Pak. J. Agri. Sci., Vol. 52(4), 915-922; 2015
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
http://www.pakjas.com.pk

PROSPECTS OF ZINC SOLUBILIZING BACTERIA FOR ENHANCING GROWTH OF MAIZE

Azhar Hussain[1], Muhammad Arshad[1,*], Zahir Ahmad Zahir[1] and Muhammad Asghar[2]

[1]Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad-Pakistan;
[2]Department of Biochemistry, University of Agriculture, Faisalabad-Pakistan.
[*]Corresponding author's e-mail: arshad_ises@yahoo.com

Zinc (Zn) is an important micronutrient and its adequate supply is considered indispensable for growth, development and normal functioning of plants. Recent reports reveal that more than 70% of Pakistani soils are Zn deficient. $ZnSO_4$, containing 33% Zn, is commonly used, but only 4-8% of the total applied Zn is available to plants while remaining gets fixed into soil. Contrarily, zinc oxide is a cheaper and insoluble source which contains 80% Zn. However, zinc solubilizing bacteria could be used to solubilize insoluble sources of zinc (ZnO). Keeping in view the above stated problem, the present study was conducted for isolation, screening, identification and characterization of efficient zinc solubilizing bacteria for improving growth of maize. Several bacteria were isolated from rhizosphere of maize through dilution plate technique. The selected bacterial isolates, capable of solubilizing ZnO, were further screened for their plant growth promoting activity under axenic conditions. Out of ten bacterial isolates, AZ6 was found best strain on the basis of maximum zinc solubilization potential and growth promotion of maize. The selected bacterium was identified as *Bacillus sp.* AZ6 (Accession # KT221633). *Bacillus sp.* AZ6 had different growth promoting attributes and also ability to produce organic acids.
Keywords: Zinc solubilization, *Bacillus sp.*, organic acids, insoluble sources, growth promotion

INTRODUCTION

Zinc is an important micronutrient for human beings, animals as well as crops. Zn is an important component of different enzymes catalyzing many metabolic reactions in plants. The essential processes of life in plants are influenced by Zn, such as (a) nitrogen metabolism i.e. nitrogen and protein uptake quality; (b) photosynthesis i.e. synthesis of chlorophyll and carbon anhydrase activity (c) resistance against biotic and abiotic stresses i.e. resistant against oxidative damage (Alloway, 2004). Zinc also plays a significant role in plant resistance against diseases, photosynthesis, cell membrane integrity, protein synthesis, pollen formation (Gurmani *et al.,* 2012) and enhances the level of antioxidant enzymes and chlorophyll within plant tissues (Sbartai *et al.,* 2011). Moreover, Zn is critical as a co-factor for the activity of more than 300 enzymes (Mccall *et al.,* 2000). In addition, Zn is required for the production of phytohormones such as absicic acid, auxin, gibberellins and cytokinins and its deficiency results in an impairment of growth of plant cells. Therefore, Zn deficiency in plants seriously affects various vital processes occurring within plants (Imran *et al.,* 2014; Younas *et al*., 2014).

Pakistani soils are generally alkaline and calcareous in nature and are more prone to Zn deficiency, because these soils are intrinsically low in available Zn (Tinker and Lauchli, 1984). In Indo-gagnetic plains 50-70% agricultural soils are Zn deficient (Alloway, 2008). Moreover, in these soils Zn also precipitates or sorbs in unavailable forms (Khoshgoftarmanesh *et al.,* 2004). Zinc deficiency also becomes a major problem due to salt stress in arid and semi-arid regions of Pakistan. It is assumed the most common deficient element of alkaline calcareous soils (Rashid and Ryan, 2008). Factors contributing in the unavailability of Zn in Pakistani soils are clayey, alkaline and calcareous nature of soils (Tahir *et al.,* 1991), high pH, high $CaCO_3$ contents, organic matter and phosphate (Kapoor *et al.,* 2002). Zn deficiency also significantly affects the root system including poor development of roots (Fageria, 2004).

Inorganic fertilizers are recommended as good source of Zn but they are quickly fixed on soil medium, causing poor availability to plants (Zia *et al.,* 2000). The application of zinc sulfate ($ZnSO_4$) in the form of fertilizer might decrease Zn deficiency and increase plant yield. However the soil applied $ZnSO_4$ transformed into different insoluble forms depending on the type of soil and entirely become unavailable in the environment within seven days of application (Rattan and Shukla, 1991). In calcareous soils, up to 90% of applied Zn fertilizer is adsorbed on soil colloids and precipitated (Saeed and Fox, 1977). Within 7 days of application customary application of inorganic zinc partially caters the plant need as 96–99% of applied Zn is converted into different insoluble forms depending upon the soil types and physicochemical reactions (Saravanan *et al.,* 2004).

There are many techniques through which we can enhance the fertilizer use efficiency of ZnO, because it has more amount of Zn as compared to $ZnSO_4$. The solubility of ZnO can be

increased by using nano technology, coating technique and zinc solubilizing bacteria (Bremner and Douglas, 1971).

Generally PGPR solubilize the nutrients through acidification, release of organic acids, chelation and by exchange reactions (Chang *et al*., 2005). Reduction in pH and availability of micronutrients in soil is very much sensitive to soil. A little change in soil pH may have a great impact on micronutrient mobility/solubility in soil. It has been reported that availability of Zn decreases 100 times with one unit increase in pH (Havlin *et al.,* 2005). Thus by decreasing the pH of alkaline soil, bioavailable fraction of Zn can be enhanced to an appreciable level. Rhizosphere microflora has been reported to lower the soil pH to a good extent (Wu *et al.,* 2006), which may occur due to secretion of some organic acids and protons extrusion (Fasim *et al.,* 2002)**.** Among microbes, both bacteria and fungi have shown terrific ability to improve plant Zn availability in the rhizosphere and also enhance zinc in plant parts (Subramanian *et al*., 2009). *Pseudomonas aeruginosa* has a potential to solubilize ZnO in liquid medium (Fasim *et al.,* 2002). Bacterial inoculation has also ability to increase bioavailable Zn in rhizosphere soil (Whiting *et al.,* 2001) and improves plant Zn content (Whiting *et al*., 2001; Biari *et al*., 2008).

PGPR produced siderophores (Saravanan *et al*., 2011), the derivatives of gluconic acids, e.g., 2- ketogluconic acid (Fasim *et al.,* 2002), 5-ketogluconic acid (Saravanan *et al.,* 2007), and different other organic acids for the mobilization of Zn and iron (Tariq *et al*., 2007). These bacteria can be used to solubilize insoluble sources of Zn such as ZnO and $ZnCO_3$ because most of the soils are rich in Zn contents but less in soluble Zn. *Bacillus* and *Pseudomonas spp.* have much potential to solubilize these sources in soil system for taking economically efficient Zn (Saravanan, 2003). The rhizosphere microorganism may benefit plants through different mechanisms including mobilization of nutrients, also acts as a biocontrol agent (Khalid *et al*., 2009). Plant growth promoting rhizobacteria (PGPR) have achieved worldwide fame for their agricultural benefits. These are the potential trend for the future research as well as tools for sustainable agriculture (Podile and Kishore, 2006). For the evaluation of zinc solubilizing bacteria maize has been selected because it is an important cereal crop of the world known as king of the crops.

Microbes are potential alternate that could cater plant zinc requirement by solubilizing the insoluble source of zinc (ZnO) and complex zinc in soil. Several genera of rhizobacteria belonging to *Pseudomonas spp.* and *Bacillus spp*. are reported to solubilize zinc. Keeping in view the above comprehensive facts the present study is planned with the objectives to isolate, screen, characterize and identify the zinc solubilizing bacteria. In this study we reported the zinc solubilizing ability to enhance the growth of maize seedling in axenic condition.

MATERIALS AND METHODS

Laboratory and growth room experiments were conducted in the Environmental Sciences Laboratory, Institute of Soil & Environmental Sciences University of Agriculture, Faisalabad for isolation, screening and characterization of novel zinc solubilizing bacteria for enhancing growth of maize.

***Isolation of zinc solubilizing bacteria*:** Zinc solubilizing bacteria were isolated from the rhizosphere of the maize by using dilution plate technique on nutrient agar medium. Isolates were purified by repeated streaking on Bunt and Rovira medium (Bunt & Rovira, 1955). Bacterial colonies with prolific growth were selected, purified and preserved in glycerol at -80°C.

***Zinc solubilizing potential of isolated bacteria*:** An incubation study was conducted to evaluate the zinc solubilizing potential of isolated bacteria with ZnO. The solubilization potential of bacteria was assessed both qualitatively and quantitatively under *in vitro* conditions (Saravanan *et al.,* 2003).

***Qualitative assay*:** To check the Zn solubilization ability of selected strains, bacteria were subjected to grow on Bunt & Rovira media containing 0.1% insoluble zinc compound (ZnO) as described by (Bunt and Rovira, 1955). Experiments were done in triplicate. Bacterial cultures were spot inoculated on the media using a sterile loop full of bacterial culture. The Petri plates were incubated at 28±1°C for 5 days in dark to observe clear halozone formation around the colonies. The holo diameters of colonies were measured. Zinc solublization area (cm^2) was calculated according to Saravanan *et al*. (2003).

$$\text{Area} = \pi r^2$$

***Quantitative assay*:** Basal broth medium (Bunt and Rovira, 1955) was prepared, autoclaved at 121 ^{0}C and kept in incubator at optimal temperature. After inoculation of various zinc solubilizing bacterial isolates, flasks were incubated at 28±1°C for 15 days in shaking incubator. The samples were collected on 15th day. pH of the culture medium was recorded after incubation. The aliquot of the medium was centrifuged (7000 rpm, 15 minutes) and filtered (0.22 µm). For the determination of soluble zinc content the culture supernatant was directly inject to atomic absorption spectrophotometer. The total amount of solubilized zinc was taken by comparing the solubilized Zn of the inoculated sample from the corresponding un-inoculated control and expressed as µg /mL of Zn culture (Saravanan *et al*., 2003).

Total 52 rhizobacterial colonies were selected and from them only 14 were positive for zinc solubilization (Qualitative). On the basis of halo-zone diameter, solubilizing area, pH reduction and zinc solubilizing ability, out of 14 bacterial isolates, 10 isolates were selected for further screening of bacteria for plant growth promotion activity

Growth room experiment:

***Screening of bacteria for plant growth promotion activities in growth room*:** The experiment was conducted in the growth room under axenic conditions to screen the bacteria for plant growth promotion activities. Under controlled conditions 10 different isolates of zinc solubilizing bacteria were used to check the effect of bacteria on the maize seedling. Growth and physiological parameters were studied. On the basis of growth and physiological parameters one effective growth promoting bacterial isolate was selected for identification and characterization**.**

***Identification of the selected bacterial isolate*:** The selected bacterial strain was identified through amplification, sequencing and bioinformatics analysis of its 16S rRNA gene sequence. For this purpose, crude DNA of the selected isolate AZ6 was extracted from the cell culture using proteinase K treatment (Cheneby *et al.,* 2004). The 16S rRNA sequence was amplified in a thermocycler (Eppendorf, USA) using the universal primers 27F (50'-AGA GTT TGA TCH TGG CTC AG-30') and 1492R (50'-TAC GGH TAC CTT GTT ACG ACT T-30') as previously described by Hussain *et al.* (2011). The PCR reaction was carried out using 2.5 µL crude DNA as a template following the program as already described by Hussain *et al*. (2013). The size of the amplified *16S rRNA* was confirmed by separating on 1% agarose gel along with GeneRuler 1kb DNA (Fermentas). The 16S rRNA PCR product was purified using a PCR Purification Kit (Favorgen, Taiwan) and sequenced by Macrogen (Seoul, Korea). 16S rRNA of AZ6 was compared with the known nucleotide sequences using BlastN accessed at www.ncbi.nlm.nih.gov/BLAST. The phylogenetic tree was constructed by carrying out the multiple alignments using ClustalX (Thompson *et al*., 1997) and processing the data using NJ Plot for neighbor joining method (Perriere and Gouy, 1996). The partial sequence was submitted in the GenBank database under the accession number *KT221633.*

***Characterization of selected strain*:** *Bacillus sp.* AZ6 was characterized for various growth promoting traits. *Bacillus sp.* AZ6 was evaluated for their auxins production as indole acetic acid (IAA) equivalents in the presence and absence of L-tryptophan as described by Sarwar *et al*. (1992). Auxin compounds (IAA-equivalents) were determined by spectrophotometer, using Salkowski coloring reagent. ACC-deaminase activity was accessed by following the method by Honma and Shimomura (1978); Penrose and Glick (2003). Siderophores production was measured following the method of Schwn and Neilands (1987).

Organic acids released by the bacterial isolates were measured following the method of Butsat *et al.* (2009). Organic acids (cinamic acid, Ferulic acid, caffeic acid, chlorggenic acid, syrirgic acid and gallic acid) were determined by HPLC (Shimadzu, Japan) with LC 10AT, UV-visible and SPD 10 AV,) after running the samples along with standards for organic compounds

RESULTS

***Zinc solubilizing potential of isolated bacteria*:** Total 52 rhizobacterial isolates were selected and from them only 14 were positive for zinc solubilization. Zinc solubilizing potential of bacterial isolates with insoluble source of zinc (ZnO) was assessed both qualitatively and quantitatively under *in vitro* conditions. The results of zinc solubilization potential of isolated bacteria are given below.

Table 1. Zinc solubilizing potential of isolated bacteria (quantitative & qualitative).

Bacterial isolates	**Qualitative (after 5 days)**			**Quantitative (after 10 days)**	
Name of parameter with units	**Holozone diameter (cm)**	**Colony diameter (cm)**	**Area (cm^2)**	**pH**	**Zinc Concentration (Broth media) (µg /mL)**
Control				7.07 ab	2.19 kl
AZ1	1.27 k	0.28 i	1.27 k	7.03 a	3.63 k
AZ2	1.73 f	0.39 f	2.35 f	6.73 cd	7.54 ef
AZ3	1.37 j	0.23 k	1.47 j	7.03 a	3.77 jk
AZ4	1.43 i	0.26 j	1.61 i	6.93 ab	3.97 ij
AZ5	1.30 k	0.32 h	1.33 k	6.93 ab	4.23 i
AZ6	3.13 a	0.97 a	7.69 a	4.93 f	13.55 a
AZ7	2.33 b	0.46 c	4.26 b	6.87 abc	8.31 d
AZ8	1.60 h	0.35 g	2.02 h	6.60 d	5.77 h
AZ9	2.17 c	0.53 b	3.71 c	5.43 e	9.45 b
AZ10	1.73 f	0.34 g	2.36 f	6.67 d	6.63 g
AZ11	1.90 d	0.42 de	2.84 d	6.63 d	7.76 e
AZ12	1.87 e	0.41 e	2.75 e	6.77 bcd	6.66 g
AZ13	1.93 d	0.42 d	2.91 d	6.93 ab	8.68 c
AZ14	1.67 g	0.38 f	2.18 g	6.73 cd	7.27 f

Means sharing the same letter do not differ significantly (P< 0.05).

***Qualitatively assay*:** In plate assay, the strains produced a clear solubilization halo on Bunt and Revira medium amended with insoluble zinc sources i.e. zinc oxide. Most of the bacterial isolates solubilized zinc better than control. Among the bacterial isolates maximum halo-zone diameter was observed in AZ6 (3.13 cm). The strains AZ1, AZ3, AZ4, and AZ5 showed minimum zinc solubilization while AZ2, AZ6, AZ7, AZ9, and AZ13 showed zinc solubilization ranging from 1.73 to 2.33 cm holozone**.** The maximum colony diameter was observed in bacterial isolates AZ2, AZ6, AZ7, AZ9 and AZ13. Among the bacterial isolates, maximum colony diameter was observed in AZ6 (0.97 cm) as compared to all bacterial isolates. The bacterial isolates AZ1, AZ3, AZ4, and AZ5 showed minimum colony diameter in plates. Solubilizing area was calculated and AZ6 showed maximum area as compared to other bacterial isolates.

Quantitative assay:

***Amount of solubilized zinc (µg zinc/mL)*:** Most of the bacterial isolates solubilized zinc but AZ6, AZ13, AZ9, AZ7 and AZ2 (14, 9, 9, 8 and 4 µg zinc/mL respectively) performed better than all other bacterial isolates and control treatment (2 µg zinc/mL). Among the bacterial isolates, AZ6 showed maximum zinc solubilization that was 13.55 µg zinc/mL. The bacterial isolates, AZ1, AZ3, AZ4, and AZ5 showed minimum zinc solubilizing activity as compared to other isolates (Table 1).

***pH of culture medium*:** Decrease in pH of the broth medium was observed when inoculated with zinc solubilizing bacterial isolates as compared to control. Data regarding pH decrease is represented in Table 1. Most of the bacterial strains reduced pH of the broth media and solubilized insoluble zinc, but maximum pH reduction was observed in response to AZ6. The bacterial isolates AZ1, AZ3, AZ4, and AZ5 also showed decrease in pH.

Effective zinc solubilizing bacteria for improving growth of maize seedlings (growth room study): Ten effective zinc solubilizing bacterial isolates were selected on the basis of zinc solubilization potential. The selected isolates were further screened for their growth promoting activity under axenic conditions. The results imply that the zinc solubilizing isolates significantly improved the growth of maize seedlings. The results are given below.

Table 2. Effect of zinc solubilizing bacteria on growth of maize seedlings.

Parameters	Shoot length (cm)	Root length (cm)	Shoot fresh biomass (g/plant)	Root fresh biomass (g/plant)	Shoot dry biomass (g/plant)	Root dry biomass (g/plant)
Control	20.33 i	7.70 i	1.707 i	0.43 h	0.29 f	0.12 h
AZ2	29.00 cd	10.20 de	1.940 e	0.76 f	0.30 f	0.23 f
AZ6	32.33 a	12.13 a	2.393 a	1.81 a	0.55 a	0.57 ab
AZ7	29.33 bc	10.43 cd	1.880 g	0.50 g	0.41 d	0.15 g
AZ8	26.53 f	8.70 h	1.777 h	0.80 e	0.26 g	0.25 e
AZ9	28.50 d	9.90 e	2.060 d	1.57 b	0.41 d	0.53 b
AZ10	27.16 e	9.50 f	2.143 c	1.03 d	0.45 c	0.36 c
AZ11	28.70 d	10.53 c	2.220 b	0.80 e	0.51 b	0.24 ef
AZ12	25.50 g	8.43 h	2.013 e	1.54 b	0.36 e	0.55 b
AZ13	29.76 b	11.33 b	2.037 de	1.09 c	0.40 d	0.36 c
AZ14	24.66 h	9.13 g	1.967 f	1.01 d	0.34 e	0.32 d

Means sharing the same letter do not differ significantly (P< 0.05).

Table 3. Effect of zinc solubilizing bacteria on physiology of maize seedlings (growth room study).

Parameters	Photosynthetic rate (A) (µmol CO_2 m^{-2} s^{-1})	Transpiration rate (E) (mmol H_2O m^{-2} s^{-1})	Stomatal conductance (gs) (mmol m^{-2} s^{-1})	Chlorophyll Content (Spade value)
Control	3.23 g	1.11 i	106.67 i	13.10 j
AZ2	4.23 f	1.41 g	130.33 b	31.36 b
AZ6	6.14 a	2.32 ab	135.00 a	35.73 a
AZ7	5.43 b	1.91 e	126.00 c	27.50 d
AZ8	4.65 cd	1.20 h	115.67 g	14.63 i
AZ9	4.40 e	2.03 d	122.67 de	20.23 f
AZ10	4.26 f	1.53 f	118.00 fg	15.90 gh
AZ11	4.61 d	2.10 c	125.33 cd	22.56 e
AZ12	4.20 f	1.18 h	112.33 h	15.43 hi
AZ13	4.74 c	2.19 b	127.33 c	29.30 c
AZ14	4.30 ef	1.53 f	120.67 ef	16.76 g

Means sharing the same letter do not differ significantly (P< 0.05).

***Growth parameters*:** The data showed positive effect of most of the zinc solubilizing bacteria on growth parameters of maize (shoot length, root length, fresh and dry shoot and root biomass) as compared to un-inoculated control (Table 2). The maximum shoot length was observed by AZ6, AZ13, AZ7 and AZ2. Bacterial isolates AZ6 increased the shoot length up to 59% as compared to un-inoculated control. Root length of maize seedling was also improved by most of the zinc solubilizing bacteria. It was observed that maximum root length showed by AZ6 (12.13 cm) and the minimum was observed in un-inoculated control (7.70 cm). Other growth parameters like fresh and dry shoot and root biomass were the maximum by the strain AZ6 as compared to other bacterial isolates. It was also observed that AZ6 was best growth promoting strain compared to all other strains.

***Physiological parameters*:** The data showed positive effect of most of the isolates of zinc solubilizing bacteria on physiological parameters (photosynthetic rate, transpiration rate, stomatal conductance and chlorophyll contents) of maize as compared to un-inoculated control (Table 3). The maximum photosynthetic rate was observed in bacterial isolate AZ6. Bacterial isolate AZ6 increased the photosynthetic rate by 90% more as compared control. It was observed that bacterial isolates including AZ6 improved physiological parameters of maize.

***Identification of selected strain*:** The 16S rRNA gene (1354 bp) amplified from the strain AZ6 was sequenced; the sequence was deposited in the GenBank database under the accession number *KT221633*. The BlastN analysis of the 16S rRNA amplicon indicated its maximum similarity (912) with the bacterial strains belonging to genus *Bacillus*. In-silico analysis of the 16S rRNA of the bacterial strain AZ6 was carried out by constructing the phylogenetic tree following the neighbor joining method. The bacterial strain AZ6 was observed to be phylogenetically positioned in the cluster comprising the bacterial strains belonging to the genus *Bacillus*. Following the phylogenetic relationship of the strain AZ6 with several *Bacillus sp*. (Fig. 1), this bacterial isolate was named as *Bacillus sp. AZ6*. The GeneBank accession numbers of the strains used for in-silico analysis are given in brackets, whereas, bootstrap values greater than 800% are marked as black circles.

***Characterization*:** Selected zinc solubilizing strain (*Bacillus sp.* AZ6) was characterized for IAA (Indole-3-acitic acid) production activity, ACC-deaminase activity, siderophores production and release of organic acids by the *Bacillus sp. AZ6*. ACC-deaminase activity (Table 4) of the selected zinc solubilizing bacteria was 211.34 α-ketobutyrate nmol g^{-1} biomass hr^{-1}. Bacteria had ability to produce auxin (as IAA equivalents) both in the presence and absence of L-tryptophan (Table 4). In the absence of L-tryptophan produced 12.03 µg mL^{-1} auxin. In the presence of L-tryptophan in the growth media auxin production was 35.30 µg mL^{-1}.

The production of low molecular weight, iron chelating siderophores by Zn mobilizing bacterial strain was detected on blue agar. The solubilizing bacterial strain expressed the

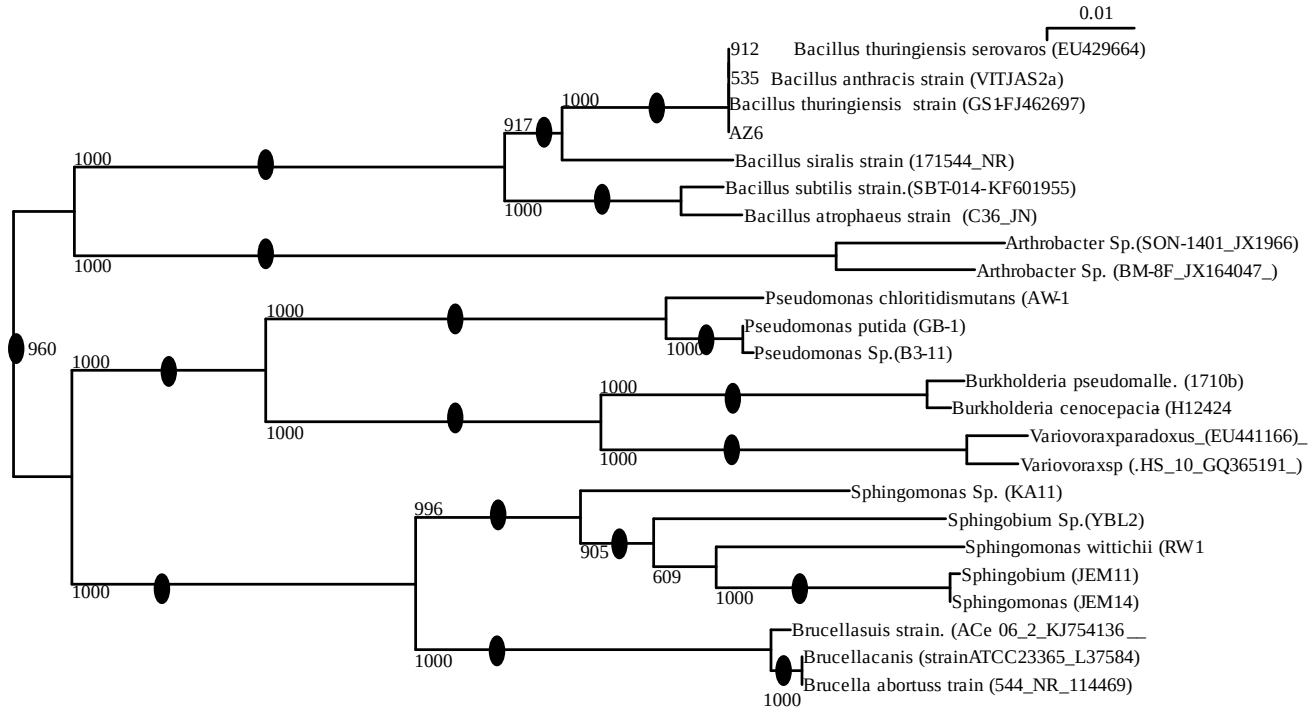

Figure 1. Neighbor-joining phylogenetic analysis resulting from the multiple alignment of 16S rRNA gene sequence of Bacillus sp. with those of other bacterial strains found in Gene Bank database.

capability to chelate Fe but with various strength. The bacterial metabolites extracted by methanol were analyzed by HPLC (Shimadzu, Japan) with LC 10AT, UV-visible and SPD 10 AV). The chromatograms of bacterial metabolites exhibited different patterns (Fig. 2) and many peaks were detected. Based on comparison with the standards, we could identify some of the compounds in the metabolites *Bacillus sp. AZ6*. Cinamic acid (9.8 mg L^{-1}), Ferulic acid (8.1 mg L^{-1}), Caffeic acid (5.9 mg L^{-1}), Chlorggenic acid (5.8 mg L^{-1}), Syrirgic acid (3.8 mg L^{-1}), Gallic acid (3.8 mg L^{-1}) were present in the metabolites of *Bacillus sp. AZ6*.

Table 4. Growth promoting characteristics of *Bacillus sp. AZ6.*

Character	Value
ACC Deaminase activity	211.34 α-ketobutyrate nmol g^{-1} biomass hr^{-1}
Auxin production	12.03 µg/mL (Without L-Tryptophan) 35.30 µg/mL (With L-Tryptophan)
Siderophore production	+++

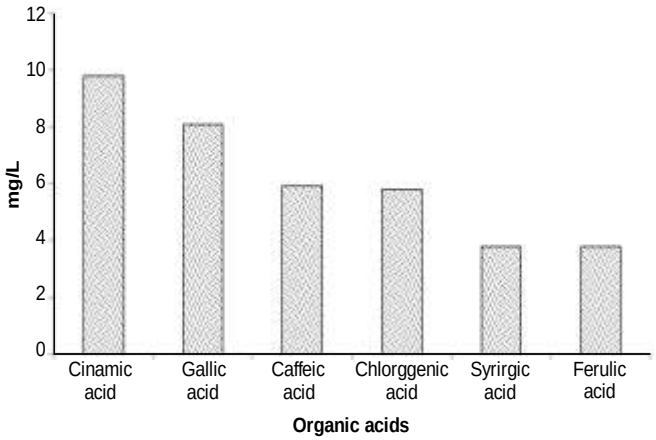

Figure 2. Bacterial metabolite profiles, detected by HPLC.

DISCUSSION

In present study the 52 bacteria were isolated from rhizosphere of maize. In plate assay, only 14 bacterial isolates showed good potential of zinc solubilizing bacteria from insoluble source zinc (ZnO). As the plate assay has some limitations, it is not considered as relatively authenticated procedure to assess the solubilization and mineralization ability of bacteria. Therefore, the bacteria showing good potential of zinc solubilizing on agar plate were further tested in a broth assay supplemented with ZnO. Similar, studies have been conducted by Sarvanan *et al.* (2003) in which *Bacillus* sp. and *Pseudomonas sp*. were screened on zinc oxide (ZnO) i.e., Zinc solubilizing bacteria isolate to solubilize zinc oxide (Di Simine *et al.*, 1998; Saravanan *et al.*, 2007; Sharma *et al.*, 2014).

The bacterial isolates capable to solubilize zinc oxide (ZnO) were further screened for their plant growth promoting activity of maize seedling under axenic conditions. In present study different zinc solubilizing bacterial isolates enhanced zinc availability to plants. Results showed that zinc solubilizing bacterial isolates significantly improved the growth and physiological parameters of maize seedling in a growth room experiment. Bacterial isolates increased significantly growth and physiological parameters of maize plant as compared to un-inoculated control. These finding are in agreement with the previous reports that the increase in growth of plant might be due to the reason that zinc solubilizing bacteria increased the solubilization of nutrients, produced different plant growth promoters and siderophores that suppressed the pathogens (Kloepper *et al.*, 1989; Arshad and Frankenbcrgcr, 1998). Zinc solubilizing bacterial strains increased the root length, root hair and surface area, due to which the availability of nutrients increased (Biswas *et al.*, 2000; Adesemoye *et al.*, 2008). According to results AZ6 improved maximum growth and physiological parameters of maize seedling. Maximum growth and physiological parameters of maize seedling as compared to all other bacterial isolates might be due to AZ6 have more growth promoting attributes of AZ6 compared to other strains.

The selected strain was later identified as *Bacillus sp.* AZ6. Previously it has been reported by Sarvanan *et al.*, 2003 that *Bacillus sp.* has ability to solubilize zinc oxide. The *Bacillus* sp. AZ6. was characterized for their plant growth promoting attributes like auxin production, ACC-deaminase activity, siderophores production and organic acid production. Our bacterial strain produced IAA, which could be useful while interactions with the plants as plant exudates have tryptophan that may help the IAA production potential of the bacteria. These results are in agreement with earlier reports in which IAA producing rhizobacteria were isolated and have beneficial impact on plant growth promotion (Ali *et al.*, 2014). In our study *Bacillus sp. AZ6* showed ACC-deaminase activity. ACC-deaminase activity of PGPR help plants to withstand stress (biotic and abiotic) by lowering the level of the stress hormone ethylene through the activity of enzyme ACC-deaminase, which hydrolyses ACC in to α-ketobutarate and ammonia instead of ethylene (Arshad *et al.*, 2007). In our study the strain expressed siderophore production. Siderophores are recognized for making iron (Fe) available to the plant and the production of the siderophores by the microorganisms can bind iron with high affinity, making the iron unavailable for the other microorganisms, and thereby limiting their growth. These results are similar with the previous results of (Gull and Hafeez 2009; Naureen *et al.*, 2009).

The organic acids produced by *Bacillus sp. AZ6* were cinamic acid, ferulic acid, caffeic acid, chlorggenic acid, syrirgic acid,

gallic acid. These acids solubilized the insoluble source of zinc by lowering pH. The organic acid production by the bacteria can directly facilitate the Zn by changing the soil colloids surface charge and reducing sorption (Jones, 1998).

***Conclusion*:** Zinc solubilizing bacterial strain *Bacillus sp.* AZ6 solubilized insoluble source of zinc (ZnO) and had the ability to improve growth of maize.

***Acknowledgement(s)*:** Authors are highly thankful to the Institute of Soil & Environmental Sciences, University of Agriculture, Faisalabad-Pakistan for providing research facilities.

REFERENCES

Adesemoye, A., H. Torbert, and J. Kloepper. 2008. Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. Can. J. Microbiol. 54: 876-886.

Ali, S.Z., V. Shya and L.V. Rao. 2014. Isolation & characterization of drought-tolerant acc deaminase & exopolysaccharide-producing fluorescent *Pseudomanas sp*. Ann. Microbiol. 64:493-502.

Alloway, B.J. 2004. Zinc in soils and crop nutrition. International Zinc Association, Brussels, Belgium.

Alloway, B.J. 2008. Zinc in soils and crop nutrition, 2nd Ed. International Fertilizer Industry Association, Paris, France.

Arshad, M. and W.T. Frankenbergedr. 1998. Plant growth regulating substances in the rhizosphere: Microbial production and functions. Adv. Agron. 62: 46-51.

Arshad, M., M. Saleem and S. Hussain. 2007. Perspectives of bacterial ACC-deaminase in phytoremediation. Trends Biotechnol. 25: 356-362.

Biari, A., A. Gholami and H.A. Rahmani, 2008. Growth promotion and enhanced nutrient uptake of maize (*Zea mays* L.) by application of plant growth promoting rhizobacteria in arid region of Iran. J. Biol. Sci. 8: 1015–1020.

Biswas, B.K., T.R. Chowdhury, G. Samanta, B.K. Mandal, G.C. Basu, C.R. Chanda, D. Lodh, K.C. Saha and S.K. Mukherjee. 2000. Groundwater arsenic contamination in Bangladesh and West Bengal, India. Environ. Health Perspect. 108:393-397.

Bremner, J.M. and L.A. Douglas. 1971. Inhibition of urease activity in soil. Soil Biol. Biochem. 3: 299-307.

Bunt, J.S. and A.D Rovira. 1955. Microbiological studies of some subantartic soils. J. Soil Sci. 6:119-128.

Butsat, N., N. Weerapreeyakul and S. Siriamornpun. 2009. Change in phenolic acids and antioxidant activity in Thai rice husk at five growth stages during grain development. J. Agri. Food Chem. 57:4566-4571.

Chang, H.B., C.W. Lin and H.J. Huang. 2005. Zinc induced cell death in rice (*Oryza sativa* L.) roots. Plant Growth Regul. 46: 261–266.

Cheneby, D., S. Perrez, C. Devroe, S. Hallet, Y. Couton, F. Bizouard, G. Iuretig, J.C. Germon and L. Philippot. 2004. Denitrifying bacteria in bulk and maize-rhizospheric soil: Diversity and N_2O-reducing abilities. Can. J. Microbiol. 50:469-474.

Di Simine, C.J. Sayer and G. Gadd. 1998. Solubilization of zinc phosphate by a strain of *pseudomonas fluorescens* isolated from a forest soil. Boil. Fert. Soils 28: 87-94.

Fageria, N. 2004. Dry matter yield and nutrient uptake by lowland rice at different growth stages. J. Plant Nutr. 27: 947-958.

Fasim, F., N. Ahmed, R. Parsons and G.M. Gadd. 2002. Solubilization of zinc salts by bacterium isolated by the air environment of tannery. FEMS Microbiol. Lett. 213: 1-6.

Gull, M. and F.Y. Hafeez. 2009. Characterization of siderophore producing bacteria as plant growth promoting and biocontrol agents In: F.Y. Hafeez, K.A. Malik and Y. Zafar (eds.), Microbial Technologies for Sustainable Agriculture. Crystal press, Islamabad Pakistan; pp.59-62. ISBN: 978-969-8189-14-3.

Gurmani, A.R., S.U. Khan, R. Andaleep, K. Waseem and A. Khan. 2012. Soil application of zinc improves growth and yield of tomato. Int. J. Agric. Biol. 14: 91-96.

Havlin, J., J.D. Beaton, S.L. Tisdale and W.L. Nelson. 2005. Soil fertility and fertilizers: An introduction to nutrient management. Upper Saddle River NJ: Pearson Prentice Hall.

Honma, M. and T. Shimomura. 1978. Metabolism of l-aminocyclopropane-1-carboxylic acid. Agri. Biol. Chem. 42:1825-1831.

Hussain, S., M. Devers-Lamrani, N. El-Azhari and F. Martin-Laurent. 2011. Isolation and characterization of an isoproturon mineralizing *Sphingomonas sp.* strain SH from a French agricultural soil. Biodegr. 22:637-650.

Imran, M., M. Arshad, A. Khalid, S. Kanwal and D.E. Crowley. 2014. Perspectives of rhizosphere microflora for improving Zn bioavailability and acquisition by higher plants. Int. J. Agri. Biol. 16: 653-662.

Jones, D.L. 1998. Organic acids in the rhizosphere– a critical review. Plant Soil 205: 25-44.

Kapoor, S., A. Kobayashi and H. Takatsuji. 2002. Silencing of the tapetum-specific zinc finger gene TAZ1 causes premature degeneration of tapetum and pollen abortion in petunia. The Plant Cell Online 14: 2353-2367.

Khalid, A., M. Arshad, B. Shaharoona and T. Mahmood. 2009. Plant growth promoting rhizobacteria (PGPR) and sustainable agriculture, pp.133-160. In: M.S. Khan, A. Zaidi and J. Musarat (eds.), Microbial Strategies for Crop Improvement. Springer-Verleg, Germany.

Khoshgoftarmanesh, A.H., H. Shariatmadari, N. Karimian, M. Kalbasi and M.R. Khajehpour. 2004. Zinc efficiency of wheat cultivars grown on a saline calcareous soil. J. Plant Nutr. 27: 1953-1962.

Kloepper, J.W., R. Lifshitz and R.M. Zablotowiez. 1989. Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol.7: 39-43.

McCall, K.A., C. Huang and A.F. Carol. 2000. Function and mechanisms of zinc metalloenzymes. J. Plant Nut. 130: 1437-1446.

Naureen, Z., A.H. Price, F.Y. Hafeez and M.R. Roberts. 2009. Identification of rice blast disease-suppressing bacterial strains from the rhizosphere of rice grown in Pakistan. Crop Prot. 2:1052-1060.

Penrose, D.M. and B.R. Glick. 2003. Methods for isolating and characterizing ACC-deaminase-containing plant growth promoting rhizobacteria. Physiol. Plant. 118:10-15.

Perriere, G. and M. Gouy. 1996. WWW-Query: an on-line retrieval system for biological sequence banks. Biochimie 78:364-369.

Podile, A.R. and G.K. Kishore. 2006. Plant growth-promoting rhizobacteria, pp. 195-231. In: S.S. Gnanamanickam (ed.), Plant-Associated Bacteria. Springer New York.

Rashid, A. and J. Ryan. 2008. Micronutrient constraints to crop production in the Near East: Potential significance and management strategies. In: B.J. Alloway (ed.), Micronutrient Deficiencies in Global Crop production. Springer, Dordrecht, Netherlands. pp.149-180.

Rattan, R. and L. Shukla. 1991. Influence of different Zn carriers on the utilization of micronutrients by rice. J. Ind. Soc. Soil Sci. 39: 808-810.

Saeed, M., R.L. Fox. 1977. Relation between suspension pH and zinc solubility in acid and calcareous soils. Soil Sci. 124: 199–204.

Saravanan, S.V., R.S. Sudalayandy and Savariappan. 2003. Assessing *in vitro* solubilization potential of different zinc solubilizing bacterial (ZSB) isolates. Brazilian J. Microbiol. 34: 121-125.

Saravanan, V.S., M.R. Kumar and T.M. Sa. 2011. Microbial zinc solubilization and their role on plants. In: D.K. Maheshwari (ed.), Bacteria in Agrobiology: Plant nutrient management. Springer, Berlin; pp.47-63.

Saravanan, V., M. Madhaiyan and M. Thangaraju. 2007. Solubilization of zinc compounds by the diazotrophic, plant growth promoting bacterium Gluconacetobacter diazotrophicus. Chemosphere 66:1794-1798.

Saravanan, V.S., S.R. Subramoniam and S.A. Raj. 2004. Assessing *in vitro* solubilization potential of different zinc solubilizing bacterial (ZSB) isolates. Brazilian J. Microb. 35: 121–125.

Sarwar, M., M. Arshad, D.A. Martins and W.T. Frankenberger. 1992. Tryptophan dependent biosynthesis of auxins in soil. Plant soil 147:207-215.

Sbartai, H., M. Djebar, R. Rouabhi, I. Sbartai and H. Berrebbah. 2011. Antioxidative response in tomato plants *Lycopersicon esculentum* L. roots and leaves to zinc. Am.-Eurasian J. Toxicol. Sci. 3: 41-46.

Schwn, B. and J. Neilands. 1987. Universal chemical assay for the detection and determination of siderophores. Ann. Biochem. 160:47-56.

Sharma, S.K., A. Ramesh, M.P. Sharma, O.P. Joshi, B. Govaerts, K.L. Steenwerth and D.L. Karlen. 2014. Microbial community structure and diversity as indicators for evaluating soil quality. In: E. Lichtfouse (ed.), Biodiversity, Biofuel, Agroforestry and Conservation Agriculture, Sustainable Agriculture Review 5. Springer Science + Business Media B.V. The Netherlands 1:317-358.

Subramanian, K., V. Tenshia, K. Jayalakshmi and V. Ramachandran. 2009. Role of arbuscular mycorrhizal fungus (*Glomus intraradices*)–(fungus aided) in zinc nutrition of maize. J. Agric. Biotechnol. Sust. Dev. 1: 29-38.

Tahir, M., M. Kausar, R. Ahmad and A. Bhatti. 1991. Micronutrient status of Faisalabad and Sheikhupura soils. Pak. J. Agric. Res. 12: 134-140.

Tariq, M., S. Hameed, K.A. Malik, F.Y. Hafeez. 2007. Plant root associated bacteria for zinc mobilization in rice. Pak. J. Bot. 39: 245-253.

Thompson, J.D., T.J Gibson, F. Plewniak, F. Jeanmougin and D.G. Higgins. 1997. The ClustalX Windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl. Acids Res. 24:4876-4882.

Tinker, P.B. and A. Lauchli. 1984. Advances in Plant Nutrition, Vol. 1. Praeger Publishers, New York; p.301.

Whiting, S.N., M. De Souza and N. Terry. 2001. Rhizosphere bacteria mobilize Zn for hyperaccumulator by *Thlaspi caerulescens*. Environ. Sci. Technol. 35: 3144-3150.

Wu, S.C., K.C. Cheung, Y.M. Luo and M.H. Wong. 2006. Effects of inoculation of plant growth promoting rhizobacteria on metal uptake by *Brassica juncea*. Environ. Pollut. 140: 124-135.

Younas, M.S., M.S. Butt, I. Pasha and M. Shahid. 2014. Development of zinc fortified chitosan and alginate based coatings for apricot. Pak. J. Agri. Sci. 51: 1033-1039.

Zia, M.S., M. Aslam, M. Baig and A. Ali. 2000. Fertility issues and fertilizer management in rice–wheat system: A review. Quart. Sci. Vision 5: 59-73.