Ameliorative effect of indole-3-acetic acid- and siderophore-producing *Leclercia adecarboxylata* MO1 on cucumber plants under zinc stress

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**ABSTRACT**

This study investigated the plant growth-promoting effects of *Leclercia adecarboxylata* MO1 for Zn stress mitigation and plant growth improvement in Zn-contaminated soil. Results demonstrated that *L. adecarboxylata* MO1 produced siderophores that could solubilize Zn and silicate, had a tolerance to elevated levels of Zn supplementation (2 and 5 mM) in growth mediums, and produced significant amounts of indole-3-acetic acid (IAA). It was also found to promote plant growth under both control conditions and Zn toxicity (2 and 5 mM). Furthermore, *L. adecarboxylata* MO1 positively regulated physiochemical attributes by decreasing hydrogen peroxide (H$_2$O$_2$) and Zn uptake in both roots and shoots, improving antioxidant systems (e.g. catalase (CAT), peroxidase (POD), polyphenol peroxidase (PPO), superoxide anion (SOA), lipid peroxidation (MDA), and glutathione (GSH)), and reducing stress-responsive endogenous abscisic acid (ABA) and salicylic acid (SA) in plants grown under Zn toxicity of 2 and 5 mM, compared with non-inoculated plants.

**INTRODUCTION**

Rapid industrialization has led to remarkable increases in metal pollutants in the environment that have even been so high to contaminate water and render the soil unfit for agricultural use (Rai et al. 2019; Francová et al. 2020; Hou et al. 2020). These increases in heavy metal toxicity in agricultural land pose a serious threat to both human and plant health (Okerefor et al. 2020), as metal toxicity directly and indirectly affects plant growth and production (Tomáš et al. 2019). Among the various heavy metals, zinc (Zn) is excessively used in different industries without recovery, which has resulted in rapid increases in Zn toxicity that has become a serious threat (Nagajyoti et al. 2010; Mishra et al. 2019).

Zn is an important trace element for all living organisms on earth (Frassinetti et al. 2006; Hanif et al. 2017); however, elevated Zn levels are extremely toxic to living organisms (Mossa et al. 2020; Okerefor et al. 2020). Its high accumulation in agricultural soil and its high absorption by plants disturb the metabolism, causes stunted growth, and reduces productivity (Kang et al. 2017; Bilal et al. 2018). Zn is not a redox element, and therefore, the generation of reactive oxygen species (ROS) when Zn levels are elevated causes oxidative stress to organisms, which is one of the primary reasons why elevated Zn levels eventually evoke plant defense systems (Pandey et al. 2009; Lin and Aarts 2012). Nutritional imbalance, production of stress response hormones, chlorosis, and poor growth are also important features of Zn toxicity (Rout and Das 2009; Nagajyoti et al. 2010).

It is well recognized that soil microbes, particularly plant growth-promoting rhizospheric bacteria (PGPR), can influence plant growth and be used for toxic metal remediation (Kong and Glick 2017; Jan et al. 2019). PGPR mobilize heavy metals by solubilizing and acidifying rhizospheres and expanding root surfaces (Hayat et al. 2010; Pii et al. 2015). PGPR can both increase and decrease the bioavailability of metallic nutrients (Khan and Bano 2018; Uzoh and Babalola 2020). Recently, microbes have been reported to promote plant growth and decrease heavy metal bioavailability and absorption by plants, which has resulted in the safer production of crops under heavy metal toxicity conditions (Kang et al. 2017; Bilal et al. 2018; Shahzad et al. 2019a). Among vegetable crops, cucumber is one of the top 10 vegetable crops with significant economic importance and nutritional value; it is used for food and medicinal purpose and is sensitive to abiotic stress (Yan et al. 2016; Kim et al. 2019). In South Korea, cucumber is an important part of daily meal and used in several traditional recipes such as soups, kimchee, and salad (Kang et al. 2014). However, cucumber yield and quality are frequently affected by different types of abiotic stresses that cause a decline in cucumber output (Wang et al. 2012; Kang et al. 2014; Nadeem et al. 2016; Al-Harbi et al. 2018; Chen et al. 2020; Kartik et al. 2020). Accordingly, several authors have reported about the use of plant growth-promoting microbes for enhancing the growth and mitigating the abiotic stress in cucumber (Wang et al. 2012; Kang et al. 2014; Nadeem et al. 2016; Kartik et al. 2020).

Zinc-solubilizing bacteria are a type of PGPR that produce organic acids, which sequester zinc cations and...
decrease soil pH (Kumar et al. 2019). It has also been reported that anions may contribute to zinc chelation (Kour et al. 2019; Kumar et al. 2019). Zinc solubilization requires siderophore production and proton oxidoreductive chelating ligands (Chung et al. 2005; Kumar et al. 2019). Leclercia adecarboxylata MO1 is a PGPR used in the present study that has been isolated from tomato rhizospheres and has also been reported to potentially promote plant growth and mediate salinity stress (Kang et al. 2019b). In this study, we evaluated L. adecarboxylata MO1 for Zn resistance, indole-3-acetic acid (IAA) production, siderophore production, plant growth influence, metal accumulation, antioxidant enzyme activities, and stress response-induced abscisic acid (ABA) regulation under Zn toxicity conditions.

Materials and methods

Bacterial growth conditions

The plant growth-promoting L. adecarboxylata MO1 used in the present study was previously isolated and reported for plant growth promotion and salinity stress reliance (Kang et al. 2019b). In the present study, L. adecarboxylata MO1 was grown in Luria–Bertani (LB) broth at 28°C in a shaking incubator at a speed of 120 rpm.

Siderophore production, silica and zinc solubilization

L. adecarboxylata MO1 was incubated on a chrome azurol S agar (CAS) plate as previously described by Kim et al. (2017) to evaluate its siderophore-producing potential. Briefly, an actively growing single colony of L. adecarboxylata MO1 was streaked onto a CAS plate and incubated at 28°C for 7 days. The formation of yellow zones around the streaked bacteria was considered as a positive sign for siderophore production. The zinc- and silicate-solubilizing abilities of L. adecarboxylata MO1 were examined by Kang et al. (2017). Briefly, L. adecarboxylata MO1 was grown on Zn+-solubilizing medium (10.0 g L⁻¹ glucose, 2.5 g L⁻¹ magnesium trisilicate [Mg₃O₈Si₃], and 15 g L⁻¹ agar) as described by Goteti et al. (2013) and on silicate-solubilizing medium (10 g L⁻¹ glucose, 2.5 g L⁻¹ magnesium trisilicate [Mg₃O₈Si₃], and 15 g L⁻¹ agar) as described by Kang et al. (2017) and incubated at 28°C for 7 days. Clear zone formations were considered to indicate the zinc- and silicate-solubilizing properties.

Stress resistance

The Zn⁺ stress tolerance capabilities of L. adecarboxylata MO1 were evaluated according to a method reported by Shahzad et al. (2019a). Briefly, L. adecarboxylata MO1 was grown in 100 mL LB (Luria–Bertani) broth supplemented with 2 and 5 mM Zn⁺ and incubated at 28°C in a shaking incubator at a speed of 120 rpm for 5 days. After 5 days, the cell growth was measured by determining the cell density at OD₅₆₀ using a spectrophotometer.

IAA production under Zn toxicity

IAA production in the culture medium of L. adecarboxylata MO1 was evaluated under normal conditions, and Zn toxicity (2 and 5 mM) was quantified after 3 days according to a previously described protocol (Shahzad et al. 2019b). Briefly, the L. adecarboxylata MO1 culture was centrifuged after 3 days to separate cells into a free culture, which was further acidified to a pH of 2.8 and supplemented with 40 µL [D5]-IAA as an IAA internal standard. The acidified and standard supplemented free culture cells were extracted, methylated, and injected into a GC–MS SIM column for identification and quantification of IAA.

Plant–microbe interaction under Zn toxicity

The plant growth-promoting potential and stress-mediating potential of L. adecarboxylata MO1 under Zn⁺ toxicity were also evaluated. Cucumber seeds purchased from Danong Co. (Korea) were surface-sterilized (in 5% sodium hypochlorite) for 10 min, thoroughly rinsed with autoclaved, double-distilled water, and then germinated in germination trays filled with autoclaved, horticultural soil under greenhouse conditions (30°C ± 2°C). The horticultural soil was composed of peat moss (10%–15%), coco peat (45%–50%), perlite (35%–40%), and zeolite (6%–8%), along with NO₃⁻ (−0.205 mg g⁻¹), NH₄⁺ (−0.09 mg g⁻¹), P₂O₅ (−0.35 mg g⁻¹), and K₂O (−0.1 mg g⁻¹). Macronutrients consisted of NH₄⁺~90 mg kg⁻¹, NO₃⁻~205 mg kg⁻¹, P₂O₅~350 mg kg⁻¹, and K₂O~100 mg kg⁻¹. The soil pH was 5–7, the bulk density was ≤0.3 mg/m³, and the EC (ds/m) was ≤1.2. Germinated seeds were transferred after 1 week to plastic pots filled with autoclaved horticultural soil. At 15 days, 20 mL of the bacterial suspension (diluted in sterile distilled water to a final concentration of 10⁶ CFU/mL) was administrated to the plants. After 7 days of bacterial inoculation, 50 mL of ZnSO₄ (2 and 5 mM) was applied to the plants daily for 2 weeks. After this 2-week stress period, the growth parameters of shoots, root length, biomass, and chlorophyll contents (SPAD) were measured at the time of harvest. The harvested plants were immediately stored at −80°C.

Determination of Zn content in plants

The zinc contents in the shoots and roots of L. adecarboxylata MO1-inoculated and -non-inoculated plants grown under 2 and 5 mM Zn⁺ stress were extracted and quantified according to the method described by Shahzad et al. (2019a) using inductively coupled plasma mass spectrometry (ICP-MS; Optima 7900DV Perkin–Elmer, USA).

Hydrogen peroxide (H₂O₂)

Hydrogen peroxide (H₂O₂) generation in leaves was determined by 3,3’-diaminobenzidine (DAB) staining, as described by Yu-Na et al. (2020) and Torres et al. (2002).

Catalase

Catalase activity (CAT) (EC 1.11.1.6) in L. adecarboxylata MO1-inoculated and -non-inoculated plants was evaluated according to the method described by Bilal et al. (2018). Briefly, 200 mg of plant leaves were ground in 50 mM Tris HCl (pH 7.0), 3 mM MgCl₂, 1 mM EDTA, and 1.0% PVP and then centrifuged to obtain the supernatant. To the
supernatant, 0.5 mL of 0.2 M H₂O₂ in 10 mM phosphate buffer (pH 7.0) was added, and the resulting absorbance was measured at 240 nm wavelength. The CAT was calculated using a standard curve.

**Estimation of peroxidase (POD) and polyphenol peroxidase (PPO) levels**

Peroxidase (POD) (EC-Number 1.11.1.7) and polyphenol peroxidase (PPO) (EC-Number 1.10.3.1) levels in *L. adecarboxylata* MO1-inoculated and -non-inoculated plants under normal and Zn stress (2 and 5 mM) conditions were examined and calculated according to the protocol reported by Bilal et al. (2018). Briefly, 500 mg of plant samples were homogenized in a 0.1 M potassium phosphate buffer (pH 6.8) and then centrifuged at 5000 rpm for 15 min at 4°C to obtain the supernatant. Next, 100 μL of the supernatant was mixed with a reaction mixture (0.1 M potassium phosphate buffer (pH 6.8), 50 μL pyrogallol (50 μM), and 50 μL H₂O₂ (50 μM)) and incubated for 5 min at room temperature (25°C) to initiate reaction. The initiated reaction was stopped with the addition of 5% H₂SO₄ solution. The formation of purpurogallin in result of reaction was determined by measuring the absorbance at 420 nm. Similarly, polyphenol peroxidase (PPO) was estimated using the same reaction mixture of POD, excluding H₂O₂, and the resulting reaction was measured at 420 nm wavelength (Khan et al. 2019c).

**Superoxide anion (SOA)**

Superoxide anion (SOA) levels in *L. adecarboxylata* MO1-inoculated and -non-inoculated plants under normal and Zn stress conditions were measured according to the method described by Khan et al. (2018) and Khan et al. (2019b). Briefly, 200 mg of samples was homogenized in an extraction solution (0.05% (w/v) NBT and 10 mM sodium azide (Na₃N) in a 0.01 M phosphate buffer (pH 7.0)) and then incubated at room temperature for 60 min. After incubation, the extraction solution was heated at 85°C in a preheated water bath for 15 min and then cooled and vacuum-filtrated. The absorbance of the filtered solution was used to estimate the reduction of exogenously supplied nitroblue tetrazolium (NBT) to determine the amount of superoxide anions at 580 nm, which was calculated using the following formula:

\[
\text{%Scavenging} = \frac{(A_{580 \text{ Control}} - A_{580 \text{ Sample}})}{A_{580 \text{ Control}}} \times 100
\]

**Lipid peroxidation**

Lipid peroxidation was measured as malondialdehyde (MDA) (EC) production in *L. adecarboxylata* MO1-inoculated and -non-inoculated plants under normal and Zn stress (2 and 5 mM) conditions according to the method described by Bilal et al. (2020). Briefly, 500 mg of plant samples was extracted in 10 mM phosphate buffer (pH 7) and then centrifuged to separate the supernatant. To the resulting supernatant, a reaction mixture (0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of 20% acetic acid (pH 3.5), and 1.5 mL of 0.81% thiobarbituric acid aqueous solution) was added and then incubated in preheated boiling water for 60 min. The heated samples were then cooled down to room temperature. Next, 5 mL of butanol:pyridine (15:1 v/v) was added to the reaction mixture, and the resulting upper layer (MDA) was removed and measured at 532 nm wavelength.

**Glutathione (GSH)**

Glutathione (GSH) (EC 1.8.4.4) level was measured according to the method described previously by Shahzad et al. (2019a). Briefly, 0.2 g of samples was ground and homogenized in 3 mL of 5% trichloroacetic acid (TAC) and then centrifuged to collect the supernatant. Next, 0.1 mL of the supernatant was mixed with 3 mL of 150 mM monosodium phosphate buffer (pH 7.4) and 0.5 mL of Ellman’s reagent. The solution mixture was incubated at 30°C for 5 min, and the absorbance was measured at 412 nm.

**Abscisic acid**

The endogenous ABA content in *L. adecarboxylata* MO1-inoculated and -non-inoculated plants grown under 2 and 5 mM Zn⁺ toxicity was extracted and quantified according to the method described by Shahzad et al. (2016). Briefly, 0.5 g of freeze-dried plant samples was extracted with 95% isopropanol and 5% glacial acetic acid and supplemented with 20 ng of [(±)−3,5,5,7,7,7-d₆]-ABA as an internal ABA standard. The final extracted samples were methylated with diazomethane for gas chromatography-mass spectrometry (GC–MS) analysis using a selected ion monitoring (SIM) 6890N network GC system and the 5973 network mass-selective detector (Agilent Technologies, Palo Alto, CA, USA). The monitored responses to ions at m/z of 190 and 162 for Me-ABA and 194 and 166 for Me-[2H₆]-ABA were obtained using the Lab-Base (ThermoQuest, Manchester, UK) data system software.

**Salicylic acid**

The salicylic acid (SA) content in the shoots and roots of *L. adecarboxylata* MO1-inoculated and -non-inoculated plants grown under 2 and 5 mM Zn⁺ toxicity was extracted and quantified according to the method described by Shahzad et al. (2017a). Briefly, 0.2 g of freeze-dried plant samples was extracted and subjected to high-performance liquid chromatography (HPLC), which was performed using a Shimadzu device outfitted with a fluorescence indicator (Shimadzu RF-10AxL) with excitation and emission wavelengths of 305 and 365 nm, respectively, and filled with a C18 reverse-phase HPLC column (HP Hypersil ODS, particle size 5 μm, pore size 120 Å, Waters). The flow rate was maintained at 1.0 mL/min.

**Statistical analysis**

Data were collected in triplicate and subjected to Duncan’s multiple range test using the SAS software (version 9.2, Cary, NC, United States) and also to the t-test using the GraphPad Prism software (version 6.01, San Diego, CA, United States) where appropriate, followed by Two-Way Anova (Supplementary Table 1). Graphical presentation of the data

\[
\text{A580 Control} - \text{A580 Sample}/\text{A580 Control} \times 100
\]
was performed using the GraphPad Prism software (version 6.01, San Diego, CA, United States).

Results

Siderophore production, silica and zinc solubilization

The siderophore production, as well as the silica- and zinc-solubilizing potential, of L. adecarboxylata MO1 in vitro showed positive results (Figure 1). After 7 days of inoculation on CAS, the Zn- and silica-supplemented agar medium exhibited siderophore production, as indicated by a color change from blue to orange, and Zn and silica solubilization, as indicated by a clear zone formation around the inoculated bacterial colony.

Stress resistance

Treatment with 2 mM Zn showed no significant difference in the growth of L. adecarboxylata MO1; however, 5 mM Zn toxicity did significantly influence L. adecarboxylata MO1 by decreasing its growth by 27% compared with control (Figure 2).

Indole-3-acetic acid production by MO1 under Zn toxicity

Indole-3-acetic acid (IAA) in the control and 2 and 5 mM Zn-supplemented culture medium of L. adecarboxylata MO1 was quantified after 3 days, respectively. The results showed that L. adecarboxylata MO1 significantly produced 11.0516 ± 0.81 µg/mL under 2 mM Zn toxicity, with 24.31% increase compared with control, which was 8.8986 ± 0.66 µg/mL. However, under higher Zn toxicity of 5 mM, L. adecarboxylata MO1 produced 6.198 ± 1.08 µg/mL IAA, with 30.28% decrease compared with control (Figure 2).

Plant growth-promoting effect and Zn stress mediation potential of MO1

Examination of the plant growth promotion potential of L. adecarboxylata MO1 revealed significant differences in plant growth attributes under normal conditions compared to those under Zn toxicity conditions (Figure 3). Under normal conditions, L. adecarboxylata MO1-inoculated plants had significantly increased shoot length (9.01%), root length (10.18%), shoot fresh weight (8.93%), and chlorophyll content (8.74%) compared to those of non-inoculated plants. However, root fresh weights and stem diameters showed nonsignificant differences, with only minor increases of 2.27% and 1.5%, respectively. (Table 1 and Figure 3)

Under Zn stress, L. adecarboxylata MO1-inoculated plants exhibited significantly higher growth parameters than non-inoculated plants. Under 2 mM Zn stress, L. adecarboxylata MO1 inoculation significantly increased the shoot length (15.57%), root length (4.85%; nonsignificant difference), shoot fresh weight (11.37%), root fresh weight (37.28%), stem diameter (19.23%), and chlorophyll content (6.92%) compared to those of non-inoculated plants. Similarly, under 5 mM Zn stress, the shoot length, root length, shoot fresh weight, root fresh weight, stem diameter, and chlorophyll content were increased by 23.02%, 16.48%, 11.91%, 52.94%, 10.52%, and 16.51%, respectively, upon L. adecarboxylata MO1 inoculation (Table 1 and Figure 3).

Figure 1. Effect of Leclercia adecarboxylata MO1 on siderophores production (a) and on solubilization of silicate (b) and zinc (c). The images are representative of three replicates.

Figure 2. The ability of Leclercia adecarboxylata MO1 to tolerate zinc (Zn) toxicity (a) and produce indole-3-acetic acid (IAA) (b) under Zn toxicity (2 and 5 mM). Columns with error bars represent mean ± SD of the data of three replicates. Columns with different letters are significantly different at the 0.05 level of probability, as identified by the DMRT test.
Estimation of Zn content

Estimation of the Zn content in *L. adecarboxylata* MO1-inoculated and -non-inoculated plants showed significant differences when grown under 2 and 5 mM Zn toxicity (Figure 4). Under normal conditions, nonsignificant differences in Zn accumulation were recorded in the roots and shoots of *L. adecarboxylata* MO1-inoculated and -non-inoculated plants. Under 2 mM Zn toxicity, *L. adecarboxylata* MO1 inoculation significantly hindered Zn accumulation in the roots and shoots by a decrease of 69.45% and 54.89%, respectively, compared to that in non-inoculated plants. A similar trend of reduced Zn accumulation by 69.22% and 67.22% was recorded in the roots and shoots of *L. adecarboxylata* MO1-inoculated plants, respectively, under 5 mM Zn toxicity compared to that in non-inoculated plants (Figure 4).

Hydrogen peroxide (H$_2$O$_2$)

The ameliorative effect of *L. adecarboxylata* MO1 inoculation on hydrogen peroxide (H$_2$O$_2$) accumulation in cucumber leaves under the three conditions was also examined (Figure 5). The results demonstrated excessive hydrogen peroxide (H$_2$O$_2$) accumulation under 2 and 5 mM Zn toxicity; however, H$_2$O$_2$ accumulation was significantly decreased by *L. adecarboxylata* MO1 inoculation. Under control conditions, no hydrogen peroxide (H$_2$O$_2$) accumulation was recorded in both inoculated and non-inoculated leaves (Figure 5).

Catalase

Catalase regulates stress responses and maintains intracellular redox states by reacting with ROS. Under normal growth conditions, the concentrations of catalase were significantly enhanced upon *L. adecarboxylata* MO1 inoculation by 41.67% compared to those in non-inoculated plants. There was a negative trend of decreased catalase concentrations in *L. adecarboxylata* MO1-inoculated plants under 2 and 5 mM Zn toxicity, with the decreases being 16.32% and 36.09%, respectively (Figure 6).

Table 1. Plant growth-promoting effect of *Leclercia adecarboxylata* MO1 inoculation under normal condition and Zn stress (2 and 5 mM) conditions.

| Treatments | Shoot length (cm) | Root length (cm) | FW (g) | DW (g) | Stem diameter (cm) | CC (SPAD) |
|------------|------------------|-----------------|--------|--------|-------------------|-----------|
| Control Condition | 37.7 ± 0.81a | 21.6 ± 1.38a | 29.1 ± 1.29a | 8.8 ± 0.66a | 6.3 ± 0.23a | 38.9 ± 1.32a |
| MO1 | 41.1 ± 1.30b | 23.8 ± 1.51b | 31.7 ± 1.53b | 9.0 ± 0.83a | 6.4 ± 0.19a | 42.3 ± 1.90b |
| 2 mM Zn Stress | 32.1 ± 1.12a | 20.6 ± 1.27a | 25.5 ± 1.58a | 5.9 ± 1.07a | 5.0 ± 0.19a | 36.1 ± 0.89a |
| MO1 | 37.1 ± 1.05b | 21.6 ± 1.38a | 28.4 ± 1.59b | 8.1 ± 0.71b | 6.2 ± 0.19b | 38.8 ± 0.84b |
| 5 mM Zn Stress | 29.1 ± 1.53a | 18.2 ± 0.80a | 23.5 ± 1.80a | 5.1 ± 1.32a | 5.7 ± 0.24a | 32.7 ± 1.25a |
| MO1 | 35.8 ± 1.27b | 21.2 ± 0.98b | 26.3 ± 1.59b | 7.8 ± 1.00b | 6.3 ± 0.14b | 38.1 ± 1.41b |

Data in rows represent mean ± SD of three replicates, and the different letters show significant difference at the 0.05 level of probability, as identified by the t-test.
Peroxidase (POD) and polyphenol oxidase (PPO)
The stress ameliorative potential of *L. adecarboxylata* MO1 was also investigated, and the results showed that under normal growth conditions, *L. adecarboxylata* MO1-inoculated and -non-inoculated plants exhibited nonsignificant differences in PPO levels. Under 2 and 5 mM Zn stress, *L. adecarboxylata* MO1 inoculation significantly reduced the PPO levels by 15.35% and 19.55%, respectively, compared to those in non-inoculated plants (Figure 6).

Superoxide anion (SOA)
The evaluation of SOA levels in *L. adecarboxylata* MO1-inoculated and -non-inoculated plants grown under normal conditions and Zn stress (2 and 5 mM) conditions revealed nonsignificant differences in PPO levels. Under normal conditions, *L. adecarboxylata* MO1 inoculation significantly increased the PPO levels by 36.05% with respect to those in non-inoculated plants (Figure 6).

Lipid peroxidation
The extent of lipid peroxidation due to metal stress was estimated by measuring the levels of malondialdehyde (MDA). Under normal conditions, nonsignificant differences in LPO levels were observed in *L. adecarboxylata* MO1-inoculated and -non-inoculated plants compared to those in non-inoculated plants. However, under 2 mM Zn stress, *L. adecarboxylata* MO1 inoculation reduced the LPO levels by 45.10% and 35.53%, respectively, compared to those in non-inoculated plants (Figure 6).

Glutathione (GSH)
Glutathione (GSH) is considered as one of the primary mechanisms of antioxidant defense against ROS. Under normal conditions, *L. adecarboxylata* MO1-inoculated and -non-inoculated plants showed nonsignificant differences in GSH levels. However, under 2 and 5 mM Zn stress, the GSH levels were significantly decreased by 18.68% and 28.01% in *L. adecarboxylata* MO1-inoculated plants, respectively, compared to those in non-inoculated plants (Figure 6).
Endogenous abscisic acid

Stress-responsive, endogenous ABA levels were significantly regulated in *L. adecarboxylata* MO1-inoculated plants under Zn toxicity (Figure 7). Under control conditions, *L. adecarboxylata* MO1 inoculation did not cause a significant difference compared with non-inoculated plants. Under Zn toxicity (2 and 5 mM), the plants accumulated a significant amount of ABA; however, *L. adecarboxylata* MO1 inoculation significantly decreased the ABA accumulation compared to that in non-inoculated plants (Figure 7). Under 2 mM Zn stress, *L. adecarboxylata* MO1 inoculation significantly reduced the ABA accumulation by 38.97% compared to that in non-inoculated plants. Similarly, under 5 mM Zn stress, the ABA levels were significantly lower (22.63%) in *L. adecarboxylata* MO1-inoculated plants than in non-inoculated plants (Figure 7).

Endogenous salicylic acid

Stress-responsive, endogenous SA levels were significantly regulated in *L. adecarboxylata* MO1-inoculated plants under Zn toxicity (Figure 7). Under control conditions, *L. adecarboxylata* MO1 inoculation resulted in nonsignificant differences compared with non-inoculated plants. Under Zn toxicity (2 and 5 mM), the plants exhibited significant accumulation of endogenous SA; however, *L. adecarboxylata* MO1 inoculation significantly decreased the SA accumulation compared to that in non-inoculated plants (Figure 7). Under 2 mM Zn stress, *L. adecarboxylata* MO1 inoculation significantly reduced the SA accumulation by 53.22% compared to that in non-inoculated plants. Similarly, under 5 mM Zn stress, the SA levels were significantly lower (72.25% decrease) in *L. adecarboxylata* MO1-inoculated plants than in non-inoculated plants (Figure 7).

Discussion

Heavy metal contamination due to rapidly increasing anthropogenic activities is a serious concern for the environment and human health, considering the transport of metals through food chain (Mishra et al. 2019; Hembrom et al. 2020). The ingestion of excessive metals may cause various
human and animal health issues (e.g. poor immunological responses and high prevalence of upper gastrointestinal diseases) (Järup 2003; Duruibe et al. 2007; Tchounwou et al. 2012). Reducing metal accumulations in the environment and avoiding agricultural contamination with metals can protect human lives (Kang et al. 2017; Rai et al. 2019). Several researchers have been attempting to develop sustainable, environmentally friendly, and manageable approaches to reduce heavy metal accumulations in agricultural soil (Wong et al. 2018; Bilal et al. 2019). One of the best alternatives for heavy metal removal is the use of plant growth-promoting microorganisms (Etesami 2018), which have been well reported to be heavy, resistant microbes that improve plant growth and mediate stress (Ayangbenro and Babalola 2017; Shahzad et al. 2019a). Microorganisms have developed various bioremediation strategies, such as phosphate solubilization and siderophore and phytohormone production (Kong and Glick 2017; Verma and Kuila 2019; Yin et al. 2019).

In the present study, we hypothesized that the previously isolated plant growth-promoting L. adecarboxylata MO1 (Kang et al. 2019b) could be used in soils with an overabundance of Zn to facilitate plant growth. We selected L. adecarboxylata MO1 because of its potential to produce siderophores and auxins, as well as its properties of Zn solubility and Zn resistance. These properties are the consequence of the potential of microbes to solubilize various metals and secrete phytohormones and siderophores not only to facilitate plant growth but also to mediate metal stresses by various physiochemical modulations (Kang et al. 2017; Kong and Glick 2017; Verma and Kuila 2019). The present study has revealed the potential of L. adecarboxylata MO1 to produce IAA, solubilize Zn, and resist elevated levels of Zn toxicity. L. adecarboxylata is metabolically diverse and can produce phytohormones, synthesize extracellular enzymes, degrade hydrocarbons, and solubilize minerals, which have been widely reported to promote plant growth and mediate various stresses (Sarma et al. 2004; Shahzad et al. 2017c; Kang et al. 2019b).

The IAA- and siderophore-producing potential of L. adecarboxylata MO1 (Figures 1 and 2) is consistent with the reports of Shahzad et al. (2017c), Kang et al. (2019b), and Kumawat et al. (2019). In addition, L. adecarboxylata MO1 was found to produce significant amounts of IAA under Zn toxicity (Figure 2). Moreover, the growth-promoting capability of L. adecarboxylata has been widely reported (Sarma et al. 2004; Shahzad et al. 2017c), and L. adecarboxylata has the potential to mitigate different abiotic stresses such as salinity stress (Kang et al. 2019b) and drought stress (Danish and Zafar-ul-Hye 2019; Danish et al. 2020). Nevertheless, the role of L. adecarboxylata in metal stress mitigation, and more specifically Zn stress, has not explored in detail, which allows for an appropriate investigation of its potential role in stress mitigation and improving stress tolerance. The plant growth-promoting effect and Zn tolerance capability of L. adecarboxylata are consistent with the reports of Kang et al. (2019b), Sarma et al. (2004), Shahzad et al. (2019a), and Han et al. (2019). They linked the plant growth-promoting potential and stress-mitigating capability of L. adecarboxylata to its distinguishing abilities of nitrogen fixation, mineral solubilization, phytopathogen inhibition, siderophore production, and indole-3-acetic acid production; this association was also observed in the present study.

Studies have reported that elevated Zn concentrations inhibit the survival of plant PGPR in Zn-contaminated soil (Vivas et al. 2006; Kang et al. 2017). Similarly, Marques et al. (2013) demonstrated the inhibition of PGPR in agricultural soil supplemented with elevated Zn levels. Therefore, L. adecarboxylata was evaluated for Zn tolerance, because the survival of microbes under stressed conditions is important to help us understand plant survival in stressed environments (Shahzad et al. 2017b). In the present study, L. adecarboxylata MO1 was found to tolerate Zn toxicity (Figure 2), which could be the reason for the survival of MO1 in Zn-supplemented soil and the promotion of plant growth under Zn toxicity (Figure 3 and Table 1). Zinc stress causes biochemical and physiological disturbances within plants by inhibiting their growth, photosynthesis, nutrient uptake, enzyme activation or deactivation, and phytohormone production (Rout and Das 2009). In the present study, cucumber plants exposed to zinc stress showed inhibited growth, root/shoot length, and biomass production (fresh/dry weight) (Figure 3 and Table 1). However, the zinc-tolerant isolate MO1 mitigated zinc toxicity in cucumber plants by enhancing the cucumber growth attributes such as root/shoot length and biomass (fresh/dry weight) (Figure 3 and Table 1). Previously, several researchers have reported about different bacteria such as Serratia, Sphingomonas, and Rhizobium sp. in maize, sedum, and lentil,
which increased the plant growth, biomass, and tolerance to zinc toxicity (Ahmad Wani et al. 2008; Chen et al. 2014; Jain et al. 2020). Similarly, chlorophyll has a vital role in photosynthesis, and its synthesis was found to be inhibited under zinc stress. However, cucumber plants inoculated with the isolate MO1 showed a markedly increased chlorophyll content compared to that in zinc-treated non-inoculated control plants (Figure 2). These results are in agreement with previous findings (Joshi et al. 2013; Islam et al. 2014; Jain et al. 2020), wherein the authors reported similar observations in maize and wheat seedlings under zinc stress.

It is important to determine the distribution and accumulation of metal uptake in the roots and shoots of plants when evaluating plant survival under metal stress (Bilal et al. 2017; Kang et al. 2017). In the present study, *L. adecarboxylata* MO1 inoculation significantly protected the plants from the inhibitory effects of higher concentrations of Zn by reducing the Zn content (Kang et al. 2017). The reduction of Zn content at higher Zn toxicity levels by this bacterium might be due to Zn removal through adsorption and desorption mechanisms, as well as solubilization and leaching mechanisms of the bacterium (Pirhadi et al. 2016; Jafarian and Ghaffari 2017). Enhanced Zn uptake triggered Fe deficiency (Lešková et al. 2017) that reduced root and shoot growth and chlorophyll content as well as chlorosis in young leaves (Marschner 2012; Lešková et al. 2017). The results of plant growth and chlorophyll content in our study under Zn stress might be due to Fe deficiency. A similar observation was made by Fukao et al. (2011) who reported that excessive Zn levels reduce chlorophyll content and cause iron deficiency (Lešková et al. 2017).

It has been reported that zinc toxicity in plants induces oxidative damage and causes cellular damage, which in turn alters the plant antioxidative systems (Rout and Das 2009; Radić et al. 2010). In general, Zn toxicity can induce several alterations in plant cells, such as binding of cell membrane proteins and enzymes to sulphhydril groups, increases in lipid peroxidation, and disturbances in essential elements (Castillo-González et al. 2018). In the present study, the induction of oxidative stress was found in plants grown in Zn-contaminated soil, which was evident by the increase in the antioxidant activities of plants (Figure 6). However, *L. adecarboxylata* MO1 inoculation reduced the antioxidant activities of plants, suggesting that improved plant tolerance under heavy metal stress can occur after rhizobacterial inoculation (Mesa et al. 2015; Ahemad 2019). Rhizobacterial bioaugmentation in the present study also diminished the activities of CAT, POD, PPO, SOA, MDA, and GSH in the plants compared to those in non-inoculated plants, which also supports the conclusion that Zn accumulation in plant roots and shoots was reduced (Figure 6). With higher metal accumulation and toxicity, an increase in ROS generation and, consequently, antioxidant enzyme activity could be expected. Although the upregulation of antioxidants in plants under stress conditions has been reported by several researchers (Kang et al. 2019a; AbdElgawad et al. 2020), our data support that *L. adecarboxylata* MO1 inoculation may contribute to the mediation of Zn stress not by increasing the antioxidants but by reducing the metal toxicity (Figure 6). For instance, *L. adecarboxylata* MO1 produced siderophores and indole-3-acetic acid. Studies have reported that siderophores released by the rhizobacterial consortium are chelators that may bind metals, thus alleviating their toxicity (Dimkpa et al. 2009a, 2009b). In the same manner, selected rhizobacteria produce indole-3-acetic acid, which has a bioprotective effect (Mesa et al. 2015). Collectively, the findings of this study suggest that *L. adecarboxylata* MO1 inoculation causes a reduction in the formation of cell-damaging free radicals, thus lessening the need of plant enzymatic defences (Dimkpa et al. 2009a, 2009b).

The regulation of stress-responsive plant endogenous hormones, specifically ABA, is well known to be a stress coping mechanism (Shahzad et al. 2017b; Khan et al. 2019a). However, higher accumulation of ABA under conditions of stress causes stomata closure, which further results in leaf senescence and photosynthesis reduction (Verma et al. 2016). Interestingly, in the present study, *L. adecarboxylata* MO1 inoculation resulted in a significant reduction of endogenous ABA levels in plants grown under Zn stress compared to those in non-inoculated plants (Figure 7). Moreover, the reduced ABA levels in *L. adecarboxylata* MO1-inoculated plants were associated with a decrease in antioxidant contents, because enhanced ROS generation led to the higher accumulation of endogenous ABA for stress mitigation (Kar 2011; Letiáño and Enguita 2016). The reduction of endogenous ABA levels is accredited to the potential of *L. adecarboxylata* MO1 to enhance stress tolerance and reduce metal toxicity in plants.

Endogenous SA is regulated in plants upon exposure to various biotic and abiotic stresses (Shahzad et al. 2017a, 2017b; Khan et al. 2020). It has been reported that SA protects plants from heavy metal-induced toxicity due to ROS production by antioxidant regulation (Liu et al. 2016; Kang et al. 2017). In the present study, endogenous SA was significantly reduced in MO1-inoculated plants under 2 and 5 mM levels of Zn compared to that in non-inoculated plants (Figure 7). In general, endogenous SA is regulated and synthesized from chorismate-derived l-phenylalanine through various reactions of enzymes initially catalyzed by phenylalanine ammonia lyase (PAL) (Vlot et al. 2009). Furthermore, Zn toxicity has been reported to enhance PAL activity, which resulted in the enhancement of endogenous SA levels in plants exposed to Zn toxicity (Luo et al. 2010; Kang et al. 2017). The inoculation of MO1 in plants grown under Zn toxicity may regulate PAL activity and subsequently decrease endogenous SA synthesis to exert its protective role in plants.

**Conclusion**

The present study has demonstrated interesting results and identified *L. adecarboxylata* MO1 as a promising alternative for plant growth promotion and also showed that MO1 application may be an effective method to mitigate metal toxicity in plants. Our results demonstrated that *L. adecarboxylata* MO1 inoculation enhanced Zn stress tolerance and reduced metal toxicity in plants by the mechanisms of Zn stress tolerance, as well as by siderophore and IAA production, which positively regulated Zn distribution in the plant tissues, regulated the antioxidant levels (H2O2, CAT, POD, PPO, SOA, MDA, and GSH), and modulated the production of stress-responsive phytohormones (ABA and SA). These findings illustrate that *L. adecarboxylata* MO1 application is a viable strategy for improving plant growth and reducing metal toxicity. These results also encourage the utilization of secondary metabolite-producing
PGPR as an alternative to enhance plant growth, improve stress tolerance, and reduce metal toxicity in plants grown in metal-contaminated fields. Further experiments are required to assess the role of the isolate MO1 under Zn stress on iron accumulation and its effect on the growth attributes of Zn-Fe-MO1 in cucumber plants at the molecular level.

Disclosure statement

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References

AbdElgawad H, Zinta G, Hamed BA, Selim S, Beemster G, Hozezin WN, Wadaan MAM, Asard H, Abueoulw W. 2020. Maize roots and shoots show distinct profiles of oxidative stress and antioxidant defense under heavy metal toxicity. Environ Pollut. 258:113705.

Ahamed M. 2019. Remediation of metaliferrous soils through the heavy metals by hormonal and stress-responses proteins modulation. J Hazard Mater. 379:120824.

Bilal S, Shahzad R, Khan AL, Kang SM, Imran QM, Al-Harrasi A, Yun BW, Lee IJ. 2018. Endophytic microbial consortia of phytohormones-producing fungus paecilomyces formosus LHLL10 and bacteria spingonomonas sp. LHKL1 to Glycerine max L. regulates physio-hormonal changes to attenuate aluminum and zinc stresses. Front Plant Sci. 9:1273.

Castillo-González J, Ojeda-Barrios D, Hernandez A, Gonzalez-Franco A, Robles-Hernandez L, López-Ochoa GR. 2018. Zinc metalloenzymes in plants. Interenciencia. 43:242–248.

Chen C, Chen X, Han J, Lu W, Ren Z. 2020. Genome-wide analysis of the WRKY gene family in the cucumber genome and transcriptome-wide identification of WRKY transcription factors that respond to biotic and abiotic stresses. BMC Plant Biol. 20:443.

Chen B, Shen J, Zhang X, Pan F, Yang X, Fung Y. 2014. The endophytic bacterium, Sphingomonas SaMR12, improves the potential for zinc phytoremediation by its host, Sedum alfredii. PLoS One. 9:e106826.

Chung H, Park M, Madhiaiyen M, Sheshadi S, Song J, Cho H, Sa T. 2005. Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. Soil Biochim. 37:1970–1974.

Danish S, Zafar-ul-Hye M. 2019. Co-application of ACC-deaminase producing PGPR and timber-waste biochar improves pigments formation, growth and yield of wheat under drought stress. Sci Rep. 9:5999.

Danish S, Zafar-ul-Hye M, Mohsin F, Hussein M. 2020. ACC-deaminase producing plant growth promoting rhizobacteria and biochar mitigate adverse effects of drought stress on maize growth. PLoS One. 15(4):e203061. doi:10.1371/journal.pone.0203615.

Dimkpa CO, Merten D, Svatos A, Bächlin G, Köthe E. 2009b. Siderophores mediate reduced and increased uptake of cadmium by Streptomyces tendae F4 and sunflower (Helianthus annuus), respectively. J Appl Microbiol. 107:1687–1696.

Dimkpa C, Weiland T, Asch F. 2009a. Plant-rhizobacteria interactions alleviate abiotic stress conditions. Plant Cell Environ. 32:1682–1694.

Duruibe J, Ogwuegbu MOC, Egwurugwu J. 2007. Heavy metal pollution in pristine environments: a review of microbial biosorbents. Int J Pollut. 260:114057.

Frassinetti S, Bronzetti GL, Calvuturwo L, Cini M, Croce CD. 2006. The role of zinc in life: a review. J Environ Pathol Toxicol Oncol. 25:597–610.

Fukao Y, Ferjani A, Tomioka R, Nasakani N, Kurata R, Nishimori Y, Fujiwara M, Maeshima M. 2011. iTRAQ analysis reveals mechanisms of growth defects due to excess zinc in Arabidopsis. Plant Physiol. 155:1893–1907.

Goteti PK, Emmanuel LDA, Desai S, Shaik MHA. 2013. Prospective zinc solubilising bacteria for enhanced nutrient uptake and growth promotion in Maize (Zea mays L.). Int J Microbiol. 2013:1–7.

Han Y-H, Yin D-X, Jia M-R, Wang S-S, Chen Y, Rathinasabapathi B, Chen D-L, Ma LQ. 2019. Arsenic-resistance mechanisms in bacterium Leclercia adecarboxylata strain AS-1: biochemical and genomic analyses. Sci Total Environ. 690:1178–1189.

Hanif MA, Nawaz H, Ayub MA, Kanwal N, Rashid N, Saleem M, Ahmad M. 2017. Evaluation of the effects of zinc on the chemical composition and biological activity of basil essential oil by using Raman spectroscopy. Ind Crops Prod. 96:91–101.

Hayat R, Ali S, Amara U, Khalid R, Ahmed I. 2010. Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol. 60:579–598.

Hembrow S, Singh B, Gupta SK, Nema AK. 2020. A comprehensive evaluation of heavy metal contamination in foodstuff and associated human health risk: a global perspective. In: Singh P, Singh RP, Srivastava V, editors. Contemporary environmental issues and challenges in era of climate change. Singapore: Springer Singapore; p. 33–63.

Hou D, O’Connor D, Igalavithana AD, Alessi DS, Luo J, Tsang DCW, Sparks DL, Yamauchi Y, Rinkele J, Ok YS. 2020. Metal contamination and bioremediation of agricultural soils for food safety and sustainability. Nat Rev Earth Environ. 1:366–381.
Islam F, Yasmeen T, Riaz M, Arif MS, Ali S, Raza SH. 2014. Proteus mirabilis alleviates zinc toxicity by preventing oxidative stress in maize (Zea mays) plants. Ecotoxicol Environ Saf. 110:143–152.

Jafarian V, Ghaffari F. 2017. A unique metallothionein-engineered in Escherichia coli for biosorption of lead, zinc, and cadmium: absorption or adsorption? Microbiology. 86:73–81.

Jain D, Kour R, Bhojia AA, Meena RH, Singh A, Mohanty SR, Rajpurohit D, Ameta KD. 2020. Zinc tolerant plant growth promoting bacteria alleviates phytotoxic effects of zinc on maize through zinc immobilization. Sci Rep. 10:13865–13865.

Jan R, Khan MA, Asaf S, Lubna Li-J, Kim KM. 2019. Metal resistant endophytic bacteria reduces cadmium, nickel toxicity, and enhances expression of metal stress related genes with improved growth of Oryza sativa, via regulating its antioxidiant machinery and endogenous hormones. Plants. 8:363.

Järup L. 2003. Hazards of heavy metal contamination. Br Med Bull. 68:167–182.

Joshi D, Negi G, Vaid S, Sharma A. 2013. Enhancement of wheat growth and Zn content in grains by zinc solubilizing bacteria. Int J Agric Environ Biotechnol. 6:363.

Kang S-M, Khan AL, Wazqas M, Asaf S, Lee K-E, Park Y-G, Kim A-Y, Shahzad R, Kang S-M, Khan AL, Lee S-m, Park Y-G, Lee W-J, Jafarian V, Ghassemi M, Järup L. 2003. Hazards of heavy metal contamination. Br Med Bull. 68:167–182.

Khan MA, Hamayun M, Iqbal A, Khan SA, Hussain A, Asaf S, Khan AL, Ullah I, Waqas M, Hamayun M, Khan AL, Asaf S, Kang S-M, Kim A-Y, Shahzad R, Kang S-M, Khan AL, Lee S-m, Park Y-G, Lee W-J, Jafarian V, Ghassemi M, Järup L. 2003. Hazards of heavy metal contamination. Br Med Bull. 68:167–182.

Khan MA, Bano A. 2018. Role of PGPR in the phytoremediation of heavy metals. J Microbiol. 35:172.

Khan MA, Hamayun M, Iqbal A, Khan SA, Hussain A, Asaf S, Khan AL, Ullah I, Waqas M, Hamayun M, Khan AL, Asaf S, Kang S-M, Kim A-Y, Shahzad R, Kang S-M, Khan AL, Lee S-m, Park Y-G, Lee W-J, Jafarian V, Ghassemi M, Järup L. 2003. Hazards of heavy metal contamination. Br Med Bull. 68:167–182.

Kumar A, Dewangan S, Lawate P, Bahadur D, Pratap S, 2019. Zinc solubilizing bacteria: a boon for sustainable agriculture. pp. 139–155.

Kumawat KC, Sharma P, Singh I, Sirari A, Gill BS. 2019. Co-existence of Leclercia adecarboxylata (LSE-1) and Bradyrhizobium sp. (LSBR-3) in nodule niche for multifaceted effects and profitability in soybean production. World J Microbiol Biotechnol. 35:172.

Leitão AL, Enguita FJ. 2016. Gibberellins in Penicillium strains: challenges for endophyte-plant host interactions under salinity stress. Microbiol. Res. 183:18–18.

Lešková A, Giehl RH, Hartmann A, Fargašová A, von Wirén N. 2017. Heavy metals induce iron deficiency responses at different hierarchical and regulatory levels. Plant Physiol. 174:1648–1668.

Lin YF, Aarts MG. 2012. The molecular mechanism of zinc and cadmium stress response in plants. Cell Mol Life Sci. 69:3187–3206.

Liu Z, Ding Y, Wang F, Ye Y, Zhu C. 2016. Role of salicylic acid in resistance to cadmium stress in plants. Plant Cell Rep. 35:719–731.

Lu Z-B, He X-J, Chen L, Tang L, Gao S, Chen F. 2010. Effects of zinc on growth and antioxidiant responses in Jatropha curcas seedlings. Int J Agric Biol. 12:1560–8530.

Marques AP, Moreira H, Franco AR, Rangel AO, Castro PM. 2013. Inoculating Helianthus annuus (sunflower) grown in zinc and cadmium contaminated soils with plant growth promoting bacteria--effects on phytoremediation strategies. Chemosphere. 92:74–83.

Marschner H. 2012. Marschner’s mineral nutrition of higher plants. Vol. 89. San Diego, CA: Academic Press.

Messa J, Mateos-Naranjo E, Caviedes MA, Redondo-Gómez S, Pajuelo E, Rodríguez-Llorente ID. 2015. Endophytic cultivable bacteria of the metal bioaccumulator spartina maritima improve plant growth but not metal uptake in polluted marshes soils. Front Microbiol. 6:1450–1450.

Mishra S, Bhargava RN, More N, Yadav A, Zainith S, Mani S, Chowdhary P. 2019. Heavy metal contamination: an alarming threat to environment and human health. In: Sobti RC, Arora NK, Kothari A, editors. Environmental biotechnology: for sustainable future. Singapore: Springer Singapore; p. 103–125.

Mossa AW, Young S, Crout N. 2020. Zinc uptake and phyto-toxicity: Comparing intensity- and capacity-based drivers. Sci Total Environ. 699:134314.

Nadeem SM, Ahmad M, Naveed M, Imran M, Zahir ZA, Crowley DE. 2019. Zinc uptake and phyto-toxicity: Comparing intensity- and capacity-based drivers. Sci Total Environ. 699:134314.

Pandey N, Pathak GC, Pandey R. 2016. Relationship between in vitro characterization and comparison of exogenous hormone application. PLOS ONE. 15:e0232228.

Pandey N, Pathak GC, El-Gayar MA, Pandey R. 2009. Zinc uptake and phyto-toxicity: Comparing intensity- and capacity-based drivers. Sci Total Environ. 699:134314.

Rodríguez-Llorente ID. 2015. Endophytic cultivable bacteria of the metal bioaccumulator spartina maritima improve plant growth but not metal uptake in polluted marshes soils. Front Microbiol. 6:1450–1450.

Rajpurohit D, Ameta KD. 2020. Zinc tolerant plant growth promoting bacteria alleviates phytotoxic effects of zinc on maize through zinc immobilization. Sci Rep. 10:13865–13865.

Rai PK, Lee SS, Zhang M, Tsang YF, Kim K-H. 2019. Heavy metals in food crops: health risks, fate, mechanisms, and management. Environ Int. 125:365–385.
Rout GR, Das P. 2009. Effect of metal toxicity on plant growth and metabolism: I. zinc. In: Lichtfouse E, Navarrete M, Debaeke P, Véronique S, Alberola C, editors. Sustainable agriculture. Dordrecht: Springer Netherlands; p. 873–884.

Sarma PM, Bhattacharya D, Krishnan S, Lal B. 2004. Degradation of polycyclic aromatic hydrocarbons by a newly discovered enteric bacterium, Leclercia adecarboxylata. Appl Environ Microbiol. 70:3163–3166.

Shahzad R, Bilal S, Imran M, Khan AL, Alosaimi AA, Al-Shwyeh HA, Almahasheer H, Rehman S, Lee IJ. 2019a. Amelioration of heavy metal stress by endophytic Bacillus amyloliquefaciens RWL-1 in rice by regulating metabolic changes: potential for bacterial bioremediation. Biochem J. 476:3385–3400.

Shahzad R, Khan AL, Bilal S, Asaf S, Lee IJ. 2017a. Plant growth-promoting endophytic bacteria versus pathogenic infections: an example of Bacillus amyloliquefaciens RWL-1 and Fusarium oxysporum f. sp. Lycopersici in tomato. PeerJ. 5:e3107.

Shahzad R, Khan AL, Bilal S, Waqas M, Kang S-M, Lee I-J. 2017b. Inoculation of abscisic acid-producing endophytic bacteria enhances salinity stress tolerance in Oryza sativa. Environ Exp Bot. 136:68–77.

Shahzad R, Khan AL, Waqas M, Ullah I, Bilal S, Kim Y-H, Asaf S, Kang S-M, Lee I-J. 2019b. Metabolic and proteomic alteration in endophytic Bacillus amyloliquefaciens RWL-1 during methanol utilization. Metabolomics. 15:16.

Shahzad R, Waqas M, Khan AL, Al-Hosni K, Kang SM, Seo CW, Lee IJ. 2017c. Indoleacetic acid production and plant growth promoting potential of bacterial endophytes isolated from rice (Oryza sativa L.) seeds. Acta Biol Hung. 68:175–186.

Shahzad R, Waqas M, Khan AL, Asaf S, Khan MA, Kang SM, Yun BW, Lee IJ. 2016. Seed-borne endophytic Bacillus amyloliquefaciens RWL-1 produces gibberellins and regulates endogenous phytohormones of Oryza sativa. Plant Physiol Biochem. 106:236–243.

Tchounwou PB, Yedjou CG, Patiloka AK, Sutton DJ. 2012. Heavy metal toxicity and the environment. Experientia Suppl. 101:133–164.

Tomáš J, Árvay J, Tóth T. 2019. Heavy metals in productive parts of agricultural plants. Journal of Microbiology, Biotechnol Food Sci. 2019:819–827.

Torres MA, Dangl JL, Jones JDG. 2002. Arabidopsis gp91phox homologues AtrbohD and AtrbohF are required for accumulation of reactive oxygen intermediates in the plant defense response. Proc Natl Acad Sci U S A. 99:517–522.

Uzoh I, Babalola O. 2020. Review on increasing iron availability in soil and its content in cowpea (Vigna unguiculata) by plant growth promoting rhizobacteria. Afr J Food Agric Nutr Dev. 20:15779–15799.

Verma S, Kulla A. 2019. Bioremediation of heavy metals by microbial process. Environ Technol Innov. 14:100369.

Verma V, Ravindran P, Kumar PP. 2016. Plant hormone-mediated regulation of stress responses. BMC Plant Biol. 16:86.

Vivas A, Biró B, Ruiz-Lozano JM, Barea JM, Azcón R. 2006. Two bacterial strains isolated from a Zn-polluted soil enhance plant growth and mycorrhizal efficiency under Zn-toxicity. Chemosphere. 62:1523–1533.

Vlot AC, Dempsey DA, Klessig DF. 2009. Salicylic acid, a multifaceted hormone to combat disease. Annu Rev Phytopathol. 47:177–206.

Wang C-J, Yang W, Wang C, Gu C, Niu D-D, Liu H-X, Wang Y-P, Guo J-H. 2012. Induction of drought tolerance in cucumber plants by a consortium of three plant growth-promoting rhizobacteria strains. PLOS ONE. 7:e52565.

Wong C, Tan LT, Mujahid A, Lihan S, Wee JLS, Ting LF, Müller M. 2018. Biosorption of copper by endophytic fungi isolated from Nopensites ampullaria. Lett Appl Microbiol. 67:384–391.

Yan S, Che G, Ding L, Chen Z, Liu X, Wang H, Zhao W, Ning K, Zhao J, Tesfamichael K, et al. 2016. Different cucumber CsYUC genes regulate response to abiotic stresses and flower development. Sci Rep. 6:20760.

Yin K, Wang Q, Lv M, Chen L. 2019. Microorganism remediation strategies towards heavy metals. Chem Eng J. 360:1553–1563.

Yu-Na K, Muhammad Aaqil K, Sang-Mo K, Muhammad H, In-Jung L. 2020. Enhancement of drought-stress tolerance of Brassica oleracea var. italica L. by newly isolated variovarox sp. YNA9. J Microbiol Biotechnol. 30(10):1500–1509.