CHROMATE-TOLERANT BACTERIA FOR ENHANCED METAL UPTAKE BY EICHHORNIA CRASSIPES (MART.)

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A total of 85 chromate-resistant bacteria were isolated from the rhizosphere of water hyacinth grown in Mariout Lake, Egypt, as well as the sediment and water of this habitat. Only 4 (11%), 2 (8%), and 2 (8%) of isolates from each of the environments, respectively, were able to tolerate 200 mg Cr (VI) L\(^{-1}\). When these eight isolates were tested for their ability to tolerate other metals or to reduce chromate, they were shown to also be resistant to Zn, Mn, and Pb, and to display different degrees of chromate reduction (28% to 95%) under aerobic conditions. The isolates with the higher chromate reduction rates from 42% to 95%, (RA1, RA2, RA3, RA5, RA7, and RA8) were genetically diverse according to RAPD analysis using four different primers. Bacterial isolates RA1, RA2, RA3, RA5, and RA8 had 16 S rRNA gene sequences that were most similar to Pseudomonas diminuta, Brevundimonas diminuta, Nitrobacteria iranicum, Ochrobactrum anthrophi, and Bacillus cereus, respectively. Water hyacinth inoculated with RA5 and RA8 increased Mn accumulation in roots by 2.4- and 1.2-fold, respectively, compared to uninoculated controls. The highest concentrations of Cr (0.4 g kg\(^{-1}\)) and Zn (0.18 g kg\(^{-1}\)) were accumulated in aerial portions of water hyacinth inoculated with RA3. Plants inoculated with RA1, RA2, RA3, RA5, RA7, and RA8 had 7-, 11-, 24-, 29-, 35-, and 21-fold, respectively, higher Cr concentrations in roots compared to the control. These bacterial isolates are potential candidates in phytoremediation for chromium removal.

KEY WORDS: rhizofiltration, chromate reduction, rhizosphere, bacteria, metal tolerance, water hyacinth

INTRODUCTION

Contamination of aquatic environments by toxic metal ions is a serious pollution problem. Metals cannot be easily degraded and cleanup usually requires chemical or
physical removal (Lasat, 2002). However, these approaches can be prohibitively expensive and often are poorly effective. Phytoremediation offers a cost-effective, nonintrusive, and safe alternative to conventional cleanup techniques.

Hexavalent chromium [Cr (VI)] compounds are used in a wide variety of commercial processes. Unregulated disposal of chromium-containing effluent in both developing and developed countries has led to the contamination of soil, sediment, surface, and groundwater (Szulczewski et al., 1997). In trace amounts, chromium is considered to be an essential nutrient for numerous organisms, but it is toxic and mutagenic at elevated levels (Cheung and Gu, 2003). Wastewater containing chromium must be treated before being discharged into the environment. To remediate the aquatic environment, toxic metal ions should be concentrated in a form that can be conveniently extracted for possible reuse or for proper disposal.

Natural resources, including plants and microorganisms, have been extensively explored for their use in metal ion removal from polluted environments (Abou-Shanab et al., 2003b, 2005; So et al., 2003; Glick, 2003). Recently, the potential of wetland plants was demonstrated in aquatic phytoremediation, a process that includes rhizofiltration, phytofiltration, and constructed wetlands (Dushenkov and Kapulnik, 2000; Zurayk et al., 2001; Bennicelli et al., 2004; Kamal et al., 2004; Maine et al., 2004). Due to the relative novelty of this technology, much of the current research is still aimed at the selection of wetland plant species with a high removal capability.

Water hyacinth (Eichhornia crassipes Mart.) is an important plant species that has attracted considerable attention due to its ability to grow in heavily polluted water together with its capacity for metal ion accumulation (Santos and Lenzi, 2000; Soltan and Rashed, 2003). Thus, water hyacinth can be used as an efficient biofilter for metal ions and has been widely used in plant-based wastewater treatment systems (Trivedy, 1998).

Alteration of the rhizosphere microbial community by inoculation of terrestrial plants can increase the feasibility and efficiency of phytoremediation (Burd et al., 2000; Whiting et al., 2001; Abou-Shanab et al., 2003a). The reduction of Cr (VI) to less toxic Cr (III) has been suggested as an additional chromosome-encoded chromate-resistance mechanism, in addition to plasmid-encoded tolerance (Cervantes and Silver, 1992; Ohtake and Silver, 1994). Bacillus sp QC1–2, a strain isolated from a Cr-polluted zone, was also selected for its ability to both tolerate chromate and reduce Cr (VI) to Cr (III) (Campos et al., 1995). The inoculation of aquatic plants to increase the efficiency of wastewater remediation has not been attempted. Therefore, in the current study, bacteria isolated from and around water hyacinth were examined for resistance to metals. Subsequently, several isolates were tested as inoculants to affect uptake of Cr, Zn, Pb, and Mn by water hyacinth.

MATERIALS AND METHODS

Site Description

Lake Mariout is a large shallow basin, southwest of Alexandria, Egypt. It covers an area of about 15,000 hectares, with an average water depth of 100 cm. The lake receives all domestic, agricultural, and industrial wastewater of Alexandria and Borg El Arab City. The drainage area contains nearly 40% of all Egyptian industry (Abou-Shanab, 2003).
Sampling and Bacterial Analysis

All plastic and glassware used in studies were washed in 2 M HNO₃ and rinsed with double-deionized water to avoid metal contamination. *Eichhornia crassipes* plants, soil sediment, and water samples were collected from Mariout Lake in sterile plastic bags and transported on ice to the laboratory. Enumeration of bacteria using a serial dilution technique was performed within 3 h of collecting samples. Plant roots were carefully removed from the sediment and a sufficient amount was blended with 30 ml of sterilized saline (0.85% NaCl) solution for 5 min. To avoid overheating, the plant roots were blended for 1 min with 5 intervals of cooling each for 5 min. (So et al., 2003). For the isolation of Cr-resistant bacteria, water, sediment, and rhizosphere soil samples were serially diluted in sterile 0.85% saline solution and 100 µl from each dilution was spread on nutrient agar amended with 25 mg L⁻¹ of Cr (VI). A filter-sterilized solution of K₂Cr₂O₇ was used as the source of Cr (VI), which was added to the sterile molten nutrient agar to prevent the problems associated with autoclaving chromate-containing solutions (Babich et al., 1982; Hassen et al., 1998; Srinath et al., 2002). Plates were incubated at 30°C for 3–5 d and the total number of bacteria was determined as colony-forming units per milliliter (CFU ml⁻¹). Eighty-five morphologically distinct colonies were selected as Cr-resistant isolates. These isolates were purified and stored at −80°C in a nutrient broth medium containing 50% glycerol until further use.

Evaluation of Chromium-Resistance

The minimal inhibitory concentration (MIC) of chromium at which no colony growth occurred was determined by the broth agar dilution method (Luli et al., 1983). The 85 bacterial isolates were individually inoculated into 25 ml nutrient broth in 100 ml conical flasks and incubated at 30°C at 150 rpm to achieve log phase cultures. Exponentially growing cultures of each bacterial isolate were spread aseptically onto nutrient agar plates containing different concentrations of Cr (VI) (50–250 mg L⁻¹). These plates were incubated at 30°C for 3–5 d. The MIC was considered to be the lowest concentration of Cr (VI) at which no growth occurred.

Assessment of Chromium Reduction

Reduction of chromium was determined by growing the 200 mg Cr (VI) L⁻¹ resistant bacterial isolates in nutrient broth supplemented with 50 mg L⁻¹ of Cr (VI) as K₂Cr₂O₇ (Aldrich, WI). Cells were grown on a shaker (150 rpm) at 30°C for 72 h and centrifuged (6000 x g) for 20 min. Chromium reduction was estimated by measuring the decrease in hexavalent chromium in the culture filtrate. Chromium (VI) content in the samples was determined by measuring optical density of the purple complex of Cr (VI) with 1, 5-diphenylcarbohydrazide at 540 nm by UV spectrophotometry (Pattanapipitpaisal et al., 2001).

Tolerance to Other Heavy Metals

Chromate-tolerant isolates were also studied for tolerance to other trace elements. Fresh overnight cultures of each isolate were inoculated aseptically on nutrient agar plates supplemented with different metals. Filter sterilized solutions of (CH₃COO)₂Pb·H₂O,
ZnCl₂, and MnCl₂ (BDH Chemicals, RA grade, UK) were used as the source of Pb(II), Zn(II), and Mn(II), which were added to the sterile molten nutrient agar to avoid binding or chelation of the metal to unspecified organic components. The metal ion concentrations tested ranged from 50 to 250 mg L⁻¹. Isolates exhibiting growth after 72 h incubation at 30°C were considered tolerant to the metal.

Random Amplified Polymorphic DNAs (RAPD-PCR) Analysis

Genomic DNA of chromate-reducing bacteria was extracted from 10-ml bacterial cultures grown overnight according to the method described by Ausubel et al. (1987). Bacterial pellets suspended in a mixture of TE buffer, SDS (10%), and proteinase K were incubated for 1 h at 37°C. NaCl (5 M) and pre-warmed solution of CTAB/NaCl (10 g CTAB [N-cetyl-N,N,N-trimethylammoniumbromide] and 4.1 g NaCl/100 mL distilled water) were added and the mixture was incubated at 65°C for 10 min. The mixture was incubated for 10 min at 65°C. The solution was extracted with 780 μL of chloroform–isoamyl alcohol (24:1) and centrifuged for 5 min. The aqueous phase was further extracted with an equal volume of phenol–chloroform–isoamyl alcohol (25:24:1). After centrifugation for 5 min, the DNA present in the aqueous phase was precipitated with 0.6 vol. isopropanol and the precipitate was washed with 70% ethanol. The DNA pellet was dried and resuspended in TE buffer. RAPD-PCR amplification was performed as described by Selenska-Pobell et al. (1996, 1998) using the primers: AP18, 5'-CACACGCACACGGAAAGA-3'; AP19, 5'-CAGGCACACGGACAGA-3' (Ralph et al., 1993) and A9B7, 5'-GGTGACCGAGGGTAACGCC-3'; A1A13; 5'-AGGCCCTTCCAGCACCCAC-3' (Williams et al., 1990). The PCR protocol was a 35-cycle PCR (initial denaturation, 95°C for 5 min; subsequent denaturation, 95°C for 30 sec; annealing temperature, 45°C for 2 min; extension temperature, 72°C for 1 min; final extension, 72°C for 10 min). The PCR products were analyzed on 1.5% agarose gel and visualized by ultraviolet illumination after staining with 0.5 μg ml⁻¹ ethidium bromide. The fingerprint patterns resulting from RAPD analysis were scored for each template DNA by recording the presence or absence of bands to construct a rectangular binary matrix. The matrix was used to derive simple matching coefficients used in a clustering analysis and the construction of dendrograms to illustrate the genetic relationships using NTSYS-PC 2.0 (Rohlfs, 1988).

16S Ribosomal RNA (rRNA) Sequencing

Oligonucleotide primers with specificity for eu-bacterial 16 S rRNA genes, primers 16Sa and 16Sb, were used to amplify the 16 S rRNA gene fragments with template DNA originating from gram-negative bacteria and using PCR protocols described by van Berkm and Fuhrmann (2000). In the case of gram-positive bacteria primers, M16Sa and M16Sb were used in combination with the PCR protocol described by Rhodes et al. (2003). Subsamples (10 μl) of the reaction mixtures were analyzed by 1% horizontal agarose gel electrophoresis to confirm the presence of products (van Berkm and Fuhrmann, 2000). PCR products were purified using QIAquick Spin columns (Qiagen Inc., Chatsworth, CA). The Applied Biosystem 3100 Genetic analyzer in combination with a Dye Deoxy Terminator Cycle Sequencing Kit (Perkin Elmer, Foster City, CA) were used for sequencing the purified PCR products, as described previously (van Berkm et al., 1996). A search of
GenBank with BLAST (Altschul et al., 1997) was used to identify named bacterial species with 16 S rRNA gene sequences similar to those of the isolates.

**Bacterial Inoculum Preparation**

Bacterial cells were grown overnight in 250-ml Erlenmeyer flasks containing 100 ml of sterilized nutrient broth on a shaker at 150 rpm at 30°C until late log phase. Bacterial cells were then harvested by centrifugation (6000 × g, 20°C, 20 min) and the pellets were washed twice with sterile distilled water. Bacterial suspensions in distilled water were adjusted to an absorbance of 0.5 at 600 nm (equivalent to approximately $7.4 \times 10^8$ CFU ml$^{-1}$). Five ml of each bacterial suspension was used for plant inoculation.

**Cultivation of Experimental Plants**

Plant samples were collected from Mariout Lake, Alexandria, Egypt, in August 2004 and were transferred to the laboratory in polyethylene bags. Plants of similar shape, size (weight of each plant, 200 ± 20 g wet mass), and height (roots, 15–20 cm; aerial parts, 20–22 cm) were selected and washed several times using tap and distilled water. Plants were cultured individually in polyethylene beakers containing 5 L of full-strength Hogland's nutrient solution (Zurayk et al., 2001) in a growth chamber at 25 ± 2°C and incubated under fluorescent lighting with an intensity of 24 μmol m$^{-2}$ s$^{-1}$ and a 12 h:12 h (light:dark) photoperiod. Four trace elements were investigated in this study including Cr ($\text{K}_2\text{Cr}_2\text{O}_7$), Mn ($\text{MnCl}_2$), Zn ($\text{ZnSO}_4 \cdot 4\text{H}_2\text{O}$), and Pb ($\text{Pb(NO}_3)_2$). Plants were supplied with 1 mg L$^{-1}$ of each metal at concentrations chosen for environmental relevance and at which most trace elements may cause some effect on plant growth (Qian et al., 1999; Zhu et al., 1999). Plants grown in a nutrient solution without added metals served as controls. Culture solutions were replaced every 2 d.

**Plant Harvesting and Preparation**

After an exposure to heavy metals of 3 wk, whole *E. crassipes* plants were harvested; their aerial parts and roots were separated, rinsed thoroughly with distilled water, and dried at 65°C. Dried plant samples were ground to pass a 20-mesh sieve. Two grams or less of milled plant matter was digested using a mixture of concentrated HCl/HNO$_3$ (4:1, v/v) (McGrath and Cunliffe, 1985). Metal concentrations were determined using an atomic absorption spectrophotometer.

**RESULTS AND DISCUSSION**

**Heavy Metal Concentrations in Water Hyacinth Plant Parts**

Concentrations of heavy metals were higher in the roots than in aerial parts of water hyacinth growing in Mariout Lake (Table 1). The co-precipitation of metals in plaques of iron and manganese occurring on the roots is one process that can explain the elevated concentrations found in the roots (Vesk and Allaway, 1997). Plaques of iron and manganese oxyhydroxides often form in waterlogged roots. Oxidation is believed to be a mechanism by which plants avoid toxicity of the reduced forms of Fe and Mn to roots under flooded conditions (St-Cyr et al., 1993).
Table 1: Concentration of heavy metals in the aerial part and roots of water hyacinth and water samples collected from Mariout Lake

<table>
<thead>
<tr>
<th>Metal</th>
<th>Root* (mg kg⁻¹ DW)</th>
<th>Aerial part* (mg kg⁻¹ DW)</th>
<th>Water (mg L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn</td>
<td>81</td>
<td>31</td>
<td>1.8</td>
</tr>
<tr>
<td>Pb</td>
<td>48</td>
<td>11</td>
<td>0.09</td>
</tr>
<tr>
<td>Cr</td>
<td>39</td>
<td>15</td>
<td>0.01</td>
</tr>
<tr>
<td>Zn</td>
<td>72</td>
<td>49</td>
<td>0.18</td>
</tr>
</tbody>
</table>

*Mean of four samples.

Compared with other metals, root concentrations of Mn were high (81 mg kg⁻¹ dry wt), followed by Zn, Pb, and Cr, in decreasing order with 72, 48, and 39 mg kg⁻¹ dry wt, respectively (Table 1). Root surfaces were often covered with a reddish-brown layer, which may have included mucilage, particulates such as clays, and a diversity of microorganisms, including bacteria, protozoans, and diatoms (Vesk et al., 1999). Concentrations of Cr, Pb, Zn, and Mn in water were very low compared to concentrations in plant tissues. Roots of water hyacinth concentrated these metals were 3900-, 533-, 400-, and 45-fold, respectively, (on a dry basis) above metal concentrations in water. Water sediment is a sink for metals and, in the anoxic zone, may contain very high concentrations of metals in a reduced state. As such, the bioavailability of the metals is low compared to terrestrial systems with oxidized soils (Weis and Weis, 2004).

**Bacterial Enumeration and Chromate Resistance**

The number of culturable bacteria in the rhizosphere of water hyacinth, 6.6 × 10⁶ CFU, was 14.2- and 9-fold higher than that in the sediment and water, respectively (Table 2). The high concentration of bacteria around the roots, i.e., in the rhizosphere, presumably occurs due to the presence of high levels of nutrients that are exuded from the roots of most plants and can be used to support bacterial growth and metabolism (Penrose and Glick, 2001). A higher percent, 42%, of chromate-tolerant bacteria were isolated from rhizosphere soils of water hyacinth compared with 4.3% and 0.9%, respectively, of the bacterial community from sediment and water. Metals affect microorganisms by reducing their number, biochemical activity, diversity, and changing the community structure (Giller et al., 1998; Ellis et al., 2003; Abou-Shanab, 2003a).

Table 2: Numbers of culturable bacteria (C.F.U) in sediment, the rhizosphere of water hyacinth, and water samples collected from Mariout Lake

<table>
<thead>
<tr>
<th></th>
<th>Rhizosphere (cfu g⁻¹ x 10⁵)</th>
<th>Sediment (cfu g⁻¹ x 10⁵)</th>
<th>Water (cfu ml⁻¹ x 10⁵)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>65.5</td>
<td>4.6</td>
<td>7.2</td>
</tr>
<tr>
<td>Cr (VI) tolerant*</td>
<td>27.7</td>
<td>0.2</td>
<td>0.07</td>
</tr>
<tr>
<td>% of Cr (VI) tolerant</td>
<td>42.3</td>
<td>4.3</td>
<td>0.9</td>
</tr>
</tbody>
</table>

*Colonies of chromate-tolerant bacteria were counted on nutrient agar plates amended with 25 mg Cr (VI) L⁻¹.
In the search for chromate-resistant bacteria, a total of 85 morphologically distinct Cr (VI)-tolerant isolates from the rhizosphere, sediment, and water (37, 24, and 24, respectively) were examined for levels of Cr (VI) tolerance. Most had an MIC less than 100 mg Cr (VI) L\(^{-1}\). Only 4 (11%), 2 (8%), and 2 (8%) of isolates originating from the rhizosphere, sediment, and water, respectively, were able to tolerate 200 mg Cr (VI) L\(^{-1}\) (Figure 1). The highest percentage of chromate-resistant bacteria were obtained from the rhizosphere, with 84%, 70%, 19%, and 11% able to grow on agar plates amended with 50, 100, 150, and 200 mg Cr (VI) L\(^{-1}\), respectively. This observation is similar to the high proportion of metal-resistant bacteria reported to persist in the rhizosphere of the hyperaccumulator Thalaspi caerulescens (Delorme et al., 2001) and Alyssum bertolonii (Mengoni et al., 2001) or Alyssum murale (Abou-Shanab et al., 2003b) grown in soil contaminated with Zn and Ni or Ni, respectively. The higher proportion of metal-resistant bacteria in the rhizosphere of these terrestrial plants was attributed to higher mobilization of metals through the production of root exudates. This may also be the case for aquatic plants such as water hyacinth.

**Heavy Metal Tolerance and Chromium Reduction**

Eight isolates tolerant to 200 mg Cr (VI) L\(^{-1}\) were also tested for their ability to tolerate different concentrations of Mn, Zn, and Pb and for their ability to reduce Cr (VI). All eight isolates were resistant to these metals (Table 3) and displayed different degrees of chromate reduction under aerobic conditions (Figure 2). The maximum metal tolerance was observed at 150–250 mg L\(^{-1}\) Mn, Pb, and Zn.

The percent of chromate reduction ranged from 28% to 95% in the presence of Cr (VI) at 50 mg L\(^{-1}\). Maximum chromate reduction was observed with RA2 and RA3 (95% and 94%, respectively) isolated from water, followed by RA7 and RA8 (86% and 83%, respectively), isolated from the water hyacinth rhizosphere. The lowest percentage of chromate reduction (28%) was observed for RA4 isolated from sediment.
Chromium exists in several oxidation states, but the most stable are trivalent Cr (III) and hexavalent Cr (VI) forms, with different chemical characteristics and biological effects (Cervantes et al., 2001). The ability of chromate-resistant bacteria to reduce Cr (VI) is widely described and is not an exclusive characteristic of specific bacterial groups or populations (Pattanapipitpaisal et al., 2001; Srinath et al., 2002; Paul and Pal, 2004; Camargo et al., 2005). Francisco et al. (2002) concluded that Cr (VI) resistance and reduction are both shared abilities, probably reflecting horizontal genetic transfer of the determinants resulting from selective pressure in environments contaminated with Cr (VI). This ability enables microbial populations to rapidly adapt and evolve in stressed environments.

**Table 3 Metal tolerance of chromate-resistant bacteria**

<table>
<thead>
<tr>
<th>Bacterial strain</th>
<th>Mn (mg L$^{-1}$)</th>
<th>Pb (mg L$^{-1}$)</th>
<th>Zn (mg L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA1</td>
<td>200</td>
<td>250</td>
<td>150</td>
</tr>
<tr>
<td>RA2</td>
<td>250</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>RA3</td>
<td>250</td>
<td>200</td>
<td>250</td>
</tr>
<tr>
<td>RA4</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>RA5</td>
<td>250</td>
<td>250</td>
<td>150</td>
</tr>
<tr>
<td>RA6</td>
<td>150</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>RA7</td>
<td>250</td>
<td>200</td>
<td>250</td>
</tr>
<tr>
<td>RA8</td>
<td>200</td>
<td>150</td>
<td>200</td>
</tr>
</tbody>
</table>

**RAPD Fingerprinting**

The six isolates—RA3, RA2, RA7, RA8, RA1, and RA5—with the highest rates of chromate reduction were selected for RAPD analysis. The selection of these isolates was made because the reduction of Cr (VI) to less toxic Cr (III) is an important step in the remediation of Cr (VI)-contaminated environments (Megharaj et al., 2003). The

![Figure 2](attachment:image.png)

**Figure 2** Screening of 200 mg Cr (VI)L$^{-1}$ resistant bacteria for chromate reduction in the presence of 50 mg Cr (VI)L$^{-1}$. 
CHROMATE-TOLERANT BACTERIA UPTAKE BY *EICHHORNIA CRASSIPES* (MART.)

**Figure 3** RAPD-PCR products from six chromate-resistant bacteria (1 = RA1, 2 = RA2, and 3 = RA3 were isolated from water; 7 = RA7, 8 = RA8 were isolated from the rhizosphere of water hyacinth; and 5 = RA5 was isolated from sediment) generated by random primers AP18, AP10, A9B7, and A1A13. Lane M, molecular marker, and size of the marker fragments are 2176, 1766, 1230, 1033, 653, 517, 453, 394, 298, 234, 154bp; Lane m, molecular size marker of Lambda DNA EcoRI digested with *HindIII*.

Development and increased availability of molecular biology techniques have made it possible to obtain information regarding the diversity of bacterial cultures isolated from different soils (Amarger *et al.*, 1994; de Oliveira *et al.*, 2000). One such technique, a polymerase chain reaction (PCR)-based assay to fingerprint genomes using random amplification of polymorphic DNA (RAPD), is useful for differentiating between bacterial isolates (Welsh and McClelland, 1990).

RAPD analysis of the six isolates led to a minimum of one and a maximum of eight discrete visible bands ranging in size from 0.2 to 2.6 kb (Figure 3). The banding patterns were markedly different with each primer. PCR with primer A1A13 was the most informative, since the highest numbers of polymorphisms were observed among the isolates. Primers AP10 and AP18 were uninformative because no polymorphisms could be detected among isolates RA1, RA7 and RA8 (Figure 3). No PCR products were obtained using primer A9B7 with RA1, but the five other isolates could be distinguished from each other using this primer. The dendrogram derived from combining the RAPD dataset showed that bacterial strains RA1 and RA8 shared 90% similarity and RA7 shared 85% similarity with RA1 and RA8. From the RAPD patterns of the three remaining isolates, it was concluded that these were more diverse, with similarities below 85% (Figure 4).

**16S rRNA Sequence Analysis**

More than 1400 bp of the 16S rRNA genes of isolates RA1, RA2, RA3, RA5, and RA8 were sequenced. From the 16S rRNA sequences, it was concluded that RA1, RA2, RA3, RA5, and RA8 were closely related to *Pseudomonas diminuta*, *Brevundimonas diminuta*,...
Nitrobacter iranccium, Ochrobactrum anthropi, and Bacillus cereus, based on 99%, 99%, 99%, 97%, and 99% sequence similarities, respectively.

Effect of Bacterial Inoculation on Metal Uptake by Water Hyacinth

Water hyacinth inoculated with RA7, RA5, RA3, RA8, RA2, and RA1 had 35-, 29-, 24-, 21-, 11-, and 7-fold higher Cr concentrations in roots, respectively, as compared with uninoculated plants. Isolates RA3 and RA1 increased Cr phytoaccumulation in plant aerial parts by 16- and 1.8-fold, respectively, compared with the uninoculated control (Figure 5).

Chromium is a highly toxic, nonessential metal for microorganisms and plants. The hexavalent form of the metal, Cr (VI), is considered a more toxic form than the relatively innocuous and less mobile Cr (III) form. The presence of Cr in the environment has selected microbial and plant variants that are able to tolerate high levels of Cr compounds. Zaranyika and Ndadwada (1995), Qian et al. (1999), and Maine et al. (2004) reported that chromium accumulated to the greatest concentrations in plant roots and to the lowest levels in plant shoots. Also, Zurayk et al. (2001) found that Cr was predominantly accumulated in the root, where Cr concentrations were 10 to 100 times larger than corresponding shoot values.

Inoculation of water hyacinth with RA1 and RA5 increased Mn accumulation in aerial parts and root tissues by 1.2- and 2.3-fold, compared to the uninoculated control (Figure 5). The highest Pb concentration in aerial parts (112 mg kg⁻¹) was attained by water hyacinth inoculated with RA8. Isolate RA3 increased Zn uptake by 3.5- and 1.1-fold in aerial parts and root, respectively, compared with the uninoculated control. Manganese has received little attention with respect to uptake and accumulation by wetland plant species (Qian et al., 1999), but may be stimulated in the presence of microorganisms (Barber and Lee, 1974). Several floating wetland plant species may also accumulate appreciable amounts of Pb (Chigbo et al., 1982; Muramoto and Oki, 1983). Information on bacterial stimulation of Zn uptake has been reported in the terrestrial environment (Whiting et al., 2001), but we are unaware of similar reports for aquatic plants. Water hyacinth plants growing in all of the above treatments looked quite healthy, with green leaves during the period under study (3 wk). Therefore, water hyacinth may be useful for the phytoremediation of waters...
containing low concentrations of metals by removing elements from water and storing them in a nontoxic form.

Bacteria associated with plant roots may have profound effects on plant growth and nutrition through a number of mechanisms, such as N₂ fixation, production of phytohormones, siderophores, and the transformation of nutrient elements. The improvement of interaction between plants and beneficial rhizosphere microorganisms can enhance the biomass production and tolerance of plants to heavy metals and are considered to be an important component of phytoremediation technologies (Whiting et al., 2001; Abou-Shanab et al., 2003a, 2003b; Glick, 2003; So et al., 2003; Belimov et al., 2005).
CONCLUSIONS

In the present study, a high proportion of chromate-resistant bacteria were observed to be present in the rhizosphere of water hyacinth compared with water and sediment. A select few could tolerate very high concentrations of K₂Cr₂O₇ up to 200 mg L⁻¹ and exhibited multiple metal (Mn, Pb, and Zn) resistances. These isolates also displayed variation in rates of chromate reduction. The potential for chromate-resistant bacteria to enhance chromium uptake from wastewater has not been previously documented. The presence of these bacteria in the rhizosphere would potentially enhance the conversion of the more soluble form of chromate Cr (VI) to a less toxic and less mobile form Cr (III). The higher chromium concentration in the root of water hyacinth than in the shoots suggests that the reduced form exists mostly bound to organic acids produced by rhizosphere microorganisms and that complexation with organic compounds is involved in facilitating Cr availability. Therefore, it may be possible to develop inoculation technologies that could improve metal uptake by water hyacinth.

ACKNOWLEDGEMENTS

The authors would like to thank Mrs. Eman Ahmed, Mubarak City for Scientific Research and Technology Applications, Environmental Biotechnology Dept., Genetic Engineering Institute, Egypt, for her valuable technical assistance. Sincere thanks also to Elia Patric, Soybean Genomics and Improvement Lab, USDA, Beltsville, MD, USA, for offering every possible help.

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