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Isolation and characterization of endophytic bacterium LRE07 from cadmium hyperaccumulator *Solanum nigrum* L. and its potential for remediation

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Abstract Valuable endophytic strains facilitating plants growth and detoxification of heavy metals are required because the application of plant–endophyte symbiotic system is a promising potential technique to improve efficiency of phytoremediation. In this study, endophytic bacterium LRE07 was isolated from cadmium hyperaccumulator *Solanum nigrum* L. It was identified as *Serratia* sp. by 16S rRNA sequence analysis. The endophytic bacterium LRE07 was resistant to the toxic effects of heavy metals, solubilized mineral phosphate, and produced indoleacetic acid and siderophore. The heavy metal detoxification was studied in growing LRE07 cells. The strain bound over 65% of cadmium and 35% of zinc in its growing cells from single metal solutions 72 h after inoculation. Besides the high removal efficiencies in single-ion system, an analogous removal phenomenon was also observed in multi-ions system, indicating that the endophyte possesses specific and remarkable heavy metal remediation abilities.

Keywords Endophyte · Hyperaccumulator · Heavy metal · Detoxification · Phytoremediation

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Introduction

With the development of high social economic status and improvement of living standards, heavy metal contamination becomes a serious worldwide problem. Heavy metals can be accumulated by living organisms, which are finally enriched by human beings through food chains (Chen et al. 2008). Consequently, it is very urgent to remove heavy metals from environment. Several physicochemical methods have been used to remove them from metal-polluted soils, sediments, and water resources (Canet et al. 2002; Esalah et al. 2000; Toles and Marshall 2002; Zouboulis et al. 1997). Although great progress has been achieved by employing physicochemical methods, they are usually restricted due to the complicated operation procedures and high cost.

Phytoremediation has been considered as a low-cost, environmentally friendly technology for the remediation of contaminated sites (Salt et al. 1998; Singh et al. 2003). Hyperaccumulating plants have attracted intensive interests for phytoremediation technologies of heavy metals treatment. However, three disadvantages restrict the wide application of phytoremediation for heavy metal contamination. (1) It is difficult to find metal hyperaccumulators. (2) As hyperaccumulating plants show the tendency of

accumulating specific metal, their efficiencies would be restricted in dealing with multi-metals pollution. (3) The application of phytoremediation is time consuming and seasonal. As a result, efforts have been devoted to enhancing the phytoremediation capacity of plants using either transgenic methods or endophytes (Doty 2008).

Much attention has been paid on endophytic bacteria due to its potential application in phytoremediation (Newman and Reynolds 2005). Endophytes are the microbes inhabiting the interior of plant tissues without causing harm to the host (Kuklinsky-Sobral et al. 2004). Endophytes can facilitate plant growth and increase plant resistance to pathogens, drought, and even herbivores (Bottini et al. 2004; Saikkonen et al. 2006; Taghavi et al. 2010). Some endophytes are diazotrophic and can provide fixed nitrogen to the host plant (Reinhold-Hurek and Hurek 1998). Simultaneously, the plant provides a ready-made environment for endophytic bacteria so that the biotic and abiotic stresses against colonization of the desired endophytes would be reduced (Newman and Reynolds 2005). In the last few years, a lot of research clearly demonstrates the utility of using natural or engineered endophytes to enhance phytoremediation (Doty 2008). However, majority of the studies have focused on biodegradation of organic pollutants (Barac et al. 2004; Van Aken et al. 2004). The applications of natural or engineered endophytic bacteria for improving phytoremediation of heavy metals have been delayed because of lack of valuable strains having the heavy metal resistance and detoxification capacities.

The objective of this study was to obtain such valuable strain from cadmium hyperaccumulator *Solanum nigrum* L. (Wei et al. 2005) for phytoremediation. As an additional aim, the endophyte was evaluated in relation to its potential for plant growth promotion by investigating indoleacetic acid (IAA) production, mineral phosphate solubilization, and siderophore production. The endophytic bacterium LRE07 isolated from cadmium hyperaccumulator *S. nigrum* L. was studied specifically for its capacity of heavy metals solubilization, resistance, and sequestration. The endophyte is expected to be a promising bioresource for enhancing the phytoremediation efficiency in heavy metal pollution due to the unique properties mentioned above.

Materials and methods

Preparation of reagents and medium

All reagents used were of analytical grade and were purchased from Shanghai Pharmaceutical Co. Ltd. in China. The deionized distilled water used in the experiment was obtained from a Milli-Q system (Millipore, USA). The standard heavy metal ions stock solutions were obtained by nitrification of high pure powder of metals. All solutions were diluted by distilled deionized water from the stock solution. Putative endophytic bacterial strains which were isolated from plants were maintained and activated in Luria-Bertani (LB) (Demuth et al. 1990) medium comprising of 10 g tryptone, 5 g yeast extract, and 5 g NaCl per liter of water. The pH of the medium was adjusted to 7.2–7.4.

Analysis techniques

Heavy metals analysis

The exact concentrations of stock solution, diluted solution, or filtrate of heavy metal ions in the following experiments were determined by flame atomic absorption spectrometry (Hitachi, Japan).

Growth detection of LRE07

Growth of strain LRE07 in batch cultures was determined by measuring optical density of the culture liquid at 600 nm with a UV-visible Spectrophotometer (Varian Cary300, USA). All the data were analyzed with Excel or mapped with Origin 6.0.

Isolation and conservation of endophytic bacteria

Putative endophytic bacterial strains were isolated from surface-sterilized *S. nigrum* L., a cadmium hyperaccumulator, collected at the sewage discharge canal bank of Zhuzhou Smeltery (27°52′N, 113°05′E). A total of six plants were collected. Collected plants were put into plastic pots and processed the following day. Root, stem, and leaves of each plant were analyzed separately. Endophytic bacteria were isolated after removing epiphytes by surface disinfection using serial washing in 70% ethanol for 3 min, sodium hypochlorite solution (2% available Cl^-) for 3 min, and rinsed three times in sterilized distilled water (Barzanti et al. 2007). The disinfection process was checked by plating a 100-μl sample of the sterile distilled water used in the final rinse onto LB medium and incubating the plates at 28 °C for 2–14 days. After surface disinfection, the leaf, stem, or root tissue was cut and triturated in 10 ml of sterile phosphate buffer saline in a 50-ml flask maintained at 28 °C and agitated at 150 rpm for 1 h, after which, appropriate dilutions (100 μl) were plated onto LB medium and incubated at 28 °C for 2–14 days. After incubation, colonies were picked off the plates, inoculated on LB agar slants, incubated at 28 °C for 2 days, and stored at 4 °C.

Detection and identification of the isolated bacterium by 16S rDNA analyses

The genomic DNA of endophytic bacterium LRE07 was extracted, and 16S rDNA was amplified in polymerase chain reaction (PCR) using the genomic DNA as template and bacterial universal primers, 27 F (5′-GAGTTTGATCACTGGCTCAG-3′) and 1492 R (5′-TACGGCTACCTTGTTACGACTT-3′) (Byers et al. 1998). Amplification was performed for 30 PCR cycles with denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1.5 min. The amplified DNA was purified with TaKaRa Agarose Gel DNA Purification Kit (TaKaRa, China), and sequencing was performed at TaKaRa Biotechnology Company, Limited (Dalian, China). The 16S rDNA sequence was compared against the GenBank database using NCBI Blast program. The strain was deposited in CCTCC (China Center for Type Culture Collection) under accession number AB 2010339.

Characterization of endophytic bacteria LRE07

Indoleacetic acid (IAA) production

IAA production was analyzed using a modification of the qualitative method developed by Bric et al. (1991). The strain was plated onto LB agar medium amended with 5 mM of L-tryptophan, overlaid with a nitrocellulose membrane, and incubated at 28 °C for 24 h. After bacterial growth had occurred, the membrane was removed from the plate and treated with Salkowski reagent (2% (*w*/*v*) 0.5 M $FeCl_3$ in 35% perchloric acid) for 15 min at room temperature; bacteria producing IAA were identified by the presence of a red halo on the membrane.

Mineral phosphate solubilization activity

Mineral phosphate solubilization activity was assayed according to Kuklinsky-Sobral et al. (2004). Briefly, isolates were plated onto LB agar medium containing inorganic phosphate (constituents (grams per liter) agar, 15; glucose, 10; NH_4Cl, 5; NaCl, 1; $MgSO_4 \cdot 7H_2O$, 1; $Ca_3(PO_4)_2$, 0.8; pH 7.2) and incubated at 28 °C for up to 48 h. Solubilization of mineral phosphate was characterized by a clear halo around bacterial colonies with phosphate solubilization capacity.

Siderophore production

Siderophore production was measured by using the ternary complex chrome azurol S/Fe (III)/hexadecyl-trimethyl-ammonium bromide on supernatants of culture fluids according to the method of Schwyn and Neilands (1987) on LB medium with 0.25 mM Fe (III).

Heavy metals resistance of LRE07

The minimal inhibitory concentration (MIC) of heavy metals at which no growth of the strain took place was observed in triplicate in LB liquid medium with series concentrations of Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, and Cr^{6+} (1–15 mM). The isolate was inoculated into 100 ml LB medium in a 250-ml conical flask and incubated at 28 °C, 150 rpm for at least 1 week (Guo et al. 2010). The bacterial growth was determined by OD_{600} and monitored every 24 h. MIC was considered to be the lowest concentration of heavy metal at which completely inhibiting bacterial growth in LB medium.

Heavy metal solubilization tests

The heavy metal solubilization test was conducted based on the concentration changes of two indissoluble heavy metals in the absence and presence of the strain. Inoculum of the isolate was prepared from logarithmic phase cell suspensions, and the composition in the incubation medium was LB medium supplemented with 500 mg/l of Pb as $PbCO_3$ or Cd as $CdCO_3$ (solubility products of $PbCO_3$ and $CdCO_3$, 7.4×10^{-14} and 1.0×10^{-12} mol/l, respectively (Lide 2010)). Triplicate test tubes containing 10 ml of sterile medium were inoculated with 0.1 ml of cells (1.0% inoculum, *v*/*v*). Noninoculated Pb- or Cd-amended medium and inoculated Pb- or Cd-free medium were made as controls. The test tubes were incubated at 28 °C under agitation (150 rpm). Initial (0 h) and final (48 h) metal concentrations and pH were monitored. Selected samples were transferred to Eppendorf tubes and centrifuged for 10 min at 6,000 rpm. The cadmium and lead concentrations in the supernatant were determined with the methods mentioned above.

Heavy metal binding assay

Single-metal system

Cells of LRE07 were exposed separately in simulated wastewater containing LB medium and heavy metals. Bioreactors consisted of 250-ml conical flasks equipped with lateral tubing for sample collection and were closed by a rubber stopper. Bioreactors contained 100 ml of liquid LB medium supplemented with 20 and 50 mg/l of Cd^{2+}, Cr^{6+}, Pb^{2+}, Cu^{2+}, and Zn^{2+}. Each flask was inoculated with 1 ml of cells (1.0% inoculum, *v*/*v*). The inoculum was prepared from log-phase cell suspensions and exhibited a final optical density at 600 nm (OD_{600}) of 1.0 (i.e., approximately 1.0×10^9 cells/ml). Bioreactors were incubated at

28 °C under agitation (150 rpm). Three-milliliter culture solution was collected periodically for analyses. Control experiments were carried out with noninoculated flasks or flasks inoculated with the bacteria but without heavy metals. The heavy metal concentrations and OD_{600} in the samples were determined with the methods mentioned above. The experiments were done in triplicate.

Multi-heavy metal system

The ability of strain LRE07 to adsorb heavy metal in multi-ions system was investigated by inoculating log-phase cells with simulated wastewater comprising of 100 ml of liquid LB medium and multi-heavy metal ions (Cd^{2+}, Cr^{6+}, Cu^{2+}, Pb^{2+}, and Zn^{2+}). Bioreactors were incubated at 28 °C under agitation (150 rpm). Samples were taken at initial and stationary phases of growth (48 h after inoculation). Noninoculated flasks were tested simultaneously as control. The heavy metal concentrations and pH value in the samples were determined with the methods mentioned above. Experiments were conducted in triplicate.

Results

A carmine-pigmented bacterium, named as strain LRE07, was selected from the root of cadmium hyperaccumulator *S. nigrum* L. (cadmium content is 187.84 mg/kg DW). The result of surface sterilization protocol in removing epiphytic microorganism suggested that LRE07 was an endophyte. The bacterial strain was propagated routinely on LB solid medium at 28 °C. Standard staining procedures and microscopic observations revealed that it was a Gram-negative, nonsporulating, rod-shaped bacterium. The isolated strain was identified as *Serratia* sp. by using 16S rDNA analysis. The sequence was deposited in Genbank (*Serratia nematodiphila* LRE07, accession no. GU270854).

Plant growth-promoting characteristics of LRE07

The potential plant growth-promoting ability of endophytic bacterium LRE07 was checked by studying its ability to produce IAA, siderophores, and mineral phosphate solubilization. After being treated with Salkowski reagent for 15 min, an obvious red halo was present on the membrane removed from LB medium in the IAA study. In the siderophore production study, an orange annulus appeared around the red colony of endophytic bacterium LRE07. A clear halo was observed in the LB agar medium supplemented with inorganic phosphate. These observations indicate that the endophytic bacterium LRE07 had the ability to produce IAA and siderophore as well as to solubilize mineral phosphate.

Heavy metal resistance and solubilization of LRE07

The endophytic bacterium LRE07 showed a high degree of resistance to heavy metals, especially to Cu, Cd, and Cr. The MIC of the strain in the liquid LB medium containing heavy metal ions was 10 mM Cu^{2+}, 8 mM Cd^{2+}, 12 mM Cr^{6+}, 4 mM Pb^{2+}, and 5 mM Zn^{2+}, respectively. The order of the toxicity of the metals to strain LRE07 was found to be Pb>Zn>Cd>Cu>Cr.

In heavy metal solubilization test, as shown in Table 1, the concentration of water-soluble Cd was obviously ($p<0.05$) increased and that of Pb was reduced by half after inoculating endophytic bacterium LRE07 for 48 h, while no distinct changes occurred in the controls. Simultaneously, pH value was significantly increased in both Cd and Pb solutions, while the pH values of controls were stable between 6.1 and 6.3.

Heavy metal binding characteristics of LRE07

The endophytic bacterium LRE07 was cultured separately in simulated wastewater comprising of liquid LB medium and two concentrations (20 and 50 mg/l) of five metal ions (Cd^{2+}, Cr^{6+}, Pb^{2+}, Cu^{2+}, and Zn^{2+}). Bacterial biomass (monitored by the OD_{600}) grown in LB medium showed typical growth curves with similar lag phases in the presence of heavy metals (Figs. 1 and 2 and Figs. S1, S2, and S3). However, the specific growth rates (μ_{max}) were different from those of the controls without heavy metals. In addition, the bacterial biomass inoculated 72 h later was decreased in the medium containing heavy metals as compared to the metal-free control, reducing from 2.37 to the value ranging from 2.35 to 1.86. The heavy metals affected the kinetic parameters of endophytic bacterium LRE07, and their inhibitory influences on μ_{max} and biomass were dependent upon the metal concentrations (Figs. 1 and 2 and Figs. S1, S2, and S3).

Based on the results obtained in the detoxification assays, the heavy metals removal tests can be divided into two groups. (1) Cd^{2+} and Zn^{2+} were bounded partially by endophytic bacterium LRE07 growing cells within 72 h from treating simulated wastewater (Figs. 1 and 2). The specific detoxification rates observed were 65.6±1.5%, 47.6±3.0%, 34.2±1.3%, and 36.8±2.8% for the initial dose for 20 mg/l Cd^{2+}, 50 mg/l Cd^{2+}, 20 mg/l Zn^{2+}, and 50 mg/l Zn^{2+}, respectively. Mass balance for Cd^{2+} and Zn^{2+} of two concentrations (20 and 50 mg/l) is presented in Table 2. The removal of Zn^{2+} occurred 40 h after inoculation, while Cd ions were bounded with the growth of the strain. (2) The second group consisted of three heavy metal ions, Cu^{2+}, Cr^{6+}, and Pb^{2+}, in which no significant sorptions of the metals were observed within 72 h.

Table 1 Effect of endophytic bacterium LRE07 on solubilization of heavy metals (Pb and Cd) and pH in the solutions

Time (h)	Cd				Pb			
	Concentrations, μg/l		pH		Concentrations, μg/l		pH	
	Cells	Control	Cells	Control	Cells	Control	Cells	Control
0	314.6±15.3	315.7	6.2±0.3	6.2	737.2±78.1	750.5	6.1±0.2	6.2
48	840.7±21.1	342.4	8.5±0.2	6.2	303.4±51.3	786.7	8.6±0.1	6.2

Results are presented as means± standard deviations

To determine whether the selective metal-binding character of endophytic bacterium LRE07 was affected by competing ions, the strain was grown on LB medium supplemented with five metal ions (Table 3). An analogous heavy metal adsorption character of strain LRE07 was observed in multi-ions system. The concentrations of Cd^{2+} and Zn^{2+} in simulated wastewater were obviously decreased 48 h after incubation, while minute decrease was detected for copper and chromium (Table 3). However, the lead concentration was reduced evidently, differing from the phenomena observed in single heavy metal ions. As shown in Table 3, the concentrations of cadmium were decreased by 40.28±2.16%, lead 32.80±0.25%, and zinc 24.28±0.15% after incubating LRE07 in the metal mixture for 48 h.

Discussion

Endophytes are bacteria inhabiting within plant tissues in contrast to rhizospheric bacteria living on or around the plant surface. Several mutually beneficial effects exist in complementary niches of endophyte and its host (Lodewyckx et al. 2002). Recently, attentions have been attracted to the role of endophytic bacteria in phytoremediation. With the aid of biotechnology and genetic engineering, the relationship between endophytes and plants has been exploited for bioremediation of a wide range of organic pollutants such as 2,4-dichlorophenoxyacetic acid (Germaine et al. 2006), toluene (Taghavi et al. 2005), and mono- and dichlorinated benzoic acids (Siciliano et al. 1998) using natural or engineered endophytic bacteria. However, very few reports today have attempted to address heavy metal remediation using this relationship. Idris et al. (2004) reported that the endophytes of Ni hyperaccumulator *Thlaspi goesingense* showed higher tolerance to nickel concentrations than the rhizospheric isolates. Functional analysis of endophytes revealed several characteristics that potentially support the uptake of the heavy metal by the plant and the reduction of stress symptoms. However, the nickel detoxification and plant growth promotion capacity of these bacteria were not mentioned in this report. Valuable endophytes are necessary for the application of the plant–endophyte symbiotic system in situ bioremediation in a variety of environments. The required endophytes to improve the efficiency of phytoremediation should possess the following properties: (1) facilitate the growth and biomass yield of the host plant, and (2) have metal resistance and sequestration systems to lower

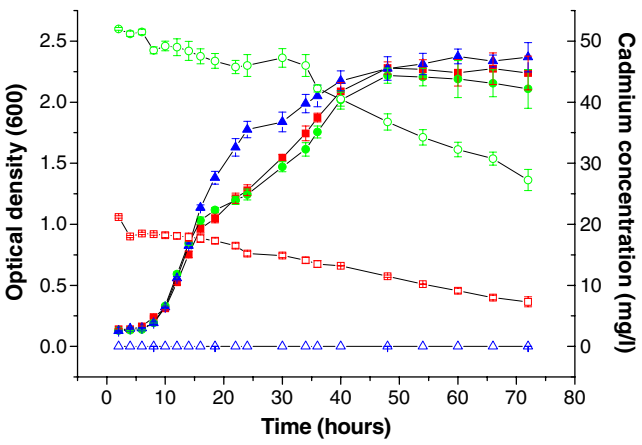

Fig. 1 Growth and cadmium removal by pure cultures of endophytic bacterium LRE07. *Solid symbols* are optical density. *Empty symbols* are the cadmium concentrations in the culture supernatant. *Red squares*, 20 mg/l; *green circles*, 50 mg/l; *blue triangles*, control. Standard deviations are indicated as *bars*. Data are the average of three experiments

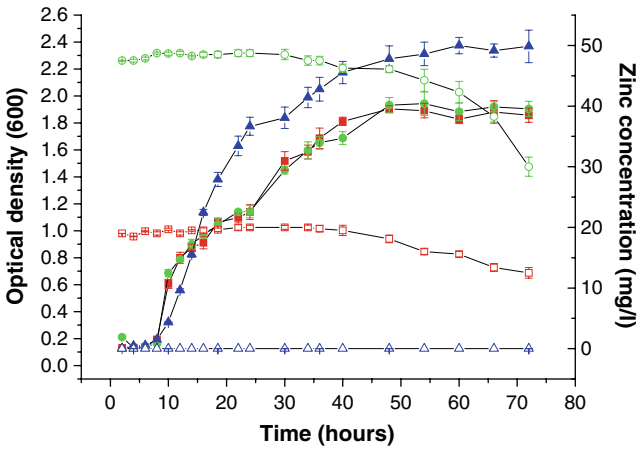

Fig. 2 Growth and zinc removal by pure cultures of endophytic bacterium LRE07. *Solid symbols* are optical density. *Empty symbols* are the zinc concentrations in the culture supernatant. *Red squares*, 20 mg/l; *green circles*, 50 mg/l; *blue triangles*, control. Standard deviations are indicated as *bars*. Data are the average of three experiments

Table 2 Mass balance for two concentrations (20 and 50 mg/l) Cd^{2+} and Zn^{2+}

Solution	Concentrations recovered after treatment with							
	Cd^{2+} (20 mg/l)		Cd^{2+} (50 mg/l)		Zn^{2+} (20 mg/l)		Zn^{2+} (50 mg/l)	
	Cells	Control	Cells	Control	Cells	Control	Cells	Control
Final	7.29±0.82	20.56	27.25±1.73	52.68	12.5±0.87	21.58	30±1.58	48.25
Bacterial cell	14.24±1.54	0.26	23.54±1.22	0.08	8.24±0.52	0.14	16.57±1.02	0.22
Mass balance	21.53±2.32	20.82	50.79±2.95	52.76	20.74±1.39	21.72	46.57±2.60	48.45

Cd^{2+} and Zn^{2+} treated with endophytic bacterium LRE07 growing in LB liquid medium after 72 h of exposure. Control experiments were carried out with noninoculated flasks. Results are presented as means±standard deviations

metal phytotoxicity and affect metal translocation to the shoot of plant.

In this study, a carmine-pigmented bacterium, named as strain LRE07, was selected from the root of cadmium hyperaccumulator *S. nigrum* L. The isolated strain was identified as *Serratia* sp. by using 16S rDNA analysis. It has been shown to be related to *Serratia marcescens*, a widely distributed *Serratia* bacterium frequently associated with water, soil, and foods (Buchanan and Gibbons 1974). Members of the genus *Serratia* are known to be common inhabitants of the rhizosphere of plants and have even been described as endophytic bacteria invading inner tissues of plants (Benhamou et al. 2000; Tan et al. 2001). However, to the best of our knowledge, this is the first time to indicate the *Serratia* sp. bacterium in endophytic association with hyperaccumulator.

The endophytic bacterium LRE07 had the ability to produce IAA and siderophore and could solubilize mineral phosphate. IAA is a plant hormone and widespread among bacteria–plant associated systems. Patten and Glick (2002) reported that bacterial IAA stimulated the development of the root system of the host plant, and Zaidi et al. (2006) reported the contribution of IAA for promoting metal accumulation indirectly by increasing plant biomass. Siderophores are organic molecules that show high affinity for Fe (III) ions, but they can also form complexes with other bivalent heavy metal ions that can be assimilated by the plant (Evers et al. 1989). Siderophore produced by bacteria may help the plant to reduce heavy metal toxicity by increasing the supply of iron to the plant (Burd et al. 2000). Phosphorus is one of the most important plant nutrients, and a large portion of inorganic phosphates applied to soil as fertilizer is rapidly immobilized after application and becomes unavailable to plants (Kuklinsky-Sobral et al. 2004). Therefore, mineral phosphate solubilization of endophytic bacteria is important to enhance phosphate availability to their host plant. The characters mentioned above suggested that endophytic bacterium LRE07 was capable of facilitating the plant growth and the biomass yield.

The bioavailability of heavy metal in the soils is a crucial factor of effective phytoextraction. Studies have evidenced that the plant-associated bacteria can solubilize insoluble heavy metals (Sheng et al. 2008; Jiang et al. 2008). The study showed that the insoluble Cd was mobilized during the growth of endophytic bacterium LRE07, indicating that the endophyte had the specific capacity of solubilizing cadmium and therefore increased the bioavailability of cadmium for its host.

The endophyte was tolerant to high concentrations of heavy metals, especially to Cu, Cd, and Cr. The toxicity order of metals to strain LRE07 was found to be Pb>Zn>

Table 3 Concentration variation in multi-ion system treated with pre- and post-addition of endophytic bacterium LRE07

Heavy metal ions	Pre-addition of LRE07		Post-addition of LRE07		Detoxification rates, %
	Cells, mg/l	Control, mg/l	Cells, mg/l	Control, mg/l	
Cr^{6+}	10.79±0.03	10.65	10.58±0.14	10.82	2.24±1.32
Cu^{2+}	10.32±0.02	10.35	10.14±0.04	10.38	2.33±0.34
Zn^{2+}	9.74±0.1	9.75	7.38±0.02	9.74	24.26±0.15
Pb^{2+}	9.98±0.02	10.0	6.73±0.03	10.02	32.80±0.25
Cd^{2+}	10.01±0.11	9.85	5.94±0.22	9.95	40.28±2.16

Multi-ions system was treated with endophytic bacterium LRE07 growing in LB liquid medium. Detoxification rate is expressed as a percentage of the initial dose. Control experiments were carried out with noninoculated flasks. Results are presented as means±standard deviations

Cd>Cu>Cr. This result was different from the studies screening multi-resistant bacteria from polluted soil (Vullo et al. 2008), where cadmium is the most toxic metal for strains. This phenomenon maybe related to the colonized environment of endophytic bacterium LRE07. The endophyte was inhabited in the root interior of cadmium hyperaccumulator *S. nigrum* L. (cadmium bioaccumulation capacity is above 100 mg/kg DW (Xiao et al. 2010)), where the selective pressure of plants had its maximum effect on the bacterial populations (Siciliano et al. 2001).

Concerning heavy metal detoxification, the endophytic bacterium LRE07 was able to bind heavy metals selectively in single- or multi-metal system. The strain bound over 65% of cadmium and 35% of zinc in its growing cells from single metal solutions 72 h after inoculation, while nearly no sorption of copper, lead, or chromium was observed (Figs. 1 and 2 and Figs. S1, S2, and S3). The declined removal efficiencies of cadmium and zinc in multi-heavy metal system may be the result of ion competition. The binding mechanism of cadmium and zinc in LRE07 was different. The removal of zinc occurred mainly 40 h after inoculation, which can be mainly attributed to the increasing biomass and some metabolism functionings with the capacity of sequestrating heavy metals. However, the cadmium ions were bound with the strain growing in the wastewater, which indicated that bioaccumulation of LRE07 was responsible for cadmium decrease. The process of Cd^{2+} binding in LRE07 may play an important role in the bioaccumulation of its host plant. Because cadmium is not an essential trace element for organisms, the selective adsorption of endophytic LRE07 may be the result of plant selective influence on the endophytes. Plants could recruit bacteria that contain genotypes specific for toxicant degradation into the rhizosphere and root interior, and this selection should be contaminant specific (Siciliano et al. 2001). Hyperaccumulators accumulate huge amounts of heavy metals and can therefore provide a specific environment for screening endophytic bacteria that could be adapted to survive in high metal concentrations. Endophytic bacteria living in hyperaccumulators may transport, transform, or deposit heavy metal ions during the process of accumulating metals in inner tissues of plants.

In conclusion, the endophytic bacterium LRE07 isolated from cadmium hyperaccumulator *S. nigrum* L. tissues was capable of facilitating plant growth and biomass yield. Furthermore, the characterization studies showed that the isolate could resist high concentrations of heavy metals and bind metals selectively. These observations indicate the great potential role of the endophytic bacterium LRE07 for cadmium phytoremediation. Further work will address the effect of selected bacterium on plant growth and the uptake of heavy metals by the plant as well as the mechanisms involved.

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