

Feasibility of enhanced phytoextraction of Zn contaminated soil with Zn mobilizing and plant growth promoting endophytic bacteria

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Received 21 July 2012; accepted 27 November 2012

Abstract: Three bacterial endophytes of *Sedum alfredii*, VI₈L₂, II₈L₄ and VI₈R₂, were examined for promoting soil Zn bioavailability and Zn accumulation in *S. alfredii*. Results showed that three strains were re-introduced into *S. alfredii* rhizosphere soils under Zn stress and resulted in better plant growth, as roots biomass increased from 80% to 525% and shoot biomass from 11% to 47% compared with the uninoculated ones. Strains IV₈L₂, II₈L₄ and IV₈R₂ significantly increased shoot and root Zn concentrations in the ZnCO₃ contaminated soil. Inoculation with strain IV₈L₂ resulted in 44% and 39% higher shoot and root Zn concentrations, while strain IV₈R₂ significantly decreased shoot Zn concentration in the Zn₃(PO₄)₂ contaminated soils. In the aged contaminated soil, isolates IV₈L₂, II₈L₄ and IV₈R₂ significantly increased root Zn concentration, but decreased shoot Zn concentration of *Sedum alfredii*. It suggested that endophytes might be used for enhancing phytoextraction efficiency.

Key words: endophytic bacteria; Zn; bioavailability; phytoremediation; *Sedum alfredii*

1 Introduction

Zinc is the second most abundant transition metal in organisms after iron (Fe), and the only metal presented in all six enzyme classes (oxidoreductases, transferases, hydrolases, lyases, isomerases) [1]. Though Zn toxicity in crops is far less widespread than Zn deficiency, Zn toxicity occurs in soils contaminated by mining and smelting activities, in agricultural soils treated with sewage sludge, and in urban soils enriched by anthropogenic inputs of Zn [2]. Phytoextraction, using metal-accumulating plants to transport and concentrate heavy metal from the soil into their harvestable biomass, has been proposed as an environmentally friendly and low-input remediation technique [3,4]. Current literature suggests that metal bioavailability in soils is decisive for the success of phytoextraction in field [5]. In soils, metals exist as a variety of chemical species and the fraction of metals in soil solution, consisting of free hydrated ions, water-soluble organic and inorganic

complexes and metals sorbed on dissolved organic matter are the most bioavailable form [6]. Unfortunately, low bioavailability is a major factor limiting phytoextraction efficiency in field, because only a very small portion of heavy metal is present in soil solution or exchangeable from soil colloids [7].

Heavy metal-resistant bacteria are ubiquitous in the environment, and their frequency is often increased in contaminated environment. Hyperaccumulators accumulate huge amounts of heavy metals and provide a specific environment for bacterial endophytes, which have to tolerate high concentrations of certain heavy metal [8]. Many metal-resistant endophytic bacteria have been isolated from various hyperaccumulators such as *Alyssum bertolonii*, *Thlaspi caerulescens*, *Thlaspi goesingense* and *Nicotiana tabacum* [8–11]. Bacteria have developed different heavy metal tolerance mechanisms involving exclusion, biosorption, precipitation or bioaccumulation both in external and intracellular spaces. These biochemical processes can influence the mobility and bioavailability of heavy metal

in soils [12]. Some studies demonstrated that certain metal resistant bacteria increased heavy metal bioavailability through acidification, producing iron chelators, siderophores, organic acids, or mobilizing metal phosphates [13,14]. Besides their role in changing metal bioavailability, some bacterial endophytes can promote plant growth by similar mechanisms as plant growth promoting rhizobacteria (PGPR), including nitrogen fixation, phosphate solubilization, IAA production, and production of siderophore. Further, 1-aminocyclopropane-1-carboxylate (ACC) deaminase producing bacteria play an important role in the alleviation of heavy metal stress in plants [9,11,15,16]. Therefore, the efficiency of phytoextraction can be enhanced by careful application of such microbes possessing the ability of increasing soil metal bioavailability and promoting plant growth. In this study, the major aims of this study were to evaluate the ability of three Zn-mobilizing endophytic bacteria for enhancing plant growth and Zn uptakes in hyperaccumulator *sedum alfredii* (*S. alfredii*) for improving the efficiency of phytoremediation of Zn-contaminated soils.

2 Experimental

2.1 Soil characterization and preparation

Two artificially Zn contaminated soils and one aged contaminated soil were used in this experiment. The artificially ZnCO_3 and $\text{Zn}_3(\text{PO}_4)_2$ contaminated soils were prepared as follows: A clean soil was collected from the farm of South China Agricultural University. The basic properties of the soil samples were pH (1:2.5 w/v water) 5.30, organic matter of 90.7 g/kg and total Zn of 74.3 mg/kg. Fine powder of ZnCO_3 or $\text{Zn}_3(\text{PO}_4)_2$ was mixed thoroughly with 10 kg soil to given 500 mg/kg of Zn and incubated at room temperature for 2 months for metal stabilization. The aged contaminated paddy soil was contaminated with Zn, Cd, Cu and Pb due to surface irrigation with the wastewater of Dabaoshan mine in

Guangdong Province of China. The basic properties of the aged contaminated soil samples were pH (1:2.5 w/v water) 3.68, total Zn of 332 mg/kg, total Cd of 0.54 mg/kg, total Cu of 407 mg/kg and total Pb of 490 mg/kg. Soil samples were air-dried, ground, and passed through a 2 mm sieve for the bacterial solubilization of soil Zn experiment, or through a 5 mm-sieve for the pot experiment.

2.2 Bacterial strains and preparation of inoculums

Endophytic bacteria VI_8L_2 , II_8L_4 and VI_8R_2 , identified as *Pseudomonas veronii*, *Pseudomonas fluorescens* and *Pseudomonas fluorescens* respectively, were isolated from the leaves and roots of Zn/Cd hyperaccumulator *S. alfredii* previously described by LONG et al [17]. Strains VI_8L_2 , II_8L_4 and VI_8R_2 were the best at solubilizing ZnCO_3 and $\text{Zn}_3(\text{PO}_4)_2$ compound under *in vitro* condition. Strains VI_8L_2 and VI_8R_2 can tolerate up to 15 mmol/L Zn, and strain II_8L_4 can tolerate to 8 mmol/L Zn in LB solid medium. In addition, strains VI_8L_2 , II_8L_4 and VI_8R_2 show plant growth promoting activity, such as IAA production, solubilization of $\text{Ca}_3(\text{PO}_4)_2$ and production of siderophores (Table 1).

The bacteria were grown in LB broth in a shaking incubator at 30 °C and 160 r/min for 36 h. Immediately prior to inoculation, the bacterial culture was centrifuged at 5000 r/min and 4 °C for 10 min, the pelleted cell was washed with sterilized physiological saline three times. Bacterial inoculation was prepared by re-suspending the pelleted cells in sterile distilled water to get an inoculums density of about $3 \times 10^8 \text{ mL}^{-1}$.

2.3 Bacterial solubilization of soil Zn

Bacteria were cultured in SMS medium at 28 °C for 48 h, then centrifuged (10000 r/min) at 4 °C for 5 min. The supernatant including the bacterial metabolite was used to extract Zn from the three tested soils, using sterile SMS medium and deionized water as control. 10 mL cell supernatant, SMS medium or water was

Table 1 Endophytic bacteria used in this study

Strain	Origin	Closest described relative	Accession No.	bp	Similarity ^a / %	Zn tolerance level ^b / (mmol·L ⁻¹)	IAA production ^c / (μg·mL ⁻¹)	P solubilization ^d / mm	Siderophores production ^e / mm	Solubilization zone ^f /mm	
										ZnCO ₃	Zn ₃ (PO ₄) ₂
IV_8L_2	Leave	<i>Pseudomonas veronii</i>	AB494445.1	1451	100%	15	30.2 ± 1.9	3.8 ± 0.7	8.3 ± 2.1	7	8
II_8L_4	Leave	<i>Pseudomonas fluorescens</i>	EF602564.1	1480	99%	8	32.2 ± 6.1	11.8 ± 1.0	3.3 ± 1.5	18	11
IV_8R_2	Root	<i>Pseudomonas fluorescens</i>	AF094725.1	1461	99%	15	45.6 ± 5.6	3.9 ± 0.1	5.6 ± 1.1	17	3

^a Similarity is at the nucleotide level; ^b The data are reported as the maximal tolerable concentration of ZnSO_4 in the LB agar plates; ^c Indole acetic acid concentration in the liquid growth medium after bacteria growth of 2 d; ^d The soluble P in the liquid growth medium after bacteria growth of 7 d; ^e The clear zone size formed by solubilization of insoluble phosphate on Pikovskaya's agar plates; ^f The clear zone size formed by solubilization of insoluble ZnCO_3 or $\text{Zn}_3(\text{PO}_4)_2$ on SMS agar plates.

added to 2 g soils. Soil suspension was vibrated at 25 °C for 2 h, then centrifuged at 4000 r/min for 15 min. Zinc concentrations in the extracted solutions were determined by AAS.

Next, batch studies were carried out to evaluate the effect of bacterial inoculation on soil Zn availability. Bacterial inoculums of 0.8 mL (ca. 3×10^8 mL⁻¹) were added to 2 g sterilized soils (steamed at 100 °C for 1 h on three consecutive days) in the 50 mL centrifugal tube, using sterilized water as a control. Three replicates were used for each treatment. All tubes were weighed and placed on an incubator at 30 °C. After 3 weeks, the tubes were again weighed to compensate for evaporation of water. 5 mL of 0.01 mol/L CaCl₂ was added to each tube to extract the soil water-soluble (i.e. labile and bioavailable) Zn [18]. The soil suspensions were shaken at 200 r/min for 2 h, then centrifuged at 4000 r/min for 10 min and filtered. The concentration of Zn in the filtrate was determined by AAS.

2.4 Influence of endophytic bacteria on *S. alfredii* growth and Zn uptake

Pot experiment was used to study the effects of the strains on plant growth and Zn uptake of *S. alfredii* using the above soils. Each pot contained 1.0 kg of sterilized soil (steamed at 100 °C for 1 h on three consecutive days). Three replicates were made for each treatment. Healthy and equal-sized stems of *S. alfredii* were surface sterilized with 0.1% (w/v) HgCl₂ for 2 min, and were subsequently washed with sterilized deionized water 4 times. Two plants were transplanted to each pot.

For inoculation, bacterial suspensions (50 mL/pot) or sterile deionized water (as the control) were sprinkled on the soil surface for 10 d after transplanting. Plants were grown in a glass greenhouse under natural lighting and day/night temperature of 22/18 °C. The soil was moistened with sterilized deionized water and maintained at about 60% of the water holding capacity. After growth of 90 d, shoots were excised approximately 1 cm above the soil surface. The whole soil plus root system was placed onto a clean plastic sheet, and roots were carefully picked up manually. Shoots and roots were carefully washed with tap water and rinsed three times with deionized water. Growth parameters such as fresh mass and dry mass of the plants were measured. The contents of Zn, Fe, N and P in root and shoot tissues were also determined. Root and shoot Zn and Fe concentrations were determined by AAS after dry ashing at 550 °C. Total N and P in root and shoot were measured using automatic gerhardt kjeldahl determination device and vanadium molybdate yellow colorimetric method, respectively, after plant samples were digested with 5 mL concentrated H₂SO₄ and 1 mL

H₂O₂. Soil pH was measured using a digital pH meter in a 1:2.5 suspension of soil-to-water ratio, and the available Zn concentrations in the rhizosphere soil were extracted with 0.01 mol/L CaCl₂ and were analyzed by AAS.

2.5 Statistical analysis

All the values expressed were means \pm S.D. (standard deviation) of the three replicates. Analysis of variance and the Student–Newman–Keuls test ($P < 0.05$) were used to compare treatment means. All the statistical analyses were carried out using SAS 9.0.

3 Results

3.1 Effect of bacteria on soil Zn mobility

The metabolites of the three bacterial isolates strongly enhanced Zn extraction from soils (Fig. 1). The total Zn extracted from the ZnCO₃ contaminated soils by the metabolites of strains VI₈L₂, II₈L₄ and VI₈R₂ were 206%, 141% and 94% higher than the sterile water, and were 134%, 89%, and 49% higher than the axenic SMS broth, respectively. The total Zn extracted from the Zn₃(PO₄)₂ contaminated soils by the metabolites of strains VI₈L₂, II₈L₄ and VI₈R₂ were 229%, 134%, and 77% higher than the sterile water, and were 168%, 91%, and 45% higher than the axenic SMS broth, respectively. The total Zn extracted from the aged contaminated soil by the metabolites of strains IV₈L₂, II₈L₄ and IV₈R₂ increased by 43%, 29%, 9.3% compared with sterile water, and increased by 23%, 12%, 12% compared with the axenic SMS broth, respectively (Fig. 1).

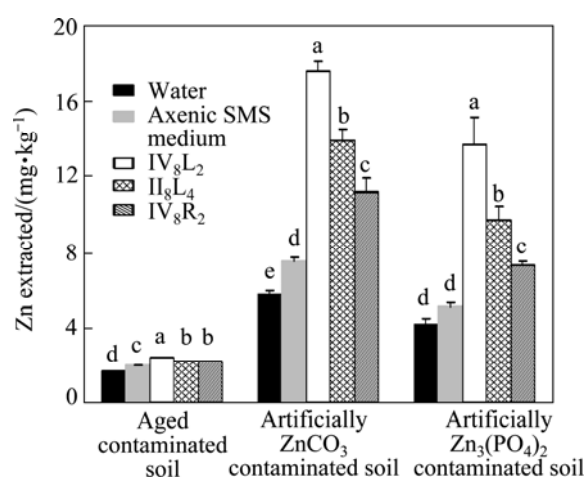


Fig. 1 Ability of bacterial metabolite to extract Zn from artificially ZnCO₃ and Zn₃(PO₄)₂ contaminated soils and aged contaminated soils (Each value is the mean of triplicates. Error bars represent standard deviation. For the same soil, significant differences according to least significant difference at $P=0.05$ levels are indicated by different letters a–e)

Re-inoculation strains IV₈L₂, II₈L₄ and IV₈R₂ to the ZnCO₃ contaminated soils increased the CaCl₂-extractable Zn concentrations by 51%, 14% and 81% compared with the control after 21 d, respectively. Strains IV₈L₂ and II₈L₄ significantly increased the CaCl₂-extractable Zn concentrations in the Zn₃(PO₄)₂ contaminated soils, while strain IV₈R₂ decreased the CaCl₂-extractable Zn concentration. However, CaCl₂-extractable Zn concentrations in the aged contaminated soils inoculated with strains IV₈L₂, II₈L₄ and IV₈R₂ were remarkably lower than the non-inoculated control (Table 2).

3.2 *S. alfredii* growth

S. alfredii grew healthy in the ZnCO₃ and Zn₃(PO₄)₂ contaminated soils, but was severely inhibited in the aged contaminated soil due to adverse acid soil pH condition. Inoculation with strains IV₈L₂, II₈L₄ and IV₈R₂ all promoted the growth of *S. alfredii* in the aged contaminated soil and the ZnCO₃ contaminated soil. Strain IV₈R₂ was the most effective strain for plant

growth promotion, shoot and root biomasses of *S. alfredii* growing on the aged contaminated soils and the artificially ZnCO₃ contaminated soils were 73%, 89% and 46%, 524% higher than the non-inoculated plants, respectively (Table 3). In the Zn₃(PO₄)₂ contaminated soils, strains IV₈L₂, II₈L₄ and IV₈R₂ significantly increased shoot biomass, but did not stimulate root growth (Table 3).

The re-inoculation with strains IV₈L₂, II₈L₄ and IV₈R₂ affected Fe, P and N uptake by *S. alfredii* (Table 3). In the ZnCO₃ contaminated soil, root Fe concentrations of *S. alfredii* inoculated with strains IV₈L₂, II₈L₄ and IV₈R₂ increased by 27%, 69% and 67% compared with the non-inoculated control, respectively. However, those strains decreased shoot N concentrations. In the Zn₃(PO₄)₂ contaminated soils, strains IV₈L₂, IV₈L₄ and IV₈R₂ significantly increased shoot Fe and P concentrations, but had no significant effects on root Fe concentrations and shoot N concentrations. In the aged contaminated soils, shoot and root Fe concentrations, shoot N and P concentrations in *S. alfredii* inoculated

Table 2 Effects of bacterial inoculation on 0.01 mol/L CaCl₂-extractable Zn concentration in soil

Bacteria strain	Artificially ZnCO ₃ contaminated soil		Artificially Zn ₃ (PO ₄) ₂ contaminated soil		Aged contaminated soil	
	Zn concentration/(mg·kg ⁻¹)	Bacterial effect [#] /%	Zn concentration/(mg·kg ⁻¹)	Bacterial effect/%	Zn concentration/(mg·kg ⁻¹)	Bacterial effect/%
Control	0.57±0.02 ^d		0.62±0.02 ^c		10.24±0.10 ^a	
VI ₈ L ₂	0.86±0.02 ^b	50.9	2.34±0.14 ^a	277	6.97±0.24 ^c	-31.9
II ₈ L ₄	0.65±0.02 ^c	14.0	0.96±0.04 ^b	54.8	8.93±0.21 ^b	-12.8
VI ₈ R ₂	1.03±0.04 ^a	80.7	0.40±0.04 ^d	-35.5	9.42±0.15 ^b	-8.0

Each value is the mean of triplicates±standard deviation. For the same soil type, significant differences according to least significant difference at *P*=0.05 levels are indicated by different letters. # means the effect of bacterial inoculation on the 0.01 mol/L CaCl₂ extractable Zn concentration in soil compared with the inoculated control.

Table 3 Effects of inoculation with Zn solubilizing bacteria on shoot and root dry matter, Fe, N and P uptake by *S. alfredii*

Soil type	Treatment	Dry mass/(g·pot ⁻¹)		Fe concentration/(g·kg ⁻¹)		Shoot N concentration/(g·kg ⁻¹)	Shoot P concentration/(mg·kg ⁻¹)
		Shoot	Root	Shoot	Root		
Artificially ZnCO ₃ contaminated soil	CK	2.94±0.46 ^b	0.08±0.01 ^d	0.140±0.007 ^a	3.018±0.031 ^c	4.095±0.652 ^a	13.05±0.707 ^b
	IV ₈ L ₂	4.09±0.28 ^a	0.30±0.00 ^c	0.134±0.011 ^a	3.838±0.251 ^b	3.384±0.440 ^{ab}	12.78±1.160 ^b
	II ₈ L ₄	4.03±0.14 ^a	0.36±0.02 ^b	0.099±0.009 ^b	5.102±0.454 ^a	3.161±0.140 ^b	13.20±0.139 ^b
	IV ₈ R ₂	4.31±0.12 ^a	0.50±0.03 ^a	0.148±0.012 ^a	4.894±0.548 ^a	3.221±0.391 ^b	15.33±1.079 ^a
Artificially Zn ₃ (PO ₄) ₂ contaminated soil	CK	3.24±0.06 ^b	0.28±0.02 ^{ab}	0.110±0.005 ^b	4.338±0.373 ^a	2.974±0.273 ^a	14.91±1.223 ^b
	IV ₈ L ₂	3.73±0.10 ^a	0.26±0.01 ^{bc}	0.144±0.020 ^a	4.392±0.660 ^a	3.270±0.361 ^a	19.34±0.826 ^a
	II ₈ L ₄	3.98±0.11 ^a	0.29±0.00 ^a	0.138±0.013 ^a	4.456±0.313 ^a	3.063±0.044 ^a	21.97±1.194 ^a
	IV ₈ R ₂	3.80±0.17 ^a	0.23±0.01 ^c	0.144±0.018 ^a	5.024±0.472 ^a	3.101±0.277 ^a	20.50±0.621 ^a
Aged contaminated soil	CK	0.53±0.02 ^c	0.10±0.01 ^b	0.205±0.029 ^c	7.375±0.948 ^c	4.669±0.288 ^b	1.341±0.141 ^c
	IV ₈ L ₂	0.71±0.00 ^b	0.19±0.00 ^a	0.484±0.083 ^a	11.75±1.495 ^a	5.984±0.956 ^a	4.310±0.707 ^b
	II ₈ L ₄	0.71±0.08 ^b	0.10±0.02 ^b	0.375±0.041 ^b	9.133±0.439 ^b	5.404±0.167 ^{ab}	4.226±0.802 ^b
	IV ₈ R ₂	0.92±0.05 ^a	0.18±0.00 ^a	0.325±0.097 ^b	10.41±1.285 ^{ab}	6.331±0.326 ^a	5.217±0.505 ^a

All the values are mean of triplicates ± SD. For the same soil type, significant differences according to least significant difference at *P*=0.05 levels are indicated by different letters.

with strains IV₈L₂, II₈L₄ and IV₈R₂ were all greatly higher compared with the control, strain IV₈L₂ was the best in stimulating Fe uptake and strain IV₈R₂ was the best in stimulating N and P uptake.

3.3 Mobilization of soil Zn to *S. alfredii*

Re-inoculation strains IV₈L₂, II₈L₄ and IV₈R₂ to the ZnCO₃ contaminated soils increased shoot Zn concentrations by 52%, 20% and 47% and root Zn concentrations by 85%, 64% and 74%, respectively, compared with the uninoculated plants. The maximum shoot (10.7 g/kg) and root Zn (2.4 g/kg) concentrations were observed in plant inoculated with strain IV₈L₂ (Fig. 2). In the Zn₃(PO₄)₂ contaminated soils, shoot and root Zn concentrations in *S. alfredii* inoculated with strain IV₈L₂ were 45% and 39% higher than the control, respectively. Strain II₈L₄ had not great effect on shoot and root Zn concentration. However, strain IV₈R₂ significantly reduced shoot Zn concentration (Fig. 2). In the aged contaminated soils, strains IV₈L₂, II₈L₄ and IV₈R₂ significantly increased root Zn concentration by 82%, 99%, and 63%, respectively, but decreased shoot Zn concentrations.

In addition, in the artificially ZnCO₃ contaminated

soils inoculated with strains IV₈L₂, II₈L₄ and IV₈R₂, CaCl₂-extractable Zn concentrations in rhizosphere soils of *S. alfredii* significantly increased, compared with the uninoculated control. This was associated with a drop of pH in the rhizosphere soil (Table 4). In the artificially Zn₃(PO₄)₂ contaminated soils, inoculation with strains IV₈L₂, II₈L₄ and IV₈R₂ resulted in significant decrease of soil pH, isolate IV₈L₂ increased CaCl₂-extractable Zn concentrations by 1.5-fold compared with the uninoculated rhizosphere soils, but isolate II₈L₄ or IV₈R₂ had no significant effects on CaCl₂-extractable Zn concentrations (Table 4). In the aged contaminated soils, CaCl₂-extractable Zn concentrations in the rhizosphere of *S. alfredii* inoculated with strains IV₈L₂, II₈L₄ and IV₈R₂ were greatly lower than that of the control, but there was no significant difference in pH between bacteria inoculated and uninoculated soils (Table 4).

4 Discussion

Recently, endophytic bacteria associated with heavy metal hyperaccumulators have attracted attention of several investigators due to their potential applications for assisting phytoremediation of heavy metal

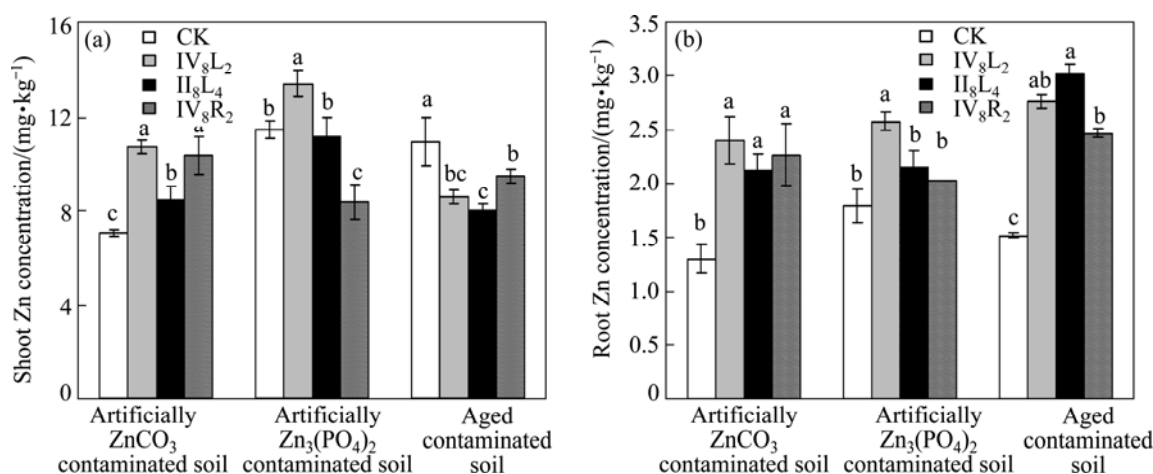


Fig. 2 Effects of inoculation with Zn solubilizing bacteria on Zn uptake by *S. alfredii* (Each value is the mean of triplicates. Error bars represent standard deviation. For the same soil type, significant differences according to least significant difference at $P < 0.05$ levels are indicated by different letters)

Table 4 Effects of bacterial inoculation on soil pH and 0.01 mol/L CaCl₂ extractable Zn concentration in rhizosphere soils of *Sedum alfredii*

Bacteria strain	Artificially ZnCO ₃ contaminated soil		Artificially Zn ₃ (PO ₄) ₂ contaminated soil		Aged contaminated soil	
	pH	CaCl ₂ extractable Zn concentration/(mg·kg ⁻¹)	pH	CaCl ₂ extractable Zn concentration/(mg·kg ⁻¹)	pH	CaCl ₂ extractable Zn concentration/(mg·kg ⁻¹)
Control	7.61±0.01 ^a	0.26±0.03 ^b	7.36±0.06 ^a	0.42±0.06 ^b	3.68±0.00 ^b	3.35±0.18 ^a
IV ₈ L ₂	7.49±0.03 ^b	0.38±0.01 ^a	7.16±0.06 ^b	1.09±0.10 ^a	3.68±0.01 ^b	2.63±0.09 ^b
II ₈ L ₄	7.58±0.01 ^{ab}	0.41±0.04 ^a	7.15±0.06 ^b	0.31±0.04 ^b	3.65±0.02 ^b	2.26±0.06 ^b
VI ₈ R ₂	7.36±0.06 ^c	0.39±0.03 ^a	7.06±0.03 ^b	0.29±0.02 ^b	3.80±0.03 ^a	2.21±0.30 ^b

Each value is the mean of triplicates±standard deviation. For the same soil type, significant differences according to least significant difference at $P = 0.05$ levels are indicated by different letters.

contaminated soil [9,18,19]. Pot experiment found that the inoculation of bacteria IV₈L₂, II₈L₄ and IV₈R₂ promoted the growth of *S. alfredii*, especially on the aged contaminated soil and the artificially ZnCO₃ contaminated soil (Table 3). Generally, endophytic bacteria can benefit plant growth by phosphate solubilization, IAA or siderophore production, nitrogen fixation, and prevention of the growth or activity of plant pathogens [4,5]. The increase in plant growth caused by these three endophytic bacteria may be attributed to the production of IAA, increasing P and Fe supply, because strains IV₈L₂, II₈L₄ and IV₈R₂ all had the intrinsic ability of production of IAA and siderophore, and solubilization of phosphate (Table 1). Furthermore, pot experiment results proved that strains IV₈L₂, II₈L₄ and IV₈R₂ significantly enhanced the assimilation of Fe, P and N by *S. alfredii*. For example, strains IV₈L₂, II₈L₄ and IV₈R₂ increased root Fe concentrations of *S. alfredii* growing on the artificially ZnCO₃ contaminated soil by 27%, 69% and 67%, respectively; shoot Fe and P concentrations in *S. alfredii* growing on the artificially Zn₃(PO₄)₂ contaminated soils inoculated with strains IV₈L₂, II₈L₄ and IV₈R₂ were significantly higher than the non-inoculated plant; shoot and root Fe concentrations, shoot N and P concentrations in *S. alfredii* inoculated with strains IV₈L₂, II₈L₄ and IV₈R₂ were greatly higher than that of the control, when grown on the extremely acid aged contaminated soils (Table 3). This result is in agreement with other studies, which have also proved that plant growth promoting rhizosphere or endophytic bacteria can assist plant establishment on contaminated soils by improving nutrient uptake by plant. For example, BARZANTI et al [10] reported that 83% of bacterial isolates recovered from *Alyssum bertolonii* could produce siderophores and promote the plant growth under Ni stress. The inoculation with metal-resistant bacterial *Bacillus weihenstephanensis* SM3 increased the fresh mass and dry mass of *Helianthus annuus* by 47% and 23% in Ni contaminated soil and by 35% and 16% in Cu contaminated soil, respectively, compared with non-inoculated plants [4]. The increase in plant growth caused by strain SM3 may be attributed to the solubilization of phosphate and production of IAA. SHENG et al [14] reported that Pb-resistant endophytic bacteria *Pseudomonas fluorescens* G10 and *Microbacterium* sp. G1, which can produce IAA and siderophores, not only increased root elongation of inoculated rape seedlings, but also increased root dry mass by 23%–37% and shoot dry mass by 12%–29%.

Low bioavailability of soil metals is one limiting factor for the success of phytoextraction in field [5]. Soil metal bioavailability is mediated by many interacting factors associated with soil properties, metal characteristics and effects of plant roots and the

associated microbial community [8]. Although the uptake and accumulation of metals by plants can be enhanced by addition of chemical chelates, such as EDTA, EDDS, TNT, EDGA and citric acid, these expensive compounds can increase the metal-leaching risk and impart negative effects on plant growth or soil structure [20]. Certain soil microorganisms can increase solubility and change speciation of metals through producing organic ligands, exuding metabolites (e.g. organic acids, microbial siderophores), reducing soil pH, and/or solubilizing metal-phosphates [21]. The present study shows that the bacterial metabolites of strains IV₈L₂, II₈L₄ and IV₈R₂ extracted much higher Zn from the artificially ZnCO₃ and Zn₃(PO₄)₂ contaminated soils and the aged contaminated soils than those extracted by axenic SMS broth and water (Fig. 1). Production of H⁺ and organic acids by rhizosphere organisms appear to be the most significant mechanism for metal mobilization. For example, SARAVANAN et al [13] reported the production of 5-ketogluconic acid, a major gluconic acid derivative product that aids the solubilization of different Zn compounds by endophyte *G. diazotrophicus* under in vitro conditions. MAJEWSKA et al [22] found that increases in microbially-produced citric acid, acetic acid, catechol siderophores, and Fe-chelators may have contributed to cadmium mobilization within soils, decreasing the pH from 6.5 to 5 after 48 h. The production of oxalic acid, tartaric acid, formic acid and acetic acid had a significant correlation ($P < 0.01$) with the concentrations of Cd and Zn mobilized from CdCO₃ and ZnO by rhizosphere bacteria associated with a Cd/Zn hyperaccumulator *S. alfredii* [21].

We also found that the CaCl₂-extractable Zn concentration in the artificially ZnCO₃ contaminated soil inoculated with isolates IV₈L₂, II₈L₄ and IV₈R₂ was significant higher than that of the uninoculated control, and inoculation with strains IV₈L₂ significantly increased the CaCl₂-extractable Zn concentration in the artificially Zn₃(PO₄)₂ contaminated soil (Table 2, Table 4). These observations are in agreement with other research works. For example, ABOU-SHANAB et al [23] reported that the concentration of extractable Ni was increased from a high-Ni soil of 2.2–2.6 mg/kg when the soil was inoculated with *Microbacterium arabinogalactanolyticum* AY509224, which has the ability of producing acid and siderophore and solubilizing inorganic phosphate. These results indicate that the activity of soil bacteria would likely have a significant effect on increasing the bioavailability of metals in soils. In the pot experiment study, we also found that inoculation with strains IV₈L₂, II₈L₄ and IV₈R₂ increased shoot Zn concentrations by 20%–52% and root Zn concentrations by 64%–85% in *S. alfredii* grown on the artificially ZnCO₃ contaminated soils, respectively;

strain IV₈L₂ increased shoot concentration by 45% and root Zn concentration by 39% in *S. alfredii* grown on the artificially Zn₃(PO₄)₂ contaminated soil; strains IV₈L₂, II₈L₄ and IV₈R₂ increased root Zn concentration of *S. alfredii* grown on the Dabaoshan contaminated soil by 63%–99% (Fig. 3). These promoting heavy metal accumulation effects of bacterial inoculation were reported also by other scientists. For example, WHITING et al [24] reported that the addition of a mixed inoculum of *Microbacterium saperdae*, *Pseudomonas monteilii* and *Enterobacter cancerogenes* to surface-sterilized seeds of *Thlaspi caerulescens* increased the Zn concentration in shoots 2-fold and total Zn accumulation 4-fold compared with non-inoculated controls, respectively. Four Zn-tolerant bacteria (*Bacillus subtilis*, *B. cereus*, *Flavobacterium sp.* and *Pseudomonas aeruginosa*) significantly increased Zn concentrations in the roots and shoots of *O. violaceus* plants compared with non-inoculated plants [25].

However, we found that the uninoculated and inoculated *S. alfredii* all grew very poor on the acid aged contaminated soil, even though the bacterial inoculation with strains IV₈L₂, II₈L₄ and IV₈R₂ increased shoot and root biomass of *S. alfredii*. Mining wastewater polluted soils are often difficult for plant establishment and growth due to a combination of factors including metal toxicity, acidic pH and stressed microbial communities [26]. Therefore, how to remediate such acidic heavy metal contaminated soils by combining chemical and bioremediation needs further to investigate.

5 Conclusions

Endophytic bacteria, including IV₈L₂, II₈L₄ and IV₈R₂, can increase soil Zn bioavailability, and their intrinsic property of plant growth promoting might make them one of the most suitable choice for improving phytoremediation of Zn contaminated soils.

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促植物生长内生细菌强化植物修复锌污染土壤

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摘 要: 以超积累植物东南景天为材料, 采用盆栽试验探讨了接种内生菌 VI₈L₂、II₈L₄ 和 VI₈R₂ 对土壤 Zn 有效性、植物生长和吸收积累锌的影响。结果表明, 接种内生菌 VI₈L₂、II₈L₄ 和 VI₈R₂ 能够促进东南景天在 Zn 污染土壤中的生长, 其根系和地上部的生物量分别比对照增加了 80%~525% 和 11%~47%。在人工 ZnCO₃ 污染土壤中, 接种菌株 VI₈L₂、II₈L₄ 和 VI₈R₂ 显著增加了东南景天根系和地上部的 Zn 含量; 在人工 Zn₃(PO₄)₂ 污染土壤中, 菌株 VI₈L₂ 使东南景天地上部和根系的 Zn 含量分别比对照增加了 44% 和 39%, 但是菌株 IV₈R₂ 显著降低了东南景天地上部的 Zn 含量; 在长期被酸性废水污染水稻土壤中, 接种菌株 VI₈L₂、II₈L₄ 和 VI₈R₂ 显著增加了东南景天根系的 Zn 含量, 但降低了地上部的 Zn 含量。这表明金属抗性促生细菌可用于强化植物修复重金属污染土壤。

关键词: 内生菌; 锌; 生物有效性; 植物修复; 东南景天

(Edited by Hua YANG)