Research Paper

Characterization of metal-resistant plant-growth promoting *Bacillus weihenstephanensis* isolated from serpentine soil in Portugal

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A metal-resistant bacterial strain SM3 isolated from a serpentine soil in the north-east of Portugal was characterized as *Bacillus weihenstephanensis* based on the morphological and biochemical characteristics and on the comparative analysis of the partial 16S ribosomal DNA sequence. *Bacillus weihenstephanensis* SM3 showed a high degree of resistance to nickel (1500 mg l^{-1}), copper (500 mg l^{-1}) and zinc (700 mg l^{-1}) and also to antibiotics (ampicillin, penicillin, kanamycin and streptomycin). Strain SM3 has also exhibited the capability of solubilizing phosphate and producing indole-3-acetic acid (IAA) both in the absence and in the presence of metals (Ni, Cu and Zn). A pot experiment was conducted to elucidate the effects of strain SM3 on plant growth and uptake of Ni, Cu or Zn by *Helianthus annuus*. Inoculation with strain SM3 increased the shoot and root biomass of *H. annuus* grown in both non-contaminated and contaminated soil. Furthermore, strain SM3 increased the accumulation of Cu and Zn in the root and shoot systems. A batch experiment was also conducted to assess the metal mobilization potential of strain SM3 in soil. Inoculation with this strain increased the concentrations of water soluble Ni, Cu and Zn in soil. Metal solubilization by this bacterial strain may be an important process to promote the uptake of heavy metals by plants. This study elucidates the multifarious role of strain SM3 in plant growth promotion and its metal mobilizing potential.

Keywords: *Bacillus weihenstephanensis* / Heavy metals / IAA / *Helianthus annuus*

Received: February 26, 2008; accepted: April 28, 2008

DOI 10.1002/jobm.200800073

Introduction

The contamination of soils with heavy metals is a major environmental problem throughout the world. Soils polluted with heavy metals may threaten ecosystems and human health. The remediation of heavy metal-contaminated soils is a challenging task because metals are not easily degraded; the danger they pose is aggravated by their almost indefinite persistence in the environment. Until now, methods used for the remediation of heavy-metal contaminated soils, such as physical separation, acid leaching or electrochemical processes are not suitable for practical applications, because of their high cost and low efficiency. Thus, the development of remediation strategies for heavy metal contaminated soils is necessary. Phytoextraction and phytostabilization of metals in metal-contaminated soils have recently received considerable attention due to their low cost and high efficiency [1, 2]. Certain plants have the ability to accumulate metals of known and unknown biological functions and have been effectively utilized in ameliorating heavy metal contamination in soils. However, heavy metals at elevated levels are generally toxic to most plants, impairing their metabolism and reducing plant growth. The interface between microbes and plant roots (rhizosphere) may have a great influence both on the increase of nutrient uptake and on the decrease of metal toxicity [3]. Therefore, the potential use of alternative methods that exploit rhizosphere microbes to reduce the toxicity of metals to plants has been investigated [4–6]. Metal resistant

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rhizosphere bacteria have an exceptional ability to promote the growth of the host plant by various mechanisms, namely fixation of atmospheric nitrogen, production of siderophores, solubilization of phosphate, or production of plant growth regulators (hormones) [7]. Furthermore, different heavy metal tolerance mechanisms have also been discovered in various microbes [8, 9]: they involve exclusion, active removal, biosorption, and precipitation or bioaccumulation of metals both in external and intracellular spaces. These processes can influence the solubility and the bioavailability of a given metal to the plant, thus modifying its toxic effects. Organisms with metal tolerating ability and plant growth-promoting activities are of practical importance for both the remediation of metal-contaminated environments and for plant growth promotion.

Naturally occurring bacteria in heavy metal-contaminated soils can have acquired resistance against heavy metals [10]. Currently, serpentine areas are considered an interesting model for the study of the evolution of metal-resistant microorganisms that are completely different from those of artificially contaminated soils. Microorganisms present in serpentine soils and their interactions with serpentinophytes have attracted the attention of several researchers due to their potential biotechnological applications on bioremediation [4, 11, 12]. In recent years, new strains and genetic determinants for heavy metal-resistance derived from serpentine soils showed high level of tolerance to Ni and other heavy metals [10, 11]. However, little is known about the plant growth promoting features of serpentine bacterial isolates. Furthermore, reports on the role of metal resistant serpentine isolates on plant growth and plant uptake of metals are scarce. The present study was conducted to (i) isolate and characterize serpentine bacterial strains that are capable of tolerating heavy metals (Ni, Cu and Zn), (ii) screen isolates for auxiliary activities including, solubilization of phosphate, production of indole-3-acetic acid (IAA), mobilization of soil heavy metals and biosorption of heavy metals and (iii) elucidate the effects of inoculating metal-resistant bacteria on plant growth and uptake of Ni, Cu and Zn by *Helianthus annuus* (sunflower).

Materials and methods

Isolation and characterization of bacterial strains

Bacterial strains were isolated from a serpentine site in Bragança, north-east of Portugal, previously described by Freitas *et al.* [13]. For isolation and enumeration of microorganisms, soil samples were serially diluted in sterile distilled water and plated on Luria-Bertani (LB) agar supplemented with 50 mg l^{-1} of heavy metals as $NiCl_2 \cdot 6\ H_2O$, $CuSO_4 \cdot 5\ H_2O$ and $ZnSO_4 \cdot 7\ H_2O$ alone or in combinations. The plates were incubated at 27 °C for 48 h before screening for metal resistant colonies. To check the extent of the resistance, selected bacterial isolates were grown in LB agar containing different concentrations of Ni, Cu or Zn ranging from 100 to 2000 mg l^{-1} [14]. The bacterial strain showing the highest degree of metal resistance was selected and identified as based on morphological and biochemical features. For further characterization, genomic DNA was isolated and the 16S rRNA gene was amplified by PCR using the conserved eubacterial primers pA (5′-AGAGTTTGATCCTGGCTCAG; *Escherichia coli* bases 8–27) and pC5B (5′-TACCTTGTTACGACTT; *E. coli* bases 1507–1492) [15]. Reaction conditions were those described by Branco *et al.* [16]. Each amplification mixture (5 µl) was submitted to electrophoresis in agarose gels (1.5% w/v) in TAE buffer (0.04 M Tris acetate, 0.001 M EDTA) containing 1 mg ml^{-1} (w/v) ethidium bromide. For further sequencing, the amplified DNA was purified using the PCR purification kit (Roche Diagnostics) according to the manufacturer's instructions. Automated sequencing of the purified PCR products was performed using the dRodamina terminator cycle sequencing kit and the ABI 310 DNA Sequencer (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. The partial 16S rDNA sequences obtained were matched to nucleotide sequences present in GenBank using the BLASTn program [17]. For the construction of a phylogenetic tree, sequences of the 12 most closely related organisms with that of *Escherichia coli* were included in the comparison. Bacteria used in the construction of the phylogenetic tree with their GenBank accession numbers included *Bacillus asahii* (AB109209), *B. psychrosaccharolyticus* (AB021195), *B. circulans* (X60613), *B. cereus* (AY138278), *B. thuringiensis* (AY461762), *B. simplex* (X60638), *B. subtilis* (AF545570), *B. anthracis* (AB116124), *B. weihenstephanensis* (AM747231), *B. vulcani* (AJ293805), *B. lentus* (AB021189), *B. galactosidilyticus* (AJ535638) and *E. coli* (J01859). The phylogenetic dendrogram was constructed with the neighbour-joining method using MEGA version 3.1 [18].

Effect of metals on bacterial growth

A culture flask (250 ml) containing 20 ml LB broth supplemented with heavy metals at the concentration of 200 mg l^{-1} (Ni, Cu or Zn) was inoculated with the bacterial isolate in logarithmic growth phase. All the cultures including controls (in triplicate) were incubated at 27 °C for 28 h at 200 rpm. Bacterial growth was

monitored at definite time intervals by measuring the optical density at 600 nm.

Sensitivity to antibiotics

The antibiotic sensitivity of the bacterial strain was determined by the disc diffusion method. The bacterium was grown in LB broth at 27 °C for 24 h. A 0.1 ml fraction from the culture was plated onto LB agar plates. Antibiotic disks were placed on freshly prepared lawns of the bacterial isolate and plates were incubated at 27 °C for 24 h. The diameter of the inhibition zones was measured, and the bacterium was classified as resistant (R), intermediate (I) or susceptible (S). The antibiotics used in the disks were ampicillin (10 μg), tetracycline (30 μg), chloramphenicol (30 μg), penicillin (20 μg), kanamycin (30 μg) and streptomycin (20 μg).

Phosphate solubilization and IAA production

The phosphate solubilizing activity of the bacterial strain was quantitatively assayed in NBRIP medium [19] containing tricalcium phosphate amended with heavy metals at the concentration of 200 mg l^{-1} (Ni, Cu or Zn). The soluble phosphate in the culture supernatant was quantified as detailed by Fiske and Subbarow [20]. For IAA analysis, the bacterium was grown for 96 h in LB broth with L-tryptophan (200 mg l^{-1}) in the presence and absence of heavy metals. Quantitative analysis of IAA was performed by the method of Bric *et al*. [21].

Influence of the bacterial strain on *H. annuus* growth and metal uptake

For pot experiments the soil was collected from the Botanical Gardens, Department of Botany, University of Coimbra, Portugal. The soil was sieved (2 mm) and sterilized by steaming (100 °C for 1 h on three consecutive days). After sterilization the soil was artificially contaminated with aqueous solution of $NiCl_2 \cdot 6\,H_2O$, $CuSO_4 \cdot 5\,H_2O$, or $ZnSO_4 \cdot 7\,H_2O$ to achieve the final concentrations of 200 mg kg^{-1} soil (Ni, Cu or Zn) and left in a greenhouse for a 2 wk period (for metal stabilization). The average pH of soil was near neutral with a value of 6.44. Seeds of *H. annuus* were surface sterilized with 70% alcohol for 30 s and 1.0% NaClO for 10 min and rinsed several times with sterile distilled water. The seeds were allowed to germinate in sterilized non-contaminated soil at 25 °C and a 16/8 day/night regime. For inoculation of the seedlings, bacterial cultures were grown for 18 h, cells harvested by centrifugation (6000 rpm, 10 min), washed twice with sterile distilled water, and resuspended in biological saline (0.85% KCl). The roots of fifteen-day-old seedlings were soaked for 2 h in the bacterial culture (10^9 CFU ml^{-1}) before they were transplanted into plastic pots (eight plants pot^{-1}) containing 300 g of metal contaminated or non-contaminated soil. Each treatment had three replicates. After 15 d the plants were carefully removed from the pots and the root surface was thoroughly cleaned with distilled water. Growth parameters such as plant fresh and dry weight were measured. The accumulation of Ni, Cu and Zn in plants was quantified following the method of Freitas *et al.* [13].

Effect of the bacterial strain on the mobility of soil metals

Batch studies on the effects of the bacterial strain on the mobility of soil metals were carried out using 50 ml scaled polypropylene centrifuge tubes. The sterilized soil was artificially contaminated with Ni, Cu or Zn as detailed in the earlier section. A pure culture of the bacterium was grown in LB broth and placed on a shaker at 200 rpm and 27 °C. After 24 h, optical density (600 nm) was measured and adjusted to 1.5; the cultures were centrifuged at 6000 rpm for 10 min, washed in phosphate buffer (pH 7.0) twice, resuspended, washed in sterile water, recentrifuged, and finally resuspended in 5 ml sterile water. Small aliquots of this final washed bacterial culture (up to 1 ml) were added to 1 g of soil in centrifuge tubes. Sterile water was added to soil as an axenic control. All tubes were weighed, wrapped in brown paper and placed on an orbital shaker at 200 rpm at 27 °C. After 10 d, the tubes were again weighed to compensate for evaporation of water. Ten millilitres of sterile water were added to each tube to extract the soil water soluble heavy metals. The soil suspensions were centrifuged at 7000 rpm for 10 min and filtered. Concentrations of Ni, Cu and Zn in the filtrate were determined using an atomic absorption spectrophotometer.

Biosorption of metals by the bacterial strain

The biosorption study was carried out as described by Hernandez *et al.* [22] with some modifications. The bacterium was grown in 100 ml of LB broth until reaching 1.0 of optical density (600 nm). Cells were harvested by centrifugation at 6000 rpm for 15 min and the bacterial pellet was washed twice with sterile water. The harvested biomass was re-suspended in Eppendorf microtubes containing 1.5 ml of metal solution at the concentration of 200 mg l^{-1} (Ni, Cu or Zn). After incubation at room temperature for 6 h, cells were harvested by centrifugation under the same experimental conditions. The amount of residual metal present in the supernatant was measured by atomic absorption spectrophotometry. Total biosorbed metal values were cal-

culated by taking the difference between metal contents in the supernatant at time zero and at the time of sampling.

Results and discussion

In this investigation, the bacterial strains were isolated from serpentine soils with the objective of assessing the usefulness of metal-resistant serpentine isolate for microbial assisted phytoremediation of metal contaminated soils. In the initial screening, 35 metal resistant bacterial strains were isolated from serpentine soil. Out of the 35 isolates, strain SM3 was chosen due to its high tolerance to nickel (up to the concentration of 1500 mg l^{-1}), copper (500 mg l^{-1}) and zinc (700 mg l^{-1}). This high tolerance to heavy metals could be attributed to the fact that this bacterium was isolated from a serpentine area containing high levels of heavy metals [10, 11, 13]. Based on the morphological, physiological and biochemical characteristics (data not shown) and on the comparative analysis of the 16S rDNA sequence with those available in the database, it was concluded that the SM3 strain was related to the *Bacillus* genus. The highest sequence similarity (99%) and phylogeny based on ClustalW clearly indicates that SM3 is a strain of *Bacillus weihenstephanensis* (Fig. 1). The sequence obtained (868 bp) was submitted in the NCBI databases under the accession number AM941158.

Many studies exist concerning the metal tolerance of bacteria; however, it was difficult to make a meaningful comparison with results in the literature because of the diversity of growth media and incubation conditions reported by other authors. The growth responses of *B. weihenstephanensis* SM3 at the concentration of 200 mg metal l^{-1} of liquid cultures are given in Fig. 2. Measurements taken on the cultures incubated for 28 h were in good agreement with bacterial resistance to each metal. The order of toxicity of the metals to strain SM3 was found to be Zn > Cu > Ni. During the initial 16 h, maximum growth was observed in the control followed by the Ni treatment. Furthermore, in the presence of Cu and Zn, growth was initially inhibited. However, after a few hours, strain SM3 recovered its ability to grow in Cu and Zn polluted medium. Microorganisms isolated from natural environments contaminated with heavy metals often exhibit tolerance to multiple pollutants as they have adapted to such environments [10, 11]. Microbial metal tolerance and antibiotic resistance have been reported previously [23, 24]. It has been suggested that under conditions of imposed stress, metal and antibiotic resistance in microorganisms possibly helps them to adapt spontaneously rather than by genetic mutation and natural selection [25, 26]. Hence,

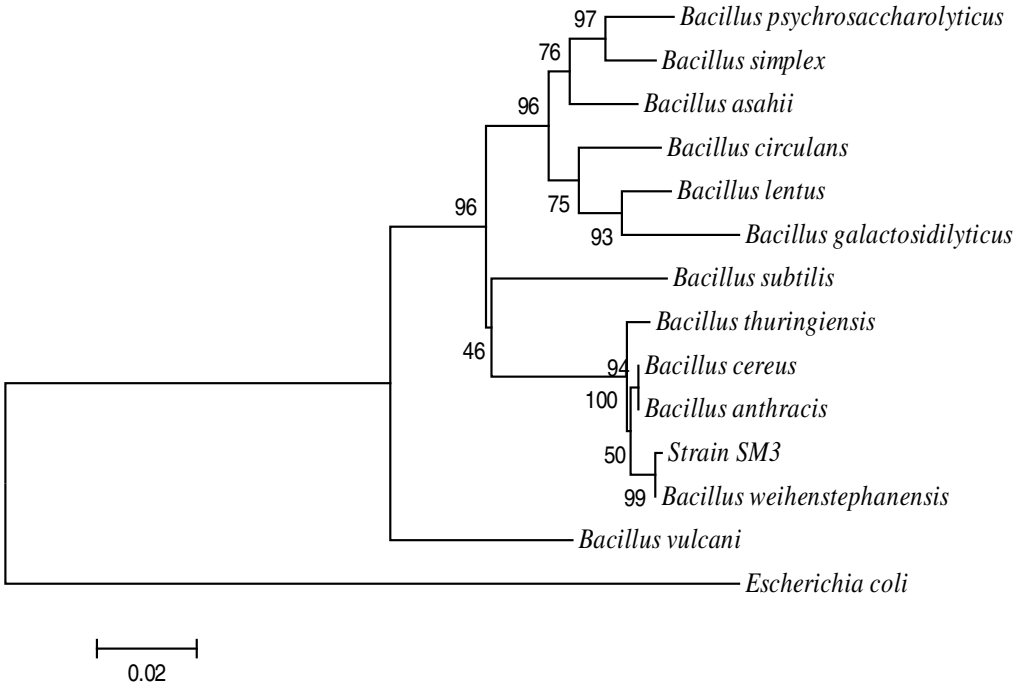

Figure 1. Phylogenetic tree showing the relationship of partial 16S rDNA gene sequences from bacterial strain SM3 isolated from serpentine soil with other related sequences from identified bacteria in the database. *Escherichia coli* was used as the out-group. The bar represents 0.02 substitutions per site.

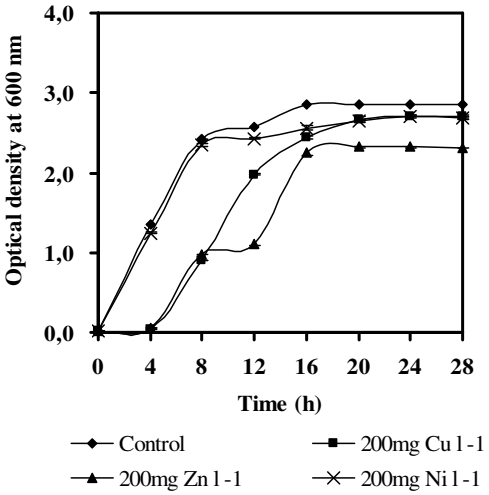

Figure 2. Growth pattern of *Bacillus weihenstephanensis* SM3 on LB medium supplemented with metals at the concentrations of 200 mg l^{-1}. Each value is the mean of three replicates. Error bars represent standard deviation.

strain SM3 was tested for the ability to grow in various antibiotic-supplemented media, and it showed resistance to ampicillin, penicillin, kanamycin and streptomycin (Table 1). The results indicate that the high degree of antibiotic resistance might be associated with heavy metal tolerance [23, 27].

The importance of soil bacteria in heavy metal tolerance and their ability to promote plant growth in a metal-contaminated environment make them the preferred choice for microbial-assisted phytoremediation studies. Certain metal resistant bacteria have been shown to improve host plant growth and development in metal-contaminated soil by mitigating toxic effects of metals on the plants [4, 28]. It has been well documented that solubilization of phosphate in the rhizosphere makes a major contribution to the growth-promoting effect of bacteria on plants [4–6]. In this work we compared the levels of phosphate solubilization by strain SM3 in the absence and presence of heavy metals (Fig. 3). The results indicate that strain SM3 utilized tricalcium phosphate as the sole source of phosphate. Further, the presence of Zn in NBRIP medium (200 mg l^{-1}) did not affect the ability of SM3 to solubilize the phosphate. However, Cu and Ni in the medium reduced the P solubilization by 17% and 43%, respectively, as compared with control. Wani *et al.* [29] have recorded similar observations in *Bacillus* spp. under chromium stress. Evidence suggests that P solubilizing bacteria are highly efficacious at dissolving calcium phosphates whose water solubility is extremely low [30]. The maximum P solubilization showed by strain SM3 in the presence of Zn could be attributed to P deficiency, which develops as a result of external interactions between zinc and phosphorus [31]. Paulson *et al.*

Table 1. Antibiotic resistance of *Bacillus weihenstephanensis* SM3.

Antibiotics	Concentration (μg)	Diameter of inhibition zone (mm)
Ampicillin	10	3 (R)
Tetracycline	30	11 (I)
Chloramphenicol	30	14 (I)
Penicillin	20	3 (R)
Kanamycin	30	8 (R)
Streptomycin	20	7 (R)

Note: (R) – resistance; (I) – intermediate.

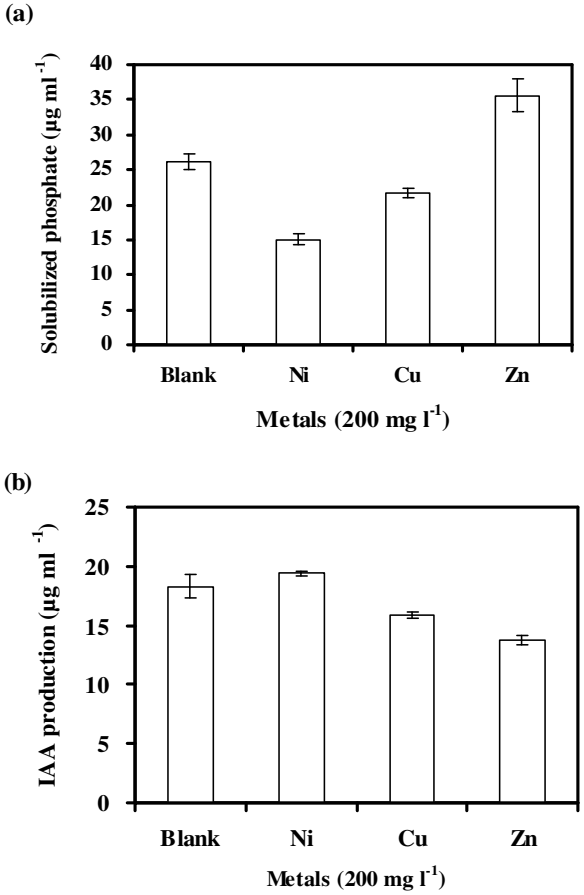

Figure 3. Phosphate solubilization (a) and IAA production (b) by *Bacillus weihenstephanensis* SM3 both in the presence and absence of metals. Each value is the mean of three replicates. Error bars represent standard deviation. Data of columns indexed by the same letter are not significantly different according to Tukey's test ($P < 0.05$).

[32] have also suggested that the elevated levels of Zn in the medium may reduce the availability of P to microorganisms. Further screening of the production of IAA by strain SM3 indicated that it utilized L-tryptophan as a precursor for growth and IAA production, and that IAA synthesis by this strain was not adversely affected by the heavy metals (Fig. 3). However, a noticeable decrease in IAA production (25%) was observed under the effect of Zn. The present study suggests that the intrinsic ability of this strain on expressing the production of IAA in the presence and absence of heavy metals could be exploited to promote the growth of plants under metal stress. Previously, metal resistant isolates belonging to different genera such as *Pseudomonas* and *Bacillus* were reported to produce IAA and aid plant growth [5, 6]. Similarly, nickel resistant *Kluyvera ascorbata* isolated from soil contaminated with nickel, lead and zinc has been reported to promote the growth of tomato (*Lycopersicon esculentum* L.), Indian mustard (*Brassica campestris*) and canola (*Brassica rapa*) [33].

The solubilization of phosphate and production of IAA by strain SM3 under metal stress indicate its inherent plant growth-promoting potential. Considering such potential, the plant growth-promoting efficiency of strain SM3 was tested in Ni, Cu and Zn contaminated soil. *Helianthus annuus* was selected for this study because this species has demonstrated ability to accumulate substantial amounts of metals in shoots and can develop substantial biomass in a very short time [34]. In the absence of heavy metals in soil, inoculation of strain SM3 showed an increase in fresh and dry weight of the plants (Table 2). However, when non-inoculated *H annuss* were exposed to heavy metal stress, plant growth was severely inhibited. For instance, in Ni contaminated soil, growth was considerably decreased, with a 39% reduction in fresh weight and 29% reduction in dry weight. In general, the higher concentrations of heavy metals exert a severe effect on root growth and function, resulting in root damage, reduction in fresh and dry weight, and a diminished uptake of water and nutrients [35, 36]. Plants inoculated with strain SM3 exhibited an increase in plant fresh and dry weight in the presence of heavy metals. For instance, in Ni contaminated soil, strain SM3 increased the plant fresh weight and dry weight by 47% and 23%, respectively, compared with non-inoculated plants. Similarly, in Cu contaminated soil, the percent increase was 35 and 16, respectively. The increase in plant growth caused by strain SM3 may be attributed to the solubilization of phosphate and production of IAA [6, 14].

Uptake of Ni, Cu and Zn by *H. annuus* root and shoot under inoculated and non-inoculated conditions in the presence of heavy metals was determined by AAS analysis (Table 2). In general, root systems accumulated considerably more Ni, Cu and Zn than shoot systems, either with or without SM3 inoculation. This could be due to the poor translocation of heavy metals from roots to shoot system [5, 37]. Inoculation of the bacterial strain SM3 increased the heavy metal accumulation in root and shoot systems. However, in the case of Ni, bacterial inoculation decreased metal accumulation in root (14%) and shoot (48%) systems. Similar observations have also been reported by Wani *et al.* [38] who found that the inoculation of *Bradyrhizobium* sp. on surface sterilized seeds of *Vigna radiate* reduced the concentration of nickel in roots, shoots and grains by 15%, 19% and 22%, respectively, compared with non-inoculated plants. The increased accumulation of Zn and Cu in the presence of strain SM3 might be due to a larger Zn and Cu uptake under acidic soil conditions,

Table 2. Influence of *Bacillus weihenstephanensis* SM3 on the plant growth and the uptake of Ni, Cu and Zn by *Helianthus annuus*.

Metal (200 mg kg^{-1} soil)	Treatment	Fresh weight (g $plant^{-1}$)	Dry weight (g $plant^{-1}$)	Metal concentration (mg kg^{-1} DW)	
				Root	Shoot
Blank	Control	0.91[a] (± 0.047)[b, c]	0.040 (± 0.004)[c]	nd	nd
	SM3	1.00 (± 0.012)[d]	0.046 (± 0.002)[c]	nd	nd
Ni	Control	0.56 (± 0.011)[c]	0.028 (± 0.003)[c]	82.2 (± 3.8)[c]	34.3 (± 1.2)[c]
	SM3	0.82 (± 0.024)[d]	0.035 (± 0.003)[c]	71.1 (± 3.8)[c]	18.0 (± 1.0)[d]
Cu	Control	0.57 (± 0.037)[c]	0.030 (± 0.002)[d]	57.8 (± 3.8)[c]	23.3 (± 2.1)[c]
	SM3	0.77 (± 0.016)[d]	0.035 (± 0.001)[c]	64.4 (± 7.7)[c]	38.0 (± 1.0)[d]
Zn	Control	0.73 (± 0.017)[d]	0.031 (± 0.003)[c]	246.7 (± 13.3)[c]	224.0 (± 10.8)[c]
	SM3	0.81 (± 0.0471)[d]	0.036 (± 0.003)[c]	300.0 (± 6.7)[c]	303.3 (± 6.4)[d]

Note: nd – not detected. DW – dry weight.
[a] Values represent average of triplicates. [b] Values in parentheses represent standard deviation. Data of columns indexed by the same letter are not significantly different between inoculated and non-inoculated plants according to the Tukey's test ($P < 0.05$).

which may develop as a result of phosphate solubilization in soil. Effects of pH on the solubility and speciation of metals are well documented [8]. Sheng and Xia [39] reported that the addition of Cd-resistant bacterial strains to *Brassica napus* grown in metal contaminated soil significantly increased the plant uptake of Cd when compared with the non-inoculated controls, as a result of pH reduction. Furthermore, Delorme *et al.* [40] hypothesized that soil acidification in the rhizosphere of *T. caerulescens* facilitates metal ion uptake by increasing metal ion mobility around the roots. Hence, a batch experiment was conducted to assess the metal mobilization potential of the strain SM3 in soil. Inoculation of strain SM3 increased the concentrations of water soluble Ni, Cu and Zn in soil after 10 d, which were 13%, 24% and 75% higher than those in the control soil, respectively (Fig. 4). The results indicate that strain SM3 facilitated the release of heavy metals, especially Cu and Zn, from the non-soluble phases in the soil. The higher water soluble Cu and Zn induced by bacterial inoculation resulted in a correspondingly higher Cu and Zn accumulation in both the shoots and roots of *H. annuus* suggesting that the bioavailability of Cu and Zn was increased by producing organic acids or specific ligands. Hence, we checked the ability of strain SM3 to produce siderophores in Fe deficient medium using CAS assay [41]. However, the strain SM3 did not produce siderophores in the absence or the presence of heavy metals (data not shown). This observation indicates that other mechanisms rather than siderophore production were involved in metal mobilization. Further work will be carried out to determine the nature of metal mobilizing compounds released by the *B. weihenstephanensis* strain SM3.

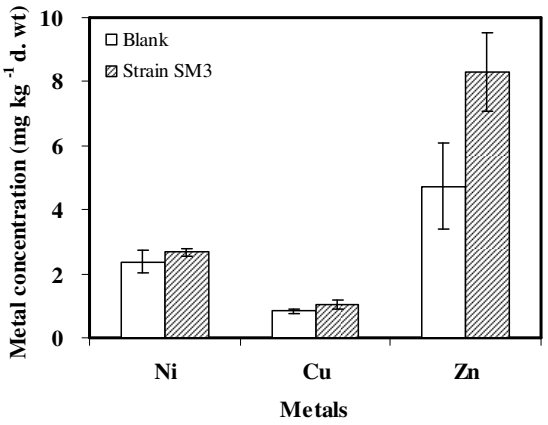

Figure 4. Effect of inoculation with *Bacillus weihenstephanensis* SM3 on the solubilization of Ni, Cu and Zn in soil. Each value is the mean of three replicates. Error bars represent standard deviation. Data of columns indexed by the same letter are not significantly different between the data sets according to Tukey's test ($P < 0.05$).

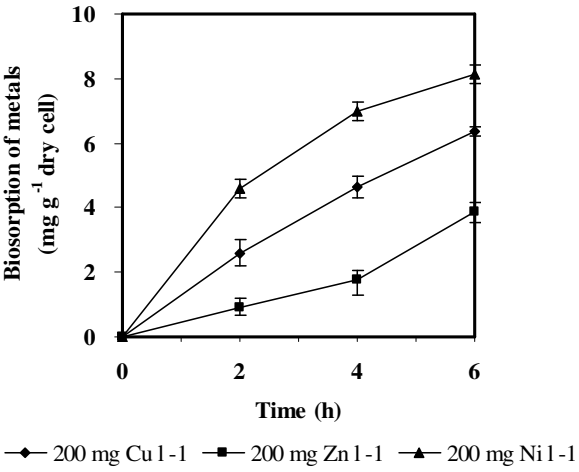

Figure 5. Biosorption of metals by *Bacillus weihenstephanensis* SM3 cells. Each value is the mean of three replicates. Error bars represent standard deviation.

Since most metal-microbe interactions are initiated at the level of metal uptake, the uptake mechanism is likely to be closely linked to the mechanism of metal resistance. The metal-resistant strain SM3 was capable of removing significant concentrations of Ni, Cu and Zn. However, the highest amount of biosorption of metals was observed with Ni (8.13 mg g^{-1} dry weight) within 6 h of incubation, while the lowest was seen with Zn (3.88 mg g^{-1} dry weight) (Fig. 5). Tobin *et al.* [42] demonstrated that molecules having smaller ionic radius can be more quickly sorbed onto a fixed surface area of sorbent. The ionic radius of Ni (0.83 Å), which is smaller than that of Cu (0.87 Å) and Zn (0.88 Å) may be responsible for its higher biosorption by the strain SM3. Several investigations have shown that relatively large quantities of metal cations are complexed by bacteria [6] and fungi [43]. With this intrinsic characteristic, strain SM3 may also contribute in reducing the phytotoxic effects of the metals by sharing the metal load due to its demonstrated ability of biosorption and bioaccumulation [40].

Our results revealed that inoculation of *B. weihenstephanensis* strain SM3 not only protects plant from heavy metal toxicity but also enhances metal accumulation (especially Cu and Zn) in plant tissues, with concurrent stimulation of plant growth. These beneficial effects caused by inoculation with *B. weihenstephanensis* SM3, together with the suggested interrelationship between the microbial heavy metal mobilization and biosorption, indicate that inoculation with metal resist-

ant serpentine isolates might have some potential to improve phytoextraction efficiency in metal contaminated soils.

Acknowledgement

M. Rajkumar thanks the Portuguese Foundation for Science and Technology (FCT) for awarding a Post-doctoral research grant (SFRH/BPD/21309/2005).

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