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RESEARCH ARTICLE

Significance of diazotrophic plant growth-promoting *Herbaspirillum* sp. GW103 on phytoextraction of Pb and Zn by *Zea mays* L.

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Abstract Microbe-assisted phytoremediation has been considered a promising measure for the remediation of heavy metal-polluted soil. The aim of this study was to assess the effect of diazotrophic plant growth-promoting *Herbaspirillum* sp. GW103 on growth and lead (Pb) and zinc (Zn) accumulation in *Zea mays* L. The strain GW103 exhibited plant growth-promoting traits such as indole-3-acetic acid, siderophores, and 1-aminocyclopropane-1-carboxylic deaminase. Treatment of *Z. mays* L. plants with GW103 significantly increased 19, 31, and 52% of plant biomass and 10, 50, and 126% of chlorophyll a contents in Pb, Zn, and Pb + Zn-amended soils, respectively. Similarly, the strain GW103 significantly increased Pb and Zn accumulation in shoots and roots of *Z. mays* L., which were 77 and 25% in Pb-amended soil, 42 and 73% in Zn-amended soil, and 27 and 84% in Pb + Zn-amended soil. Furthermore, addition of GW103 increased 8, 12, and 7% of total protein content, catalase, and superoxide dismutase levels, respectively, in *Z. mays* L. plants. The results pointed out that isolate GW103 could potentially reduce the phytotoxicity of metals and increase Pb and Zn accumulation in *Z. mays* L. plant.

Keywords Antioxidant enzymes · Diazotrophic bacteria · Heavy metals · PGPR · Phytoremediation · *Z. mays* L.

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Introduction

Soil contamination by heavy metals is a serious global environmental concern. Intensive application of fertilizers and pesticides, mine wastes, and disposal of improperly treated industrial effluents are the major sources of heavy metal pollution. The toxicity, persistence, bioaccumulation, and biomagnification attributed to heavy metals create a threat to human health (Yang et al. 2014; Batvari et al. 2016). Pb exposure could result in behavioral changes, learning disabilities, reading problems, development defects, language difficulties, mental retardation, and embryonic abnormalities (Goyer 1993). Despite being an essential micronutrient required for the normal functioning of the cells, zinc exposure, especially at quantities higher than the daily recommended level, leads to depression, lethargy, increased thirst, and neurological disorders (Kurniawan et al. 2006). Different scientific approaches have been undertaken to remove Pb and Zn from contaminated soils, each with different outcomes and with their own advantages and disadvantages.

Conventional cleanup technologies are normally too expensive and often do not yield desirable soil properties for

the reestablishment of contaminated sites (Ruttens et al. 2010). Bioremediation is considered a simple, economic, and eco-friendly technology that uses biotic communities for the remediation of contaminated soils (Govarthanan et al. 2014). Among the available bioremediation technologies, phytoremediation is more widespread because of its visual advantages and extensive applicability in different environments (Shin et al. 2012). Plants remove pollutants through one or more biologically active processes, such as extraction, transformation, stabilization, or rhizodegradation. Among these processes, phytoextraction is identified as a superior type by which plants extract heavy metals from contaminated soils (Weerakoon and Somaratne 2009). Plants belonging to *Brassica* sp., *Alyssum* sp., *Solanum* sp., *Sedum* sp., and *Helianthus* sp. have been reported to have metal phytoextraction potential (He et al. 2013; Ma et al. 2015; Zaheer et al. 2015). Recently, *Z. mays* L. was used in heavy metal phytoextraction because of its high biomass, rapid growth rate, and high metal tolerance (Tiecher et al. 2016). Vamerali et al. (2010) reviewed the application of field crops for the phytoremediation process and ranked *Z. mays* L. as the third most widely used crop species. *Z. mays* L. is a bioenergy crop, and its use in phytoremediation generates an alternative income, which is a blessing in disguise for developing countries with limited funds for the restoration of contaminated ecosystem (Meers et al. 2010).

The degree of extraction of metals in phytoextraction depends on the phytotoxicity and bioavailability of metals and on the soil physicochemical conditions (Baisak et al. 1994). To overcome these challenges, researchers seek to improve the phytoextraction process using plant growth-promoting bacteria (PGPB). PGPB can decrease phytotoxicity of metals and increase plant growth by a number of direct and indirect mechanisms (Sheng et al. 2008a,b). Moreover, certain PGPB have also been shown to alter heavy metal bioavailability to the plant by producing siderophores and organic acids (Long et al. 2011). PGPB activity depends on the ability of the bacteria to colonize the root system of plants.

Herbaspirillum sp., a gram-negative, diazotrophic bacteria, is commonly present in plant roots, rhizospheres, and oligotrophic soils. *Herbaspirillum* sp. was first isolated from cereal roots and has been reported to have plant growth-promoting activity in several economically important crops such as rice, sorghum, sugarcane, maize, and wheat (Baldani et al. 1996; Pedrosa et al. 2011). Monteiro et al. (2008) reported that *Herbaspirillum seropedicae* colonize and invade *Z. mays* L. in 30 min. The study observed a high density and substantial increase of *H. seropedicae* at lateral root junctions. Our previous study reported on the presence of four different arsenic resistance genes in the genomic DNA and the bioleaching potential of *Herbaspirillum* sp. GW103 (Govarthanan et al. 2014, 2015). Hence, the objectives of the present study were (i) to assess the potential of *Herbaspirillum* sp. GW103 in increasing plant biomass, (ii) to understand the potential of the isolate GW103 in reducing the phytotoxicity of metals, and (iii) to evaluate Pb and Zn phytoextraction potential of *Z. mays* L. in the presence of *Herbaspirillum* sp. GW103.

Materials and methods

Materials and bacterial strain

Stock solutions of $Pb(NO_3)_2$ (Daejung, Korea) and $ZnCl_2$ (Wako, Korea) were prepared by dissolving salts in nanopure water (conductivity18 $\mu\Omega\ m^{-1}$, TOC < 3 mg L^{-1}). The *Herbaspirillum* sp. GW103 strain was isolated from the rhizosphere soil of *Phragmites austrails* grown in reclaimed land (Lee et al. 2012).

Plant beneficial features

1-Aminocylopropane-1-carboxylic acid deaminase (ACCD) activity in cell lysates was assayed by determining the amount of α-ketobutyrate (α-KB) generated when the enzyme cleaves 1-aminocylopropane-1-carboxylic acid (ACC) (Honma and Shimomura 1978; Lee et al. 2016). Indole-3-acetic acid (IAA) production was measured colorimetrically (530 nm) by mixing 4 mL of Salkowski's reagent with 1 mL of cell-free supernatant (Bric et al. 1991). Siderophore secretion was measured by the "universal method" of CAS assay in iron-free succinate medium (Schwyn and Neilands 1987).

Preparation of bacterial suspension

Herbaspirillum sp. GW103 was cultured in LB broth at 25 °C for 24 h. The cells were harvested by centrifugation (5000 rpm for 5 min) and resuspended in 0.01 M phosphate buffer (pH 7.0). The concentration of the cells was adjusted to 10^8 cells mL^{-1} (OD 0.3 at 595 nm) using UV-Vis spectrophotometer (Shimadzu, Japan).

Soil and plant material preparation

Sandy loam agricultural topsoil (0–20 cm) was collected (April 2014) from an agricultural field in Iksan, South Korea. Physical and chemical properties of the sandy loam soil were as follows: pH 6.0 ± 0.07, conductivity 0.15 ± 0.09 mS m^{-1}, organic matter 4.36 ± 0.11%, sand (%) 0.54, silt (%) 0.28, and clay (%) 0.18. The soil was air-dried at room temperature, sieved through a 2-mm sieve, and was spiked with Pb and Zn solutions. In general, the heavy metal content of the contaminated soil in and around the mine area varied between 300 and 500 mg kg^{-1}. Thus, the concentration 300 mg kg^{-1} was chosen for this study. The metal-contaminated soil was moisturized for 1 week by adding

deionized water and dried in greenhouse for approximately 2 weeks. The dried soil (1.0 g) was digested with HNO_3/HCl (1:3 *v*/*v*) and 30% H_2O_2 at 125 °C, and the samples were centrifuged at 6000 rpm for 5 min. One milliliter of the supernatant was filtered through a 0.2-μm membrane and analyzed for the total metal concentration using inductively coupled plasma mass spectrometry (ICP-MS) (Leemans Labs, USA), after appropriate dilution. Mature seeds of *Z. mays* L. were thoroughly washed with distilled water. Seeds were sown in trays containing sterilized sand of about 2-in thickness and incubated at 20–22 °C in a growth chamber. The morphologically uniform seedlings were wrapped after 1 week of germination, with foam at the root–shoot junction and then transferred to experimental pots.

Phytoextraction studies and mobile fraction of metals in phytoremediated soil

The pot experiment consisted of a factorial design with two metals (Pb and Zn 300 mg kg^{-1}): (i) Pb soil with plants (Pb control), (ii) Pb soil with plants and GW103 (Pb GW103), (iii) Zn soil with plants (Zn control), (iv) Zn soil with plants and GW103 (Zn GW103), (v) Pb + Zn soil with plants (MX control), and (vi) Pb + Zn soil with plants and GW103 (MX GW103). The morphologically uniform seedlings (five seedlings/pot) were transplanted to metal-spiked soil and inoculated with 10 mL of bacterial suspension (10^8 cells mL^{-1}). Control experiments were also simulated in a similar way with sterile water. Each treatment was carried out three times, and the plants were harvested after 21 days of cultivation. The entire plants were washed with tap water, followed by washing with HCl (0.1 M) and demineralized water. Plant growth parameters such as plant biomass, shoot and root length, fresh and dry weight of root, and fresh and dry weight of shoot were analyzed. Phytoremediated soil (2 g) was mixed with 16 mL of 1 M magnesium chloride solution (pH 7), and the flasks were incubated in shaking incubator (40 rpm) at 26 °C for 1 h. Later, the samples were centrifuged at 6000 rpm for 5 min, and 1 mL of the supernatant was filtered through a 0.2-μm membrane. After appropriate dilution, the filtrate was analyzed using ICP-MS for mobile fraction of Pb and Zn.

Photosynthetic pigment analysis

Chlorophyll content in the leaf samples were estimated according to Metzner et al. (1965). Briefly, fresh leaf samples (0.2 g) were dipped into 85% (*v*/*v*) aqueous acetone solution and centrifuged at 4000 rpm for 10 min. The supernatants were collected and diluted, and absorbance was determined at 663, 645, and 480 nm by UV-Vis spectrophotometer using pure 85% aqueous acetone solution as blank.

Antioxidative enzyme assays and protein content measurement

Plant leaf and root samples were used for enzymatic analysis. Leaves and roots were grounded with mortar and pestle under liquid nitrogen. The sample was dissolved in 0.05 M phosphate buffer (maintaining pH at 7.8) and filtered through four layers of muslin cloth and centrifuged at 10,000 rpm for 10 min at 4 °C. Superoxide dismutase (SOD, E.C. 1.15.1.6) and peroxidase (POD, E.C. 1.11.1.7) activities of the extract were quantified according to Zhang (1992). Catalase (CAT, EC. 1.11.1.6) activity was determined according to Aebi (1984). The soluble protein content was analyzed by Bradford (1976) assay using Coomassie brilliant blue G-250 as dye and albumin as the standard.

Metal uptake by *Z. mays* L.

Metal accumulation in the plants was determined after shoots and roots were oven dried at 70 °C for 48 h (Li et al. 2012). The dried plant sample (0.1 g) was digested with 10 mL of concentrated HNO_3 + concentrated H_2SO_4 + $HClO_4$ (10:1:4 *v*/*v*) and kept overnight. The concentration of Pb and Zn in root and shoot was determined using inductively coupled plasma mass spectrometry (Leemans Labs, USA) after appropriate dilution. The analysis was repeated three times, and only the average values were considered.

Statistical analysis

All values reported in this experiment are mean of three independent replicates ± SD. To confirm the variability of data and validity of results, all the data were subjected to an analysis of variance (ANOVA). To determine the significant difference between treatments, the Duncan's multiple range test (DMRT) was applied to see the significance level ($P < 0.05$) wherever required. All statistical analysis was performed using SAS version 9.1.

Results and discussion

Plant growth-promoting traits of *Herbaspirillum* sp. GW103

Bacteria associated with plants are known to improve plant growth even under conditions of heavy metal stress by producing various enzymes and secondary metabolites (Ma et al. 2011). Thus, the isolate GW103 was screened for basic plant growth-promoting traits such as ACCD activity, IAA, and siderophore synthesis. In ACCD assay, the isolate GW103 produced 262.2 ± 6.17 μmol α-ketobutyrate mg $protein^{-1}$ h^{-1}. The isolate GW103 produced 9.05 ± 2.88 μg mL^{-1} of IAA in

tryptophane-supplemented Dworkin and Foster (DF) minimal media and 82.25 ± 0.88% of siderophores in iron-free succinate medium. The ACCD activity of the isolate GW103 was comparatively high when compared with other bacterial isolates. Ma et al. (2015) reported *Bacillus pumilus* E2S2 to produce 32.6 μmol α-ketobutyrate mg protein^{-1} h^{-1}. However, IAA and siderophore production by the isolate GW103 are similar to that seen in other bacterial systems. Previous studies reported similar results with *Streptomyces* KLBMP 1064, which produced 9.14 ± 0.03 μg mL^{-1} of IAA in tryptophane-supplemented DF minimal media (Qin et al. 2015), and *Pseudomonas putida*, which produced 83% of siderophores in iron-free succinate medium (Sayyed et al. 2005). However, a direct comparison of our results with other bacterial system is difficult because bacterial growth conditions, nutrient contents in the medium, reaction conditions, metabolic activity of the bacteria, and generation time of the bacteria may highly influence ACCD activity, IAA, and siderophore production. ACCD increases plant growth by decreasing the intracellular concentration of ethylene. IAA promotes plant growth by regulating plant cell division, differentiation, and root elongation. Siderophores increase the availability of Fe^{2+} ions to the plant system and, thereby, directly increase the growth rate of plants.

Plant biomass

Biomass is an important factor that determines the success of phytoextraction of heavy metals. However, the tendency of heavy metals to decrease the biomass of plants by altering the physiological and biochemical processes is well known. Thus, the effect of the isolate GW103 on the biomass of *Z. mays* L. in Pb and Zn-amended soils was evaluated, and the results are shown in Fig. 1. Compared to control, GW103-inoculated plants showed 17, 14, and 17% increase in shoot length and 16, 21, and 12% increase in root length in Pb, Zn, and MX soil. The fresh and dry weights of the plants are reported in Table 1. Evidently, some differences in *Z. mays* L. biomass were observed between the treatment and control. Compared to control plants, GW103-treated plants showed increase in fresh and dry weight of shoots (26 and 19% in Pb-amended soil, 34 and 15% in Zn-amended soil, and 80 and 115% in Pb and Zn-amended soil) and fresh and dry weight of roots (10 and 38% in the Pb-amended soil, 28 and 15% in Zn-amended soil, and 6 and 50% in Pb and Zn-amended soil). On statistical analysis, significant differences ($P < 0.05$) were observed for shoot length, root length, shoot fresh weight, shoot dry weight, and root fresh weight in all the treatments. However, significant difference was not observed for root dry weight in MX soil. Increase in plant biomass could be due to enhanced Fe availability, decreased abiotic stress, and increased root architecture (Hardoim et al. 2008). This was further supported by the increase in photosynthetic pigment content (Table 2). Guo et al. (2011) reported plant growth-promoting rhizobacterium *Burkholderia* sp. D54 to enhance the biomass of *Sedum alfredii* in Cd, Pb, and Zn-amended soils. Sheng et al. (2012) reported *Burkholderia* sp. GL12, *Bacillus megaterium* JL35, and *Sphingomonas* sp. to increase the aboveground tissue (33–56%) and root (48–83%) dry weight of maize plants in soil with high levels of Cu contamination.

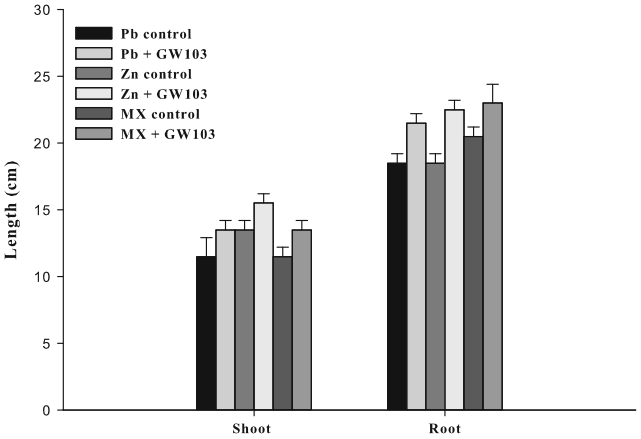

Fig. 1 Root and shoot length in control and GW103-treated *Z. mays* L. A significant increase in root and shoot length was observed in GW103-treated plants. *Bars* represent SD of three replicates. *Different letters* indicate significant differences among the treatments at $P < 0.05$

Photosynthetic pigments

Photosynthetic pigments in plants are considered as sensitive indicators of stress. Hence, chlorophyll a (Chl *a*), chlorophyll b (Chl *b*), total chlorophyll, and carotenoids were estimated in the control and GW103-inoculated plants, and the results are shown in Table 2. Chl *a* and total chlorophyll were increased in GW103-treated plants when compared to control plants, which were 10 and 2% in Pb-amended soils, 50 and 6% in Zn-amended soils, and 126 and 76% in MX soil. Statistical analysis showed significant difference ($P < 0.05$) for Chl *a* in all the treatments. However, for total chlorophyll, significant difference ($P < 0.05$) was observed only in MX soil. This response was most likely caused by the lower production of leaf fresh mass and larger amounts of pigment per unit of mass (Hewitt 1983). The results are consistent with studies that reported *Burkholderia* sp. D54 (PGPR) to increase 24% of Chl *a* content in Zn, As, Cd, and Pb-amended soils (Guo et al. 2014). However, a decrease in Chl *b* and total carotenoids was also observed in GW103-treated plants when compared to control plants, which were 14 and 3.9% in Pb-amended soil and 59 and 19% in Zn-amended soil. The decrease in carotenoids may be due to marked distortion of chloroplast ultrastructure leading to disturbed shape and inflated thylakoids (Parmar et al. 2013). The results are in agreement with the study by Guo et al. (2014) which reported a marked

Table 1 Effect of the isolate GW103 on the biomass of *Zea mays* L. in Pb and Zn-amended soils

Treatments	Shoot weight (g plant^{-1})		Root weight (g plant^{-1})	
	Fresh	Dry	Fresh	Dry
Pb control	1.34 ± 0.07 d	0.21 ± 0.07 c	1.1 ± 0.07 d	0.13 ± 0.07 b
Pb + GW103	1.7 ± 0.04 b	0.26 ± 0.06 b	1.32 ± 0.01 b	0.18 ± 0.07 a
Zn control	1.21 ± 0.06 e	0.19 ± 0.01 d	1.21 ± 0.12 c	0.13 ± 0.05 b
Zn + GW103	1.63 ± 0.14 c	0.22 ± 0.07 c	1.56 ± 0.09 a	0.11 ± 0.07 c
MX control	0.96 ± 0.02 f	0.15 ± 0.05 e	0.58 ± 0.02 f	0.08 ± 0.07 d
MX + GW103	1.75 ± 0.02 a	0.28 ± 0.04 a	0.62 ± 0.12 e	0.09 ± 0.05 d

Values are the means of three replications ± SE. *Different letters* indicate significant differences among the treatments at $P < 0.05$

decrease of carotenoids in ryegrass treated with plant growth-promoting rhizobacterium and EDTA.

Activity of antioxidant enzymes and protein contents

Heavy metal-induced oxidative stress in plant systems is well known (Baisak et al. 1994). However, plants develop several enzymatic and non-enzymatic antioxidant mechanisms to alleviate the oxidative stress. Plants eliminate excess reactive oxygen species from cells by producing these enzymatic and non-enzymatic antioxidants. Among the enzymatic antioxidants, SOD, POD, and CAT play a vital role in reducing the oxidative stress. Thus, SOD, POD, and CAT levels were analyzed, and the results are reported in Fig. 2. The SOD level of control plants and GW103-treated plants is shown in Fig. 2a. Compared to control, GW103 treatment increased SOD levels in leaves and roots at 11, 27, and 19% and 10, 21, and 11% in Pb, Zn, and MX soils, respectively. The high level of SOD in leaves is likely a consequence of electron leakage from photosynthetic electron transport chain to molecular oxygen (Liu et al. 2009). The increase in root SOD activity might reflect an enhanced superoxide radical production under chemical stress (Vafaei et al. 2013). An improved level of SOD activity may be associated as incidental support for the production of additional reactive oxygen species or overexpression of various genes encoding SOD (Malar et al. 2014). Miller et al. (2008) reported that plant systems carry multiple genes coding SOD and different SOD isoenzymes specifically targeted to different cell organelles. On statistical analysis, significant differences ($P < 0.05$) were observed for leaf and root SOD activity in Pb, Zn, and MX soils.

The activity of POD in the leaves and roots is shown in Fig. 2b. Compared with control, GW103 treatment increased the POD level of leaves and roots in Pb (43 and 44%), Zn (23 and 22%), and MX (25 and 19%) soils, respectively. However, statistical analysis showed significant differences ($P < 0.05$) only for leaf POD activity. The enhanced POD level indicates that the expression of POD genes is most likely induced by Pb and Zn stress and that *Z. mays* L. has efficient detoxification mechanisms for Pb and Zn toxicity. Zaheer et al. (2015) had reported POD in shoot and root tissues of *Brassica napus* to be significantly increased after inoculation of Cu (50 or100 μM) in the growth medium. Malar et al. (2014) reported the POD activity of *Sesbania grandiflora* to increase 100% in leaves and 82% in roots under Pb stress. Enhancement of POD activity under metal stress has been explained by its role in building up physical barrier against toxic metals entering the cells, as well as in scavenging H_2O_2 (Tewari et al. 2002).

CAT activity in control and GW103-treated plants is shown in Fig. 2c. Compared with control, the activity of enzymes in GW103-treated plant leaves was notably increased by 65, 53,

Table 2 Effect of the isolate GW103 on the chlorophyll a and b, total chlorophyll, and total carotenoids of *Zea mays* L. in Pb and Zn-amended soils

Treatments	Chlorophyll a (mg g^{-1})	Chlorophyll b (mg g^{-1})	Chlorophyll a + b (mg g^{-1})	Totalcarotenoids (mg g^{-1})
Pb control	20.6 ± 0.92 d	9.55 ± 0.62 a, b	30.1 ± 0.64 b	8.11 ± 0.32 a
Pb + GW103	22.4 ± 0.52 c	8.29 ± 0.12 b	30.5 ± 0.52 b	7.47 ± 0.13 a, b
Zn control	16.4 ± 0.67 e	10.6 ± 0.32 a	27.4 ± 0.42 c	7.26 ± 0.14 a, b
Zn + GW103	24.4 ± 0.64 b	4.31 ± 0.21 d	29.0 ± 0.43 b, c	5.51 ± 0.15 c
MX control	20.5 ± 0.26 d	7.62 ± 0.32 c	28.3 ± 0.24 c	6.54 ± 0.10 b, c
MX + GW103	47.1 ± 0.65 a	3.07 ± 0.23 d	50.3 ± 0.27 a	7.45 ± 0.11 a, b

Values are the means of three replications ± SE. *Different letters* indicate significant differences among the treatments at $P < 0.05$

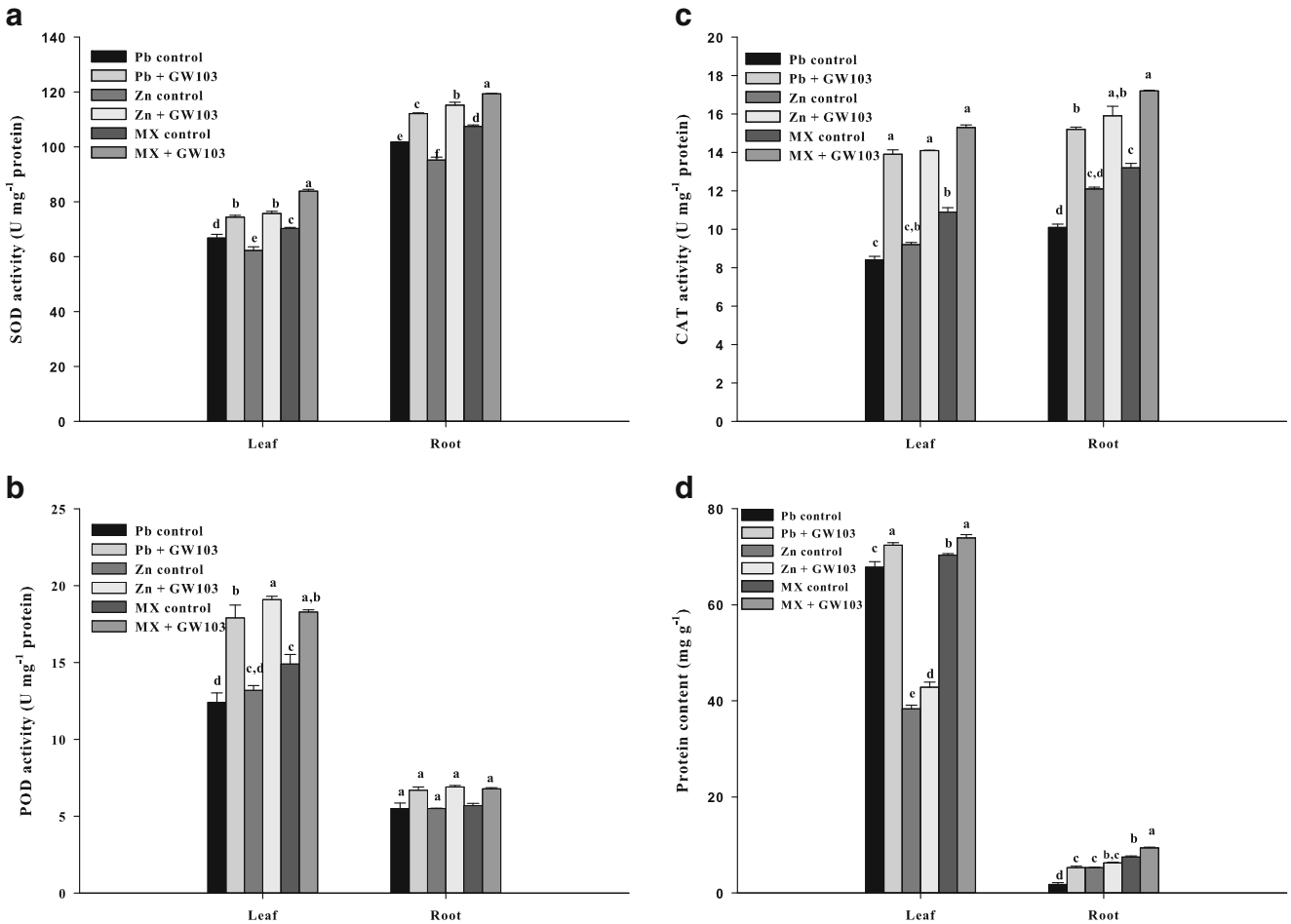

Fig. 2 Antioxidative enzyme activities in leaves and roots of control and GW103-treated *Z. mays* L. grown in Pb and Zn-contaminated soils. **a** SOD, **b** POD, **c** CAT, and **d** total protein content. *Bars* represent SD of three replicates. *Different letters* indicate significant differences among the treatments at $P < 0.05$

and 40% in Pb, Zn, and MX soils, respectively. This could be due to the high metabolic rate in leaves (Liu et al. 2009, 2012). The results are consistent with Malar et al. (2014), who reported a significant increase in CAT under Pb stress. In our experiment, catalase enzyme activity was increased under metal stress condition. Catalase is one of the major systems for the enzymatic removal of H_2O_2, and the peroxidative damage of cell walls is controlled by the potency of the antioxidative peroxidase enzyme system (Sreenvasulu et al. 1999; Velikova et al. 2000). Studies indicate that the high level of antioxidant enzymes in plants might be a powerful tool for the survival and detoxification of metals (Ali et al. 2011; Haouari et al. 2012).

Total protein content in leaves and roots of control and GW103-treated plants was estimated after 21 days of incubation, and results are shown in Fig. 2d. Compared with control, GW103 treatment significantly increased ($P < 0.05$) the protein content in leaves at 7, 12, and 5% in Pb, Zn, and MX soils, respectively. However, significant difference ($P < 0.05$) in root protein content was found only in Pb (39%) and MX (25%) soils. Islam et al. (2014) reported *Proteus mirabilis* to enhance the total protein content in shoot and roots of maize under Zn stress showing that *P. mirabilis* may delay protein degradation and maintain steady protein metabolism, thereby reducing the stress induced by ammonia-like substances and increasing the ability of plants to withstand stress (Tang et al. 2009).

Effects of GW103 on Pb and Zn uptake by *Z. mays* L.

The potential of plants to extract heavy metal ions from soil is crucial to improve phytoremediation process. Several studies report PGPR to enhance the phytoextraction rate of metals by improving the plant growth and bioavailability of metals (Prapagdee et al. 2013; Srivastava et al. 2013). Hence, the study evaluated the potential of the isolate GW103 in Pb and Zn phytoextraction, and the results are presented in Fig. 3. Compared with control plant, GW103 treatment significantly increased the metal uptake in roots and shoots of *Z. mays* L., which were 25 and 77% in Pb-amended soil, 73 and 42% in Zn-amended soil, and 84 and 27% in MX soil. Statistical

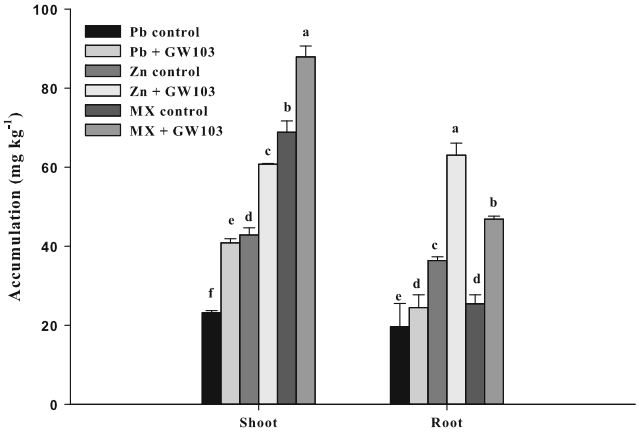

Fig. 3 Accumulation of Pb and Zn in shoots (leaves and stem) and roots of control and GW103-treated *Z. mays* L. grown in Pb and Zn-contaminated soil. *Bars* represent SD of three replicates. *Different letters* indicate significant differences among the treatments at $P < 0.05$

analysis showed that the concentration of Pb and Zn in GW103-treated plants was significantly increased ($P < 0.05$) compared with the control plants. Several reasons exist for enhanced accumulation of metals in GW103-treated plants: Metabolites of the isolate GW103 might have increased the bioavailability/solubility of Pb and Zn in the soil, the isolate might have decreased Pb and Zn phytotoxicity, or the isolate GW103 might have reduced abiotic stress in plants (Sheng et al. 2008a,b; He et al. 2013). Alternatively, the metabolites of the isolate GW103 may alter the speciation of metals, metal solubilization, and change the physical and chemical conditions of the rhizosphere region to increase the metal uptake (Ma et al. 2011; Govarthanan et al. 2016). This was supported by the results from plant growth-promoting traits where the isolate GW103 synthesizes IAA, ACCD, and siderophores. Moreover, mobile fraction of metals was increased in GW103-amended soil which was 90, 7, and 47% in Pb, Zn, and MX soils, respectively. Ma et al. (2015) reported that the increased soil metal mobility can probably be attributed to acidification and siderophore production by *B. pumilus* E2S2, which facilitated metal solubility in multimetal-contaminated soils. Also, Rajkumar et al. (2012) reported that the microbial iron-chelating siderophores can solubilize the unavailable forms of metals by complex reactions. The results are consistent with previous studies that reported the inoculation of *Pseudomonas fluorescens* G10 and *Microbacterium* sp. G16 to increase Pb accumulation in shoots and roots of rape plants (Sheng et al. 2008a,b). Ma et al. (2015) reported *Bacillus* sp. E1S2 and *Phyllobacterium myrsinacearum* RC6b to significantly enhance the accumulation of Cd and Zn in root and shoot tissues of *Sedum plumbizincicola*.

In GW103-treated plants, the concentration of Pb in shoot was comparatively higher than root system. However, the concentration of Zn was higher in the root compared with shoot system. Several reasons could be explained for the variations in Pb and Zn accumulation rate in the root and shoot systems of the *Z. mays* L.: The activity of the isolate GW103 may solubilize the Zn and thereby increases the bioavailability and accumulation of Zn in the root system, *Z. mays* L. may possess good ability to translocate Pb compared to Zn, and Pb may have high solubility in *Z. mays* L. root xylum compared to Zn. Numerous studies reported translocation rate and solubility of the metals in root xylum to vary according to the type of plant and heavy metal (Huang et al. 1997; Li and Chen 2006). Islam et al. (2014) reported that high accumulation of Zn in *Z. mays* roots was associated with solubilization of Zn by *P. mirabilis* by producing organic acids.

Conclusions

The present study attempted to enhance the phytoremediation efficiency of *Z. mays* L. using diazotrophic plant growth-promoting bacteria, *Herbaspirillum* sp. GW103. The results of the study indicate that the activities of *Herbaspirillum* sp. GW103 significantly improve the biomass and Pb and Zn phytoextration potential through several direct and indirect mechanisms such as IAA and siderophore production, ACCD activity, increased chlorophyll and protein content, and decreased metal phytotoxicity. Further studies will be needed to address the effect of *Herbaspirillum* sp. GW103 on the growth and phytoextraction potential of *Z. mays* L. in mine waste soil and tailings.

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