Creamy white	Circular	Convex	Entire
12.	ZnPTT-4	-ve	Rod	White	Circular	Raised	Undulate
13.	ZnPYY-1	-ve	Rod	White	Irregular	Convex	Curled
14.	ZnPYY-2	-ve	Rod	White	Irregular	Flat	Undulate
15.	ZnPMM-1	-ve	Rod	White	Irregular	Crateriform	Filiform
16.	ZnPMM-2	-ve	Rod	Light Yellow	Circular	Flat	Entire
17.	ZnPMM-3	-ve	Rod	Whitish Yellow	Irregular	Flat	Curled
18.	ZnPGG-1	-ve	Rod	White	Irregular	Umbonate	Undulate
19.	ZnPGG-2	-ve	Rod	White	Irregular	Convex	Entire
20.	ZnPGG-3	-ve	Rod	White	Irregular	Raised	Lobate
21.	ZnGCC-1	-ve	Rod	White	Irregular	Convex	Lobate
22.	ZnGCC-2	-ve	Rod	White	Circular	Flat	Entire
23.	ZnGCC-3	-ve	Rod	White	Irregular	Raised	Entire
24.	ZnGPP-1	-ve	Rod	White	Filamentous	Flat	Lobate
25.	ZnGPP-2	-ve	Rod	Dark Yellowish	Circular	Convex	Curled
26.	ZnGPP-3	-ve	Rod	White	Filamentous	Umbonate	Curled
27.	ZnAPP-1	-ve	Rod	White	Irregular	Raised	Curled
28.	ZnAPP-2	-ve	Rod	White	Irregular	Convex	Undulate
29.	ZnAPP-3	-ve	Rod	Light green	Circular	Umbonate	Entire
30.	ZnAUU-1	-ve	Rod	White	Circular	Raised	Curled
31.	ZnAUU-2	-ve	Rod	Yellow	Irregular	Convex	Undulate
32.	ZnAUU-3	-ve	Rod	Milky white	Circular	Flat	Entire

Table.4 Estimation of Zinc solubilization quantitatively and qualitatively for the screening of ZnSB isolates from different soil samples

S.No.	Isolate name	Soluble Zn concentration ug ml^{-1}	Zinc solubilisation		
			Zinc solubilization index (ZSI)	Zone diameter (mm)	
				Solubilization zone	Culture diameter
1.	ZnKAA-1	1.51	3.85	9.12	3.20
2.	ZnKAA-2	1.45	3.03	8.57	4.23
3.	ZnKAA-3	1.75	3.37	5.70	2.41
4.	ZnKAA-4	2.45	4.03	6.12	2.02
5.	ZnKJJ-1	1.54	3.70	9.22	3.41
6.	ZnKJJ-2	2.03	4.29	10.21	3.10
7.	ZnKJJ-3	1.61	3.00	6.00	3.00
8.	ZnKJJ-4	2.89	4.91	16.12	4.12
9.	ZnPTT-1	1.32	2.95	8.00	4.10
10.	ZnPTT-2	1.45	2.73	7.13	4.12
11.	ZnPTT-3	1.84	3.33	10.15	4.35
12.	ZnPTT-4	1.36	3.26	7.10	3.14
13.	ZnPYY-1	1.96	3.85	12.01	4.21
14.	ZnPYY-2	1.98	3.90	9.14	3.15
15.	ZnPMM-1	1.41	3.43	8.30	3.41
16.	ZnPMM-2	1.24	3.28	8.22	3.60
17.	ZnPMM-3	1.21	3.25	9.21	4.10
18.	ZnPGG-1	2.85	4.46	15.32	4.43
19.	ZnPGG-2	1.79	3.99	9.01	3.01
20.	ZnPGG-3	1.45	3.36	8.05	3.41
21.	ZnGCC-1	1.22	2.50	6.20	4.12
22.	ZnGCC-2	2.32	4.07	9.52	3.10
23.	ZnGCC-3	1.78	3.92	12.01	4.11
24.	ZnGPP-1	1.32	3.43	7.64	3.14
25.	ZnGPP-2	1.13	2.37	5.48	4.01
26.	ZnGPP-3	1.96	3.98	9.31	3.12
27.	ZnAPP-1	1.89	3.43	10.21	4.21
28.	ZnAPP-2	1.42	3.35	9.41	4.01
29.	ZnAPP-3	1.65	3.70	8.12	3.01
30.	ZnAUU-1	1.01	2.22	5.12	4.21
31.	ZnAUU-2	1.48	3.73	9.44	3.46
32.	ZnAUU-3	1.26	3.58	8.50	3.30
SE(m)		0.04		0.06	
CD(P=0.05)		0.09		0.14	
CV		1.84		1.43	

Table.5 Biochemical and physiological characterization of Zinc Solubilizing Bacterial (ZnSB) isolates collected from different soil samples

S.No.	Isolate code	Starch hydrolysis	Hydrogen sulphide test	Indole production	Catalase test	Oxidase test	Gelatine liquification	Methyl red test	Vogues Proskauer test	Citrate Utilization	Ammonia production
1.	ZnKAA-1	+	+	-	+	+	+	+	-	+	+
2.	ZnKAA-2	+	+	+	+	-	+	-	+	+	+
3.	ZnKAA-3	+	+	+	+	+	+	-	+	+	-
4.	ZnKAA-4	-	+	+	+	+	+	+	+	+	+
5.	ZnKJJ-1	+	+	+	+	+	-	+	-	+	+
6.	ZnKJJ-2	+	+	+	-	+	+	+	+	+	+
7.	ZnKJJ-3	+	+	+	+	+	+	+	+	-	+
8.	ZnKJJ-4	+	+	-	+	+	+	+	+	+	+
9.	ZnPTT-1	+	+	+	+	-	+	-	+	+	+
10.	ZnPTT-2	+	+	+	+	+	+	+	+	+	+
11.	ZnPTT-3	+	+	-	+	+	+	+	+	+	+
12.	ZnPTT-4	+	+	+	+	+	+	+	+	+	+
13.	ZnPYY-1	-	+	+	+	+	-	+	-	+	+
14.	ZnPYY-2	+	+	-	+	+	+	+	+	+	+
15.	ZnPMM-1	+	+	+	+	-	+	+	+	+	+
16.	ZnPMM-2	+	+	+	+	+	+	+	+	-	+
17.	ZnPMM-3	+	+	+	-	+	+	+	+	+	-
18.	ZnPGG-1	+	+	+	+	+	+	+	-	+	+
19.	ZnPGG-2	+	+	+	+	+	+	+	+	+	+
20.	ZnPGG-3	+	+	+	+	+	+	-	+	+	+
21.	ZnGCC-1	+	-	+	+	+	+	+	+	+	+
22.	ZnGCC-2	+	+	+	+	+	-	+	+	+	+
23.	ZnGCC-3	+	+	+	-	+	+	+	+	+	-
24.	ZnGPP-1	+	+	+	+	+	+	+	-	+	+
25.	ZnGPP-2	+	+	+	+	+	-	+	+	+	+
26.	ZnGPP-3	-	+	+	+	+	+	+	+	+	+
27.	ZnAPP-1	+	+	+	+	+	+	+	+	+	+
28.	ZnAPP-2	+	-	-	+	+	+	+	+	-	+
29.	ZnAPP-3	+	+	+	+	+	+	+	+	+	+
30.	ZnAUU-1	+	+	+	+	+	+	-	+	+	+
31.	ZnAUU-2	+	+	+	+	+	+	+	+	+	+
32.	ZnAUU-3	+	+	+	+	-	+	+	+	+	+

Plate.1 Zinc solubilization of different bacterial isolates on ZnSB medium

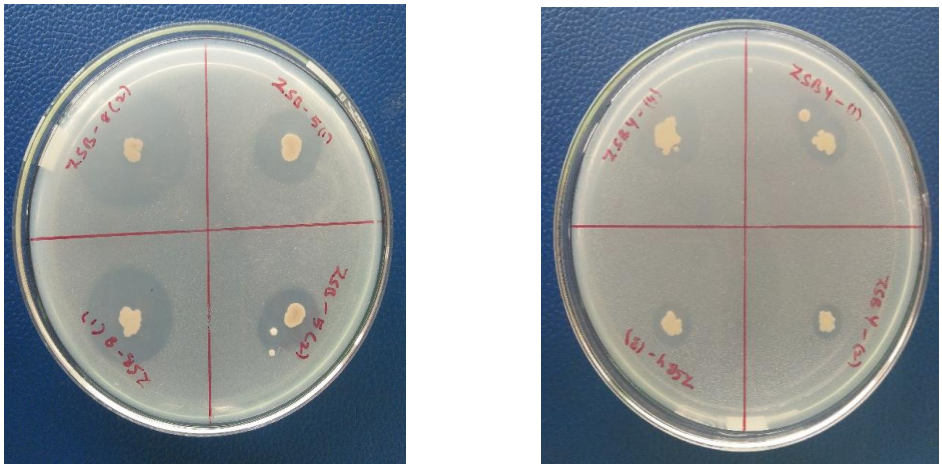


Plate.2 Zinc solubilization byZnKJJ-4bacterial isolate

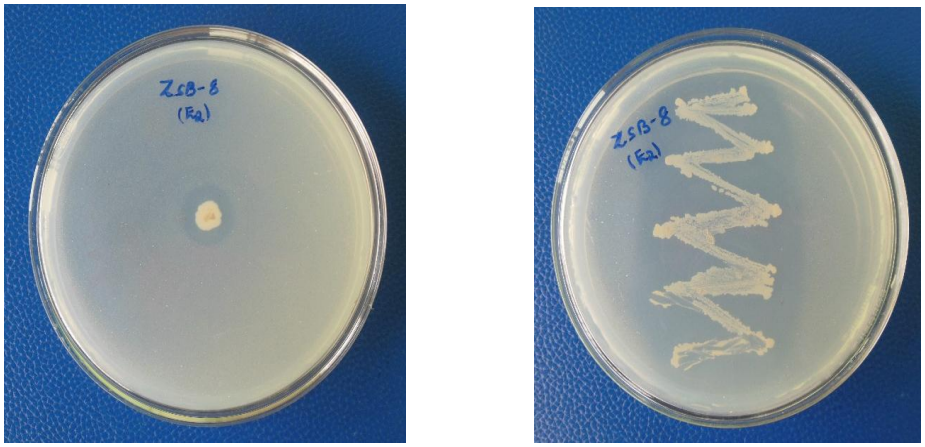


Plate.3 Zinc solubilization by ZnAUU-1bacterial isolate

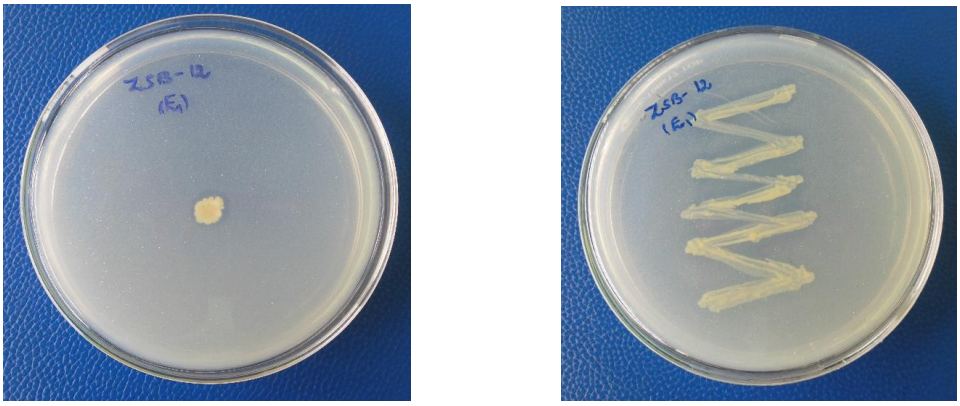
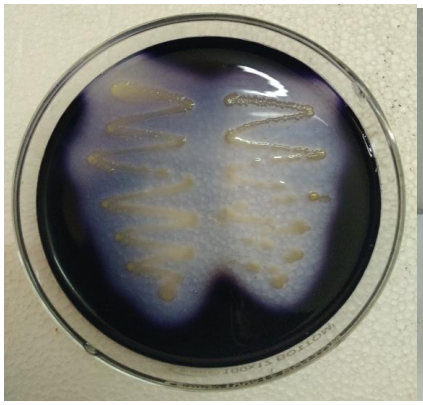
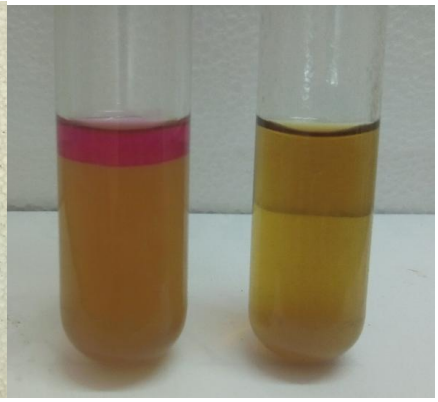


Plate.4 Different biochemical tests for characterization of ZnSB isolates



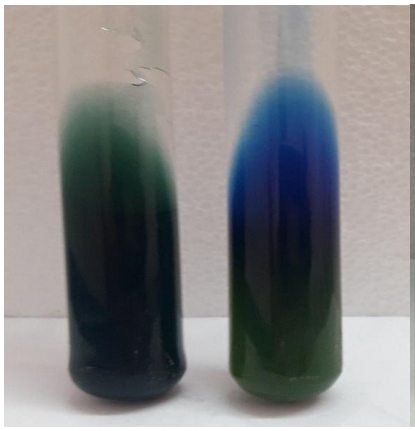
Starch hydrolysis test



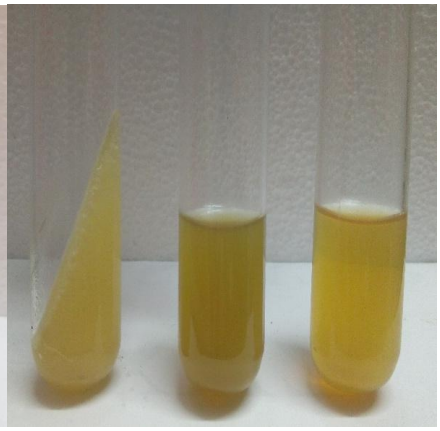
Vogues Proskauer test

Catalase test

Methyl red test



Citrate test



Gelatine test

Zinc solubulization index were also highest in ZnKJJ-4 (4.91) followed by ZnPGG-1 (4.46)and the lowest solubilization index was observed in ZnAUU-1 (2.22). This results were correlated with the Tensingh *et al.*, (2015) identified the selected strains were *Bacillus* and *Pseudomonas*. The isolated strains were characterized under *in vitro* conditions. They showed solubilization zone ranges from 2 - 5 mm at 28 - 30^{0}C. The highest solubilization was observed with *Pseudomonas putida* (5 mm) followed by *Pseudomonas flourescens* (4 mm) and the lowest solubilization was observed in *Bacillus megaterium* (2 mm).

Quantitative method in broth assay

All the thirty two zinc solubilizing isolates were able to solubilize the available zinc in zinc solubilizing broth with mineral salts medium supplemented with insoluble source of zinc phosphate at 0.1% concentration. Among them ZnKJJ-4 recorded the more available zinc content of2.89ug ml^{-1}. Second best was showed by ZnPGG-1 i.e., 2.85ug ml^{-1}.The lowest was shown by Zn AUU-1 with 1.01 ug ml^{-1}.Similar results were observed by Kajal and Desai (2015) isolated zinc solubilizing bacteria from alkaline fields. Among 309 isolates, 141 isolates were positive for zinc solubilization. Considering the quantitative aspects of solubilization efficiency the bacterial isolates ranged from (116-366%) on 0.1% zinc oxide incorporated medium. Based on solubilization efficiency five isolates with highest Zn solubilization efficiency were chosen for further tests related to multiple plant growth promoting traits.

In conclusion, zinc available in soil in various forms it was solubilized by the certain microorganisms known as zinc solubilizing microorganisms. *Pseudomonas* spp reported to be active in the zinc solubilization process. Initially zinc solubilizing bacteria was isolated and grown in respective media with insoluble source of zinc phosphate. All the isolates were characterized morphologically and biochemically. The efficient isolates were subjected to further characterization. The isolates were selected based on their good performance of PGPR characters. For Zinc solubilisation the individual isolates were able to form a solubilization zone ranged from 5.12 - 16.12 mm. The highest zinc solubilization index (4.91) was recorded in ZnKJJ-4 and lowest zinc solubilization index was observed in ZnAUU-1 (2.22).

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