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Full Length Article

Screening of Zinc Solubilizing Bacteria and their Potential to Increase Grain Concentration in Wheat (*Triticum aestivum*)

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Abstract

Zinc (Zn) is one of the most important micronutrients essential for optimum plant growth. Owing to alkaline conditions, the substantial quantity of applied inorganic Zn in soil is converted into unavailable form. In a recent decade, importance of Zn solubilizing bacteria (ZSB) has increased and these are potential candidate for improving bioavailable fraction of Zn to host plant. The objective of this study was the isolation, screening and characterization of ZSB *in vitro* and to access potential of selected ZSB to increase growth and Zn concentration in wheat. The total of 77 bacterial strains were isolated from different areas of Punjab (Shahkot, Sheikhupura, Chinniot and Gojra) of which 35 showed positive response towards Zn solubilization on Bunt and Rovira media amended with Zn source. Among these isolates 7 were selected on the basis of clear halozone and colony diameter. These isolates were then characterized on the basis of auxin production and ACC-deaminase (1-aminocyclopropane 1-carboxylicacid) activity. Among 7 isolates H-103 produced maximum colony, halo zone diameter and showed significantly higher soluble Zn (37.2 and 31.9 mg kg^{-1}) in broth amended with zinc carbonate and zinc oxide, respectively. The pH of the broth decreased in case of all strains ranging from 8.07 to 4.05 within 8 days. The results also revealed that ZSB (H-103) significantly enhanced biomass (25.4%) and Zn contents (57.7%) in wheat grains. It is concluded that the strain H-103 had ability to solubilize Zn and thus could be used as biofertilizer to improve wheat growth and Zn accumulation; however, these findings warrant further field experiments.

Keywords: Biofertilizer; Characterization; Wheat; Zinc solubilizing bacteria

Introduction

Micronutrient malnutrition is an emerging issue of developing and developed countries which result in tremendous form of hunger. Approximately more than half of the world population is facing the problem of low availability of micronutrients including Zn, Fe, I and is one of the most serious global threat and challenge to human kind (Welch and Graham, 2004). Like other Asian countries, Pakistan is also facing the problem of micronutrient malnutrition due to arid to semiarid climate, alkaline soils with low organic matter and high temperature which results in upward movement of water. After reaching the surface of soil, water evaporates leaving the salts behind and results in accumulation of salts on surface (Imtiaz *et al*., 2010). This becomes the main reason behind the high pH of soils and deficiency of nutrients especially micronutrients like Zn, Fe, Mn and Cu (Khalid *et al*., 2013). Other reasons for deficiency of micronutrients include cultivation of high yielding crops, intensive cropping system, poor recycling of crop residues, low organic matter and excessive fertilization (Hafeez *et al*., 2013). Among various micronutrients, Zn is an important micronutrient that is involved in different metabolic processes of plant including respiration, photosynthesis and assimilation of other major nutrients and thereby essential for the growth and development of plant and involved in activation of enzymes (Cakmak, 2008; Rehman *et al*., 2012). It is one of the most important micronutrients deficient among women and pre-school children, which ultimately results in hidden hunger. According to an estimate hidden hunger of micronutrients, especially zinc, iron, iodine and selenium affects more than 2 billion people worldwide (White and Broadly, 2009; WHO, 2012). In Pakistan 37% of population is suffering Zn malnutrition (Cakmak, 2008). Due to deficiency of Zn about 1/3 of total world's population becomes affected and thus considered a potent health hazard for human because it leads to thousands of deaths annually (White and Broadley, 2011). In order to mitigate the emerging health issues and to reduce the burden of diseases globally, there is need to increase nutrient concentration in grains of crop plants along with better per acre yield.

There are different strategies to combat micronutrient deficiencies that include food fortification, supplementation and enrichment (Bouis, 2003; Bouis *et al*., 2009) along with agronomic practices, genetic approaches and plant breeding.

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The agronomic practices have been found of potential significance (Reddy *et al*., 2003; Pfeiffer and McClafferty, 2008). Fertilization appears a quick methodology to rectify the nutrient deficiency but the cost of micronutrient fertilizers is high. The use of plant growth promoting microorganisms is a novel approach in this respect (Alavi *et al*., 2008). PGPR consist of beneficial microorganisms, which live in the rhizosphere of plant and promote plant growth by different mechanisms including biological nitrogen fixation, solubilization of phosphorus, synthesizing siderophores which can solubilize insoluble iron from the soil, production of phytohormones such as auxins, cytokinins and gibberellins (Glick, 2005; Jou *et al*., 2012; Ahmad *et al*., 2014; Mendoza, 2015). Bacterial auxin improve the uptake of mineral nutrient by enhancing the root exudation and also increase the lateral and adventitious rooting systems which ultimately increases bacterial population on and around the roots. The use of PGPR is an attractive way to decrease the use of pesticides, chemical fertilizers and other agro-chemicals; therefore this approach is becoming important (Rana *et al*., 2012).

In the rhizosphere of plants, bacteria release various chelating metabolites including siderophore, which are considered an important reserves of micronutrients easily available to plants like Zn and Fe (Masalha *et al*., 2000; Ahemad and Kibert, 2014). Microbial siderophore makes complexes with Zn and increases plant available fraction of it (Yehuda *et al*., 1996; Madsen *et al*., 2012). It is found that PGPR possibly produce siderophores, gluconate, or the derivatives of gluconic acids, e.g., 2-ketogluconic acid (Fasim *et al*., 2002; Salantur *et al*., 2006) 5-ketogluconic acid (Saravanan *et al*., 2007), and various other organic acids (Tariq *et al*., 2007) for the mobilization of Zn. Besides promoting plant growth, PGPR ensure the availability of nutrients to enhance the nutrient use efficiency, mitigate biotic and abiotic stresses (Arshad *et al*., 2007; Amor *et al*., 2008; Jou *et al*., 2012; Ahmad *et al*., 2014).

Thus, PGPR are a diverse group of bacteria that can be found in the rhizosphere on root surfaces as well as in association with roots (Maheshwari *et al*., 2012; Ahmad *et al*., 2008). These soil bacteria have been shown to improve plant health or increase yield can also mobilize micronutrients like Zn and Fe. Biofortification is the process by which the nutritional quality of food crops is improved through agronomic practices, conventional plant breeding, or modern biotechnology. Thus, biofortification is a approach aimed at increasing the bioavailability of micronutrients such as Zn and Fe in the staple crops of specific region (Stein, 2010). But very little information is available in this aspect. Therefore, this study investigated the PGPR having ability to solubilize indigenous Zn and improve its accumulation in edible part of crop. The specific objectives of this study were 1) the isolation, screening and characterization of Zn solubilizing bacteria and 2) to assess the potential of selected Zn solubilizing bacteria to increase growth and Zn concentration in wheat grain.

Materials and Methods

Sample Collection and Isolation of Zinc Solubilizing Bacteria

Soil samples were collected from the rhizosphere of wheat crop at tillering stage (due to maximum activity of microbes at this stage) from different areas of Punjab (Shahkot, Sheikupura, Chinniot and Gojra). These areas are known as rice belt of Punjab having rice-wheat rotation cropping system. Zinc solubilizing bacteria were isolated from rhizospheric soil by using serial dilution plate technique on Luria-Bertani (LB) agar medium. The medium contains tryptone 10, NaCl 10, yeast 5 and agar 20 (g L^{-1}) (Ahmad *et al*., 2014). Isolates were purified by repeated streaking on Bunt and Rovira medium containing (g L^{-1}): glucose 10, ammonium sulphate 1, potassium chloride 0.2, di-potassium hydrogen phosphate 0.1, magnesium sulphate 0.2, agar 15, Zn source 0.1% and pH 7 (Bunt and Rovira, 1955). Bacterial colonies with prolific growth and clear halozone were selected, purified and preserved in 20% glycerol at -40°C.

Zinc Solubilization Assay

An incubation study was conducted to check the zinc solubilizing capacity of isolated bacteria with two insoluble sources of zinc, zinc oxide (ZnO) and zinc carbonate ($ZnCO_3$). The selected bacterial isolates were exposed to Bunt and Rovira medium containing insoluble Zn source to determine Zn solubilization qualitatively (Bunt and Rovira, 1955). The growth of bacterial culture were then spotted on media with triplicates to check clear halo zone formation and incubated at 28 ± 1°C for 7 d. The colony and halo zone diameter were measured by measuring scale. Zinc-solubilization efficiency (SE) was calculated as the ratio of total diameter i.e., clearance zone including bacterial growth and the colony diameter as described by Sharma *et al*. (2014).

$$\mathrm{SE} = \frac{\text{Diameter of solubilization halozone}}{Colony\ diameter}$$

The solubilization index (SI) was calculated using the following formula (Sadiq *et al*., 2014).

$$\mathrm{SI} = \frac{\text{Colony diameter} - \text{Halozone diameter}}{Colony\ diameter}$$

The Zn solubilizing potential of the selected bacterial isolate was determined quantitatively by following the method of Fasim *et al*. (2002). The bacterial isolates were inoculated in nutrient broth by keeping them on shaking incubator at 28 ± 1°C. The Erlenmeyer flasks containing 50 mL of liquid Bunt and Rovira medium were supplemented with 0.1% each of ZnO and $ZnCO_3$ and inoculated with 1 mL aliquot of each selected rhizobacterial culture. Bunt and Rovira media supplemented with these Zn compounds and

without bacterial inoculation were served as an un-inoculated control. Flasks were placed in shaking incubator at 28 ± 1°C for 8 d. The pH of liquid broth was recorded after different time intervals and aliquots of the medium was centrifuged and filtered. For the determination of soluble Zn, the supernatant was fed to atomic absorption spectrophotometer. By subtracting the soluble Zn of the inoculated sample from the corresponding un-inoculated control, the amount of solubilized Zn was obtained and expressed as gram of Zn mL^{-1} culture.

Characterization of Bacteria

Indol-3-acetic acid (IAA) production: Auxin production was determined by lab assay in which selected isolated bacteria were tested both in the presence and absence of L-tryptophan by following the procedure as described by Sarwar *et al*. (1992). About 3 mL aliquot of bacterial culture was taken and mixed with 2 mL Salkowski's reagent (2.0 mL of 0.5 M $FeCl_3$ + 98 mL of 35% $HClO_4$). After color development, intensity of the color was measured at 535 nm by using spectrophotometer. Standard curve was drawn by measuring the intensity of the color. This standard curve was used for measuring auxin production both in the presence or absence of L-TRP.

ACC-Deaminase Activity

The ACC-deaminase activity of the selected strain was measured by the method described by Penrose and Glick (2003). The quantity of α-ketobutyrate produced is directly proportion to the ACC-deaminase activity. The number of moles of α-ketobutyrate produced by this reaction was determined by comparing the absorbance at 540 nm of a sample to a standard curve of α-ketobutyrate ranging between 0.1 and 1.5 nmol. ACC-deaminase activity expressed as α-ketobutyrate nmol g^{-1} biomass h^{-1}).

Identification of Organic Acids Using HPLC

Zn solubilizing bacteria were inoculated in test tubes containing 15 mL of LB broth medium and incubated at 28ºC for 72 h. Three biological replications were maintained for this experiment. After incubation, each sample was centrifuged at 10,000 rpm at 4ºC and the supernatant was collected. The metabolites were extracted thrice by vigorous shaking with methanol (HPLC grade) in 1:1 ratio using separating funnel. Organic acids (were determined by HPLC (Shimadzu, Japan) (Butsat *et al.*, 2009).

PCR Amplification of 16S rRNA Gene Sequencing

Most efficient bacterial isolates were identified by sequencing their 16S rRNA. Sequenced chromatogram received from the Macrogen Company; South Korea. In this technology, DNA of most efficient isolates was extracted and amplification of 16S rRNA gene was done to compare the sequenced gene with Gene Bank to obtain match.

Pot Experiment

The pot culture experiment was conducted in plastic pots (20 cm dia) filled with 9 kg of soil with three replications for each treatment. Wheat cv. Galaxy-2013 seeds treated with bacterial inoculant (H-84, H-92, H-98, H-103, H-107, H-112 and H-122) were sown along with uninoculated control and placed in glass house (30±2ºC). Statistical design was Completely Randomized Design. Pots were watered once in two days with water until 60 days. After 60 days of sowing (DAS), plants were uprooted from the pots carefully and biomass and tillers per plant were measured. Dried seed were finely ground in a grinder to amorphous powder and 1 g was taken in 150 mL conical flask containing 10 mL nitric acid (HNO_3) and perchloric acid ($HClO_4$) in 3:1 ratio and digested at 300ºC till colorless by placing on a hot plate. The extract was taken in 50 mL volumetric flask and the volume was made up to mark with distilled water. These samples were used for estimation of Zn by atomic absorption spectrophotometer.

Results

Zinc Solublizaion

Qualitative assay: All the bacterial strains were cultured on Bunt and Rovira agar plates for their potential to solubilize inorganic Zn. Out of total 77 strains, 37 bacterial strains showed positive response against Zn solubilization. The efficiency of these bacterial strains was then checked on the basis of colony and halozone diameter. Among 37 bacterial strains, 7 strains H-84, H-92, H-98, H-103, H-107, H-112 and H-122 showed maximum Zn solubilization on Bunt and Rovira media amended with ZnO and $ZnCO_3$ (Table 1).

Colony and Halozone Diameter

Among the bacterial isolates, maximum colony diameter (6.28 mm) and halozone diameter (20.25 mm) was observed in H-103 strain amended with $ZnCO_3$ and it showed significant difference from all other strains (Fig. 1). The strains H-112 and H-84 also showed more halozone diameter as compared to H-122, H-107, H-98 and H-92 in both insoluble sources of Zn. The strains having ZnO as insoluble Zn source showed slightly less colony and halozone diameter as compared to $ZnCO_3$ among all strains.

Solubilizing Efficiency and Index

The maximum solubilizing efficiency and index was observed in the strain H-103 supplemented with ZnO as insoluble Zn source (Fig. 2). The strains H-84 and H-112 also showed more solubilizing efficiency and index as compared to other strains but were statistically similar with H-103 bacterial strain.

Quantitative Assay

pH of culture medium: Inoculation of medium with zinc solubilizing bacterial strains caused a significant decrease in

Table 1: Zinc solubilization response of bacterial strains

Strain	Zinc solubilization	Strain	Zinc solubilization	Strain	Zinc solubilization
Control	-	H-1	+	H-96	-
H-65	+	H-2	+	H-58	-
H-119	+	H-8	+	H-59	-
H-11	+	H-6	+	H-60	-
H-110	+	H-92	++	H-61	-
H-91	+	H-88	+	H-62	-
H-89	++	H-75	++	H-63	-
H-15	+	H-112	+++	H-64	-
H-67	+	H-71	+	h-65	-
H-103	++++	H-122	++	H-66	-
H-38	+	H-83	+	H-67	-
H-121	++	H-87	+	H-68	-
H-84	+++	H-69	+	H-69	-
H-85	+	H-7	+	H-70	-
H-107	+++	H-78	++	H-90	-
H-5	+	H-102	+	H-91	-
H-98	++	H-76	+	H-92	-
H-3	+	H-81	+	H-93	-
H-4	+	H-90	+	H-94	-
H-12	-	H-41	-	H-95	-
H-13	-	H-42	-	H-98	-
H-14	--	H-43	-	H-97	-
H-51	-	H-44	-	H-71	-
H-52	-	H-45	-	H-72	-
H-53	-	H-46	-	H-73	-
H-54	-	H-47	-	H-74	-

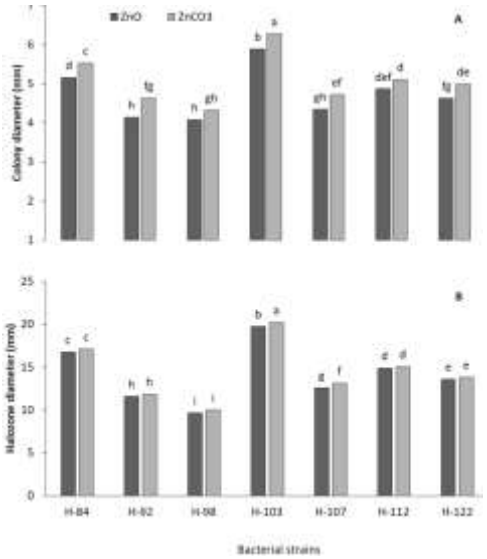

Fig. 1: Bacterial strains are showing colony (A) and halozone diameter (B) on Bunt and Rovira media
Means sharing the same letter do not differ significantly (P< 0.05)

the pH as compared to un-inoculated control (Fig. 3). The pH of the culture medium was taken after different time intervals and it was observed that pH gradually decreased with time. Maximum pH reduction (4.05) was observed in H-103 bacterial strain followed by H-84 and H-112.

Organic Acid Production by Different Bacterial Strain

All the isolates produced organic acids in the medium incorporated with insoluble source of zinc. The highest

Table 2: Amount of organic acid produced by different bacterial strains

Type of organic acid	Amount of organic acids Produced (µg mL^{-1})		
	H-103	H-84	H-112
Pyruvic Acid	83.25	80.38	87.13
Tartaric Acid	147.05	146.25	90.78
Mallic Acid	8.92	15.82	8.18
Oxaloacetic Acid	3.18	2.49	11.82

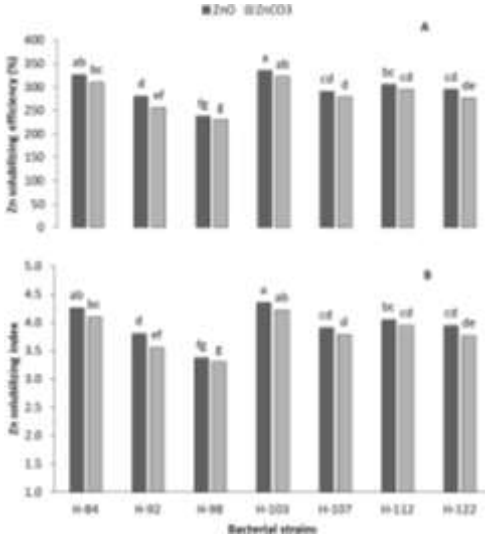

Fig. 2: Bacterial strains are showing zinc solubilization efficiency (A) and index (B) on Bunt and Rovira media
Means sharing the same letter do not differ significantly (P< 0.05)

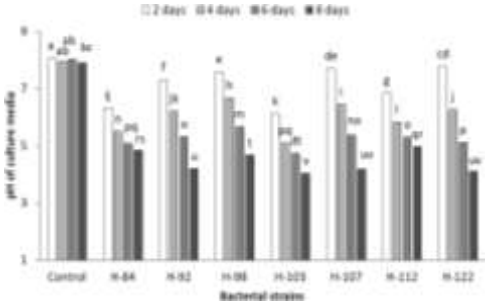

Fig. 3: Bacterial strains influence pH of the culture media
Means sharing the same letter do not differ significantly (P< 0.05)

pyruvic and oxaloacetic acids were produced by bacterial isolae H-112, tartaric acid by H-103 and mallic acid by H-84 (Table 2).

Amount of Solubilized Zinc (mg L^{-1})

All the selected bacterial strains showed Zn solubilization in liquid Bunt and Rovira medium, however, their efficiency was dependant upon Zn source (ZnO and $ZnCO_3$) (Table 3). Bacterial strains solubilized $ZnCO_3$ relatively more efficiently than ZnO. Regardless the source, the bacterial strain H-103 caused maximum Zn solubilization by 31.9 and 37.2 mg L^{-1} for ZnO and $ZnCO_3$, respectively.

Table 3: Amount of solubilized Zn (mg L^{-1}), ACC-deaminase activity of bacterial isolates and auxin production through bacterial isolates

Strain	Zinc Source		Auxin production (µg mL^{-1})		ACC deaminase Activity (α-ketobutyratenmol g^{-1} biomass $hr^{-1)}$
	ZnO	$ZnCO_3$	Without L-tryptophan	With L-tryptophan	
Control	-------	-------			
H-84	26.1±1.59 b	30.3 ±3.1 b	11.90 ±1.11 b	31 ± 1.58 b	269.36 ± 2.34 b
H-92	17.5±1.51 e	19.5 ±2.56 f	8.44 ±1.09 e	19.23 ± 1.18 e	201.34 ± 2.16ef
H-98	18.9 ±2.26 e	21.5 ±2.41 e	8.05±1.09 f	19.40 ± 1.12 e	197.56 ± 2.09 f
H-103	31.9 ± 1.93a	37.2 ±2.1 a	12.98 ±1.07 a	37.53 ± 1.42 a	285.34 ± 2.14 a
H-107	23.3 ± 2.64 c	25.6 ±2.19 d	9.33 ±1.15 d	24.57 ± 1.64 d	212.64 ± 3.01 e
H-112	24.1 ± 2.43 c	27.6 ±2.4 c	10.97±1.10 c	28.27 ± 1.41 c	254.12 ± 2.37 c
H-122	20.1 ± 2.45 d	24.6 ±1.56 d	9.07±1.09 d	20.30 ± 1.41 e	238.37 ± 2.45 d

Values are means of three replicates ± SD, means sharing similar letters don't differ significantly

Table 4: Plant traits and grain Zn contents as affected by bacterial strains

	Plant biomass (g)	Root length (cm)	No. of tillers	Total grain weight (g)	Grain Zn concentration (ppm)
Control	74.00 d	8.3 f	2.3 c	7.39 d	24.83 d
H-084	90.03 a	14.7 b	3.0 abc	11.89 ab	36.50 b
H-092	84.00 cd	11.2 de	2.7 bc	9.82 c	31.33 c
H-098	82.67 d	10.6 e	2.3 c	10.47 abc	29.00 d
H-103	92.83 a	16.1 a	3.7 a	12.43 a	39.17 a
H-107	88.67 cd	12.3 cd	2.7 bc	9.27 cd	31.50 c
H-112	89.27 ab	13.6 bc	3.3 ab	10.14 abc	34.33 b
H-122	84.23 bc	11.2 de	2.7 bc	9.88 bc	23.00 c

Values are means of three replicates, means sharing similar letters don't differ significantly

Auxin Production and ACC Deaminase Activity

All the selected bacterial strains showed auxin production, however, their potential for auxin production increased in the presence of L-tryptophan. The growth media supplemented with L-tryptophan caused maximum auxin production as IAA equivalents. The maximum auxin was produced by bacterial strain H-103 (37.53 µg mL^{-1}) followed by H-84 (31 µg mL^{-1}) and H-112 (28.27 µg mL^{-1}). Amongst all the bacterial strain, H-103 showed maximum auxin production (12.98 µg mL^{-1}) in the absence of L-tryptophan. Similarly, the maximum ACC-deaminase activity was also observed highest in case of bacterial strain H-103 (285.34 α-ketobutyrate nmol g^{-1} biomass h^{-1}).

Identification of Bacterial Isolates

Bacterial isolates H-84, H-103 and H-112 were identified. The amplification and identification made by the company revealed that isolates were closed resemblance with *Bacillus* genera (Fig. 4).

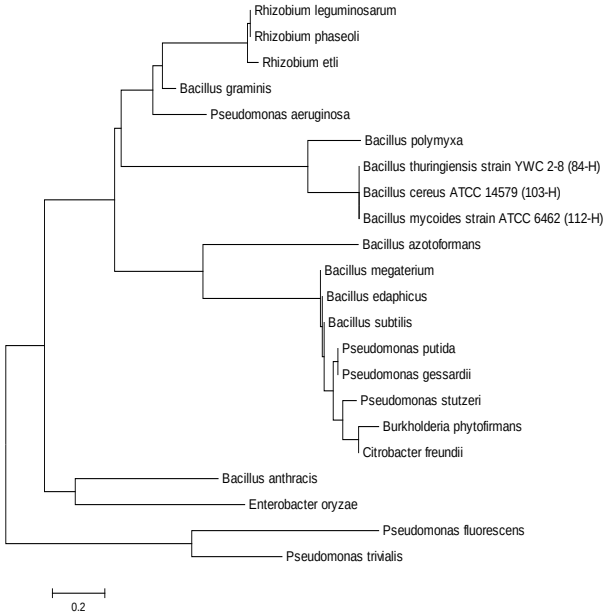

Fig. 4: Phylogenetic tree of selected bacterial strains

Plant Traits

The inoculation of bacterial strain showed improvement in fresh shoot biomass as compared to control. The inoculation of H-103, H-84 and H-112 bacterial strains performed significantly better over all other strain but maximum increase (28%) was observed with H-103 bacterial strain. Similarly, maximum root length, number of tillers per plant and total grain weight was observed in plant inoculated with bacterial strain H-103. The concentration of Zn in grains increased significantly in all the bacterial strains over control, however the maximum concentration of Zn in grains was recorded by bacterial strain H-103 (Table 4).

Discussion

The bioavailability of Zn due to solubilization by microbial strains might be a due to the proton extrusion by microbial origin possibly in a non-specific way leading to acquisition

of Zn (Ali *et al*., 2014). It had been reported that the solubilization and release of Zn compounds may be due to the production of organic acids (Agusto da Costa and Duta, 2001). Plate assay is not considered as good way to assess the solubilization and mineralization ability of bacteria due to some limitations. Therefore, the bacteria showing good potential of Zn solubilization on agar plate were further tested in broth culture amended with two insoluble Zn sources (ZnO and $ZnCO_3$). The *in vitro* quantitative assay showed that in liquid broth more Zn was solubilized as compared to un-inoculated control. The increase in the soluble Zn in medium upon inoculation can be ascribed to production of organic acids by the bacterial isolates. It is worthwhile saying that bacterial isolate H-103 caused maximum Zn solubilization in media and caused reduction in pH (4.05), thereby, it is good explanation for Zn solubilization caused by strain. The tested bacterial strain had variable response to Zn solubilization. Pawar *et al.* (2015) also found higher Zn solubilization by the isolated bacteria in the presence of $ZnCO_3$, which may be attributed to the fact that these strains isolated from calcareous soil. Pakistani soils are also calcareous in nature (Khalid *et al*., 2013) and thereby, it might be possible that the isolated bacterial strains are better adapted to these conditions and thus, presenting a higher potential for $ZnCO_3$ solubilization as compared to ZnO in the present study. The other reasons could be the higher affiliation of isolated bacteria with the carbonate particles capable of solubilizing $ZnCO_3$ which is easily degraded by the acidic exudates of bacteria. Similar findings were reported by (Bapiri *et al*., 2012) who also confirm the adherence with carbonate particles capable of solubilizing $ZnCO_3$ and it also depend upon the chemical properties of $ZnCO_3$ because it is easily affected by the exudates released by the microbes. Due to the production of organic acids, the pH reduction in broth by the isolated strains was observed in this study.

The root growth was stimulated due to the release of IAA by root exudates in the presence of tryptophan-like compounds a precursor of auxin biosynthesis (Kamilova *et al*., 2006; Khalid *et al*., 2006). The IAA produced by PGPR and its positive impact on plant growth have been reported by many researchers (Rashid *et al*., 2012; Ahmad *et al*., 2014, 2016). These strains also have good potential to produce ACC-deaminase, which helps plants to withstand both biotic and abiotic stress by lowering the production of ethylene through the activity of ACC-deaminase. This enzyme works by hydrolyzing ACC into α-ketobutyrate and ammonia instead of ethylene (Arshad *et al*., 2007).

There are different mechanisms like chelation, exchange reaction, acidification and release of organic acids through which PGPR solubilize the nutrients (Chang *et al*., 2005; Hafeez *et al*., 2005). Siderophore production (Tariq *et al*., 2007; Saravanan *et al*., 2011) and various other organic acids production by PGPR (Di Simine *et al*., 1998; Tariq *et al*., 2007) are considered as responsible mechanism for Zn and Fe solubilization. There are many processes which can influence the crop vigor and yield in soil–plant–microbe system, that's why it is thought to be very complex system (Hafeez *et al*., 2002; Pieterse *et al*., 2003). The precise mechanism through which PGPR promote plant growth is not completely understood yet.

Conclusion

Inoculation of zinc solubilizing bacterial isolates decreased rhizospheric pH, and increased auxin production and ACC deaminase activity. Better microbial activity of zinc solubilizing bacteria in rhizosphere might result in release of zinc from organic complexes by mineralization and from calcium carbonate bounded zinc by solubilization. Inoculation of selected bacterial isolates increases the Zn uptake and accumulation in wheat grains. Therefore, it might be concluded that zinc solubilizing bacteria with plant growth promoting attributes could be used for enhancing bioavailability of zinc in soil and its subsequent uptake in grains, however, to warrant this, field trial is recommended.

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