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Interactions of chromium with microorganisms and plants

Carlos Cervantes [a,*], Jesús Campos-García [a], Silvia Devars [b], Félix Gutiérrez-Corona [c], Herminia Loza-Tavera [d], Juan Carlos Torres-Guzmán [c], Rafael Moreno-Sánchez [b]

[a] *Instituto de Investigaciones Químico-Biológicas, Universidad Michoacana, Edificio B-3, Ciudad Universitaria, 58030 Morelia, Michoacán, Mexico*
[b] *Departamento de Bioquímica, Instituto Nacional de Cardiología, Mexico, D.F., Mexico*
[c] *Instituto de Investigación en Biología Experimental, Universidad de Guanajuato, Guanajuato, Gto, Mexico*
[d] *Facultad de Química, Universidad Nacional Autónoma de México, Mexico, D.F., Mexico*

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Abstract

Chromium is a highly toxic non-essential metal for microorganisms and plants. Due to its widespread industrial use, chromium (Cr) has become a serious pollutant in diverse environmental settings. The hexavalent form of the metal, Cr(VI), is considered a more toxic species than the relatively innocuous and less mobile Cr(III) form. The presence of Cr in the environment has selected microbial and plant variants able to tolerate high levels of Cr compounds. The diverse Cr-resistance mechanisms displayed by microorganisms, and probably by plants, include biosorption, diminished accumulation, precipitation, reduction of Cr(VI) to Cr(III), and chromate efflux. Some of these systems have been proposed as potential biotechnological tools for the bioremediation of Cr pollution. In this review we summarize the interactions of bacteria, algae, fungi and plants with Cr and its compounds.

Keywords: Chromium transport; Chromium toxicity; Chromate reduction; Chromium resistance; Bioremediation

Contents

* Corresponding author. Tel./Fax: +52 (43) 26-5788; E-mail: cvega@zeus.umich.mx

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1. Introduction

Chromium is a transition metal located in group VI-B of the periodic table. Although it is able to exist in several oxidation states, the most stable and common forms are the trivalent Cr(III) and the hexavalent Cr(VI) species, which display quite different chemical properties [1]. Cr(VI), considered the most toxic form of Cr, is usually associated with oxygen as chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) ions [1]. In contrast, Cr(III) in the form of oxides, hydroxides or sulfates, is much less mobile and exists mostly bound to organic matter in soil and aquatic environments. Cr(VI) is a strong oxidizing agent and in the presence of organic matter is reduced to Cr(III); this transformation is faster in acid environments such as acidic soils [1]. However, high levels of Cr(VI) may overcome the reducing capacity of the environment and thus persist as a pollutant. In addition, Cr(III) may be also oxidized to Cr(VI) in the presence of an excess of oxygen, being transformed again to the more toxic form [2].

Cr is the seventh most abundant element on earth and 21st in the crustal rocks [1]. Cr abundance in Earth's crust ranges from 100 to 300 μg g^{-1}. Soils may contain between 5 and 3000 μg of chromium per gram [3]. The world production of Cr is in the order of 10^7 tons per year; 60–70% is used in alloys, including stainless steel, and 15% is used in chemical industrial processes, mainly leather tanning, pigments and electroplating [1,4,5]. Its widespread use has converted Cr in a serious pollutant of air, soil and water [6,7]. Cr concentrations in non-polluted waters vary from 0.1 to 0.5 ppm in fresh waters and from 0.0016 to 0.05 ppm in oceanic waters [8], but levels as high as 80 ppm have been observed in paper mill effluents [9].

2. Chromium transport and accumulation

Chromate is actively transported across biological membranes in both prokaryotes [10] and eukaryotes [11,12]. Once inside the cells, Cr(VI) is reduced to Cr(III) probably via the unstable Cr(V) and Cr(IV) states ([13,14], see [15] for an opposite result). In contrast, most cells are impermeable to Cr(III) probably because it forms water insoluble compounds in non-acidic aqueous solutions [16–18].

2.1. Microbial chromium transport and accumulation

The transport of chromate through the sulfate transport system was first demonstrated in *Salmonella typhimurium* [10,19,20] and later in *Escherichia coli* [21,22], *Pseudomonas fluorescens* [23] and *Alcaligenes eutrophus* [24]. Energy-dependent Cr(VI) uptake in the cyanobacterium *Anabaena doliolum* [25] showed a biphasic behavior and Cr concentration dependence.

In contrast to other metals, which predominantly form cationic species, Cr exists mainly in the oxyanion form (i.e. CrO_4^{2-}) and thus cannot be trapped by the anionic components of bacterial envelopes [26]. However, cationic Cr(III) derivatives bind tightly to *Salmonella* lipopolysaccharides [27], *Bacillus subtilis* and *E. coli* cell walls [28], and capsular polymers of *Bacillus licheniformis* [29].

Data on chromium transport in algae are scarce [30]. Taxa differences in Cr accumulation rates have been reported: green algae retain more Cr (as well as Al and Fe) than brown or red algae [8]. Epiphytic (organisms which live on plants) algae are considered to have a high affinity for atmospheric pollutants and the ability to accumulate heavy metals from the air. Cr and Pb contents were elevated in the epiphytic alga *Pleurococcus* sp. in sites close to motorways [31].

In yeasts, Cr(VI) may enter cells via a non-specific anion carrier, the permease system, which transports different anions such as sulfate and phosphate [32]. Some chromate-resistant mutants of *Neurospora crassa* showed strongly reduced sulfate transport properties [33]. Further studies revealed that Cr(VI) toxicity was due to its specific antagonism to sulfate uptake, whereas Cr(III) toxicity resulted from antagonism with iron transport [34]. By using culture media with different sulfur sources, it was shown that the sulfate transport system is also used for chromate uptake in *Candida* sp., and to some degree in *Saccharomyces cerevisiae* and *Candida famata* [35].

2.2. Chromium transport and accumulation in plants

There are conflicting results regarding which form of Cr is taken up and accumulated by plants. The interconversion of different Cr forms is quite frequent in soil and hydroponic media during long-term experiments, making the analyses difficult. In studies with bean (*Phaseolus vulgaris* L.) and wheat (*Triticum aestivum* L.) no differences in the uptake of either Cr(III) or Cr(VI) were detected [36]. However, ^{51}Cr compounds were not analyzed in the media and thus no information on Cr interconversion was provided. Cr complexation with organic compounds is involved in facilitating Cr availability to plants [37]. For

instance, wheat (*Triticum vulgare*) plants grown in hydroponic cultures with $CrCl_3$ and oxalic acid, malate, or glycine accumulated more Cr in their roots than plants exposed to Cr alone.

Evidence for independent uptake mechanisms for Cr^{3+} and CrO_4^{2-} in plants has been presented. By using metabolic inhibitors, a substantial decrease in CrO_4^{2-} but not in Cr^{3+} uptake was observed in barley seedlings, indicating that Cr(VI) uptake depends on metabolic energy whereas Cr(III) uptake does not [38]. In contrast, an active uptake of both Cr species, slightly higher for Cr^{3+} than for CrO_4^{2-}, has also been reported in the same crop [39]. As in microorganisms, the sulfate transport system is apparently involved in CrO_4^{2-} uptake by plants, since sulfate inhibits competitively CrO_4^{2-} uptake in barley seedlings [40].

The translocation and accumulation of Cr inside the plant depends on the oxidation state of the supply [41], the concentration of Cr in the media [42], as well as on the plant species. In seven out of 10 crop species analyzed, more Cr was accumulated when plants were grown with Cr(VI) than with Cr(III) [15]. By incubating barley seedlings with $^{51}Cr^{3+}$ or $^{51}CrO_4^{2-}$, a higher content of ^{51}Cr was found in shoots when provided as CrO_4^{2-}, suggesting that Cr is transported largely by the xylem [38]. CrO_4^{2-} moves faster in the xylem than Cr^{3+} presumably because the latter is held up by electrostatic interaction with the vessel walls, as occurs for Ca^{2+} [38]. Controversial results emerged from experiments when plants were grown with either Cr(III) or Cr(VI) and only the Cr(III) form was found in their tissues [15]. These data suggest that Cr(VI) is transformed to Cr(III) mainly inside the root cells, but also in the aerial part of the plant.

Differential accumulation of Cr in plant organs has been observed. Roots accumulate 10–100 times more Cr than shoots and other tissues [15,37,38,43]. In bean, as few as 0.1% of total Cr accumulated is found in seeds, whereas roots concentrate 98% of total Cr uptake [36]. Shallari et al. [44], collecting plants growing in serpentine soils, found that *Herniaria hirusta* was a Cr high-accumulator. Also water hyacinth (*Eichhornia crassipes*) has shown a high capacity to accumulate Cr: 6 mg g^{-1} DW have been detected in roots of this plant growing in 10 ppm Cr(VI) [45]. Huge values of Cr accumulation (160–350 mg Cr/kg DW in roots and 1.6–2.0 mg Cr/kg DW in shoots) were observed for cauliflower, kale, and cabbage, plants known as sulfur-loving species, but not for peas, strawberry, or lettuce [15]. *Brassica* spp. showed a higher ability to take up and accumulate Cr and other heavy metals than other plant species [46]. The sulfate transport system may be responsible for the high Cr uptake of these plants.

3. Chromium toxicity

As previously discussed, the biological effects of Cr depend on its oxidation state: Cr(VI) is highly toxic to most organisms whereas Cr(III) is relatively innocuous [47,48]. Cr toxicity is related to the process of reduction of Cr(VI) to lower oxidation states, not necessarily to Cr(III) [49], in which free radicals are generated [50]. Reduction of Cr(VI) to Cr(III) has been reported in many biological systems; transient formation of Cr(V) is the most likely mechanism involved in Cr toxicity [49]. Cr(V) complexes are formed from Cr(VI) by physiological reducing agents such as NAD(P)H, $FADH_2$, several pentoses, and glutathione [51,52]. These complexes react with H_2O_2 to generate significant amounts of •OH radicals with no associated generation of O_2^-. The •OH radicals may trigger directly DNA alterations as well as other toxic effects [51].

Additional intracellular chromate-reducing agents are vitamins C and B_{12}, cytochrome P-450, and the mitochondrial respiratory chain [18]. Intracellular Cr(III) may be sequestered by DNA phosphate groups affecting replication, transcription and causing mutagenesis [53–56]. Oxidative damage on DNA is considered the basis of the genotoxic effects produced by Cr [57–59]. Cr(III) may also react with carboxyl and sulfhydryl groups of enzymes causing alterations in their structure and activities [17]. Modification of the DNA polymerase and other enzyme activities may be caused by the displacement of magnesium ions by Cr(III) [60].

3.1. Chromium toxicity in microorganisms

Toxic effects of Cr on bacteria and algae have been reviewed by Wong and Trevors [47]. In vivo generation of Cr(V) from Cr(VI) by the algae *Spirogyra* and *Mougeotia* has been described [14]. The growth of *Chlorella vulgaris* was unaffected by 45–100 ppm of Cr(III) or Cr(VI), whereas no growth of *Scenedesmus acutus* was detected at concentrations of Cr higher than 15 ppm [61]. However, Brady et al. [62] reported algal colony growth of *Scenedesmus* and *Selenastrum* with 100 ppm Cr(III) but not with 100 ppm Cr(VI). The mechanism underlying the different sensitivity to Cr in algae remains to be elucidated.

A lengthening in the lag growth phase induced by Cr(VI) and a decreased growth rate caused by Cr(III) has been observed in *Euglena gracilis* [63]. Inhibition of growth in *Euglena* correlates with the arrest of cells in the G-2 phase of the cell cycle and inhibition of respiration and photosynthesis [64]. Cr(VI) also induced alterations in the cytoskeleton which may be involved in the loss of motility [65]. Inhibition of photosynthesis by Cr has also been reported for *Chlorella* [47] and *Scenedesmus* [66].

Light-grown *Euglena* cells were more sensitive to Cr(VI) than dark-grown cells (Fig. 1). Lower concentrations of Cr were well tolerated in both culture conditions (Fig. 1). In estuarine algae, Cr(VI) toxicity is inversely proportional to salinity [67,8]. In *Scenedesmus*, Cr and Cu showed a synergistic effect with Cd for growth inhibition [68].

In *S. cerevisiae*, chromate toxicity was stronger in cells

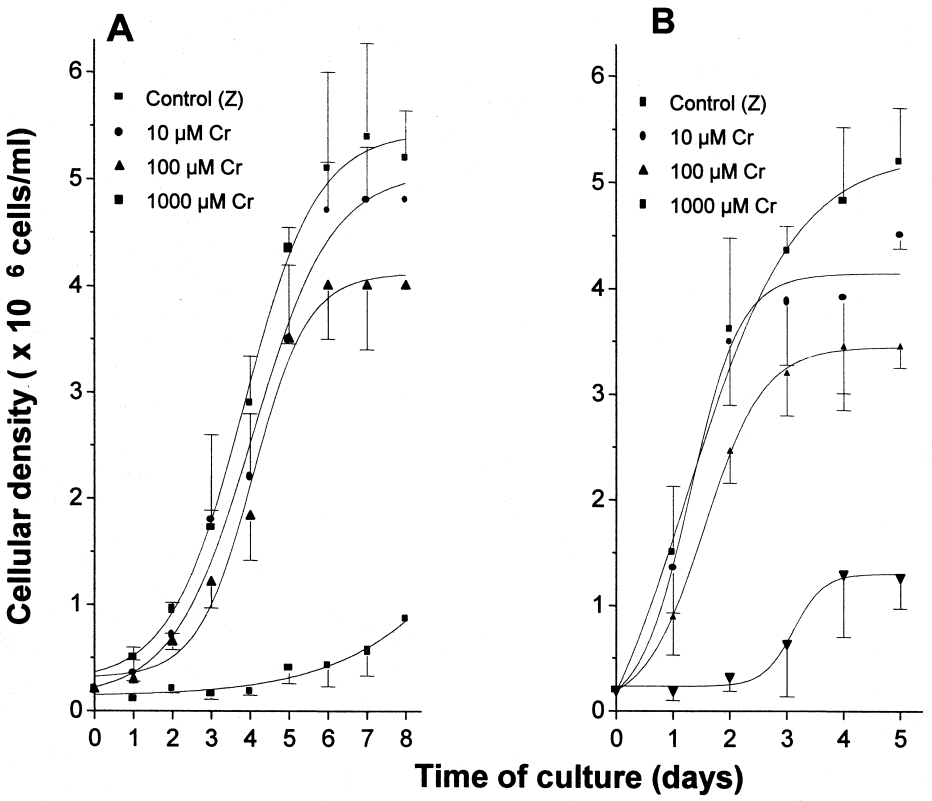

Fig. 1. Effect of chromate on the growth of *Euglena*. *E. gracilis* strain Z was cultured under white fluorescent light–dark (12 h/12 h) regime (A) or in complete darkness (B). Cultures were incubated at 25°C without shaking (A) or under orbital shaking (B) with the indicated K_2CrO_4 concentrations. Cells were counted with a Neubauer chamber ($n = 3$, mean ± S.D.). Cell viability: (A) control 98%, 10 μM 96%, 100 μM 91%, 1000 μM 76%; (B) control 99%, 10 μM 99%, 100 μM 93%, 1000 μM 81%. Devars et al., unpublished data.

grown in non-fermentable carbon sources than in those grown in fermentable substrates [69]; other effects included inhibition of oxygen uptake [69,70] and induction of *petite* mutations. These results suggest that chromate targets specifically the mitochondria of *S. cerevisiae* [69]. Additional effects of Cr in *S. cerevisiae* include gene conversion [71,72] and mutation [71–73].

3.2. Chromium toxicity in plants

Whereas Cr(VI) has been demonstrated to produce serious damage to living cells, it is considered that Cr(III) is less toxic because of its extremely low solubility, which prevents its leaching into ground water or its uptake by plants. However, studies in plants have shown that Cr(III) also produces serious problems in living tissues although at higher concentrations than Cr(VI). Barley seedlings grown with 100 μM Cr(III) showed 40% of growth inhibition whereas inhibition caused by the same Cr(VI) level reached up to 75% in shoots and 90% in roots [38].

Progressive stages of chlorosis and necrosis are symptoms of Cr toxicity in plants. Barley plants exposed to 50 ppm Cr(VI) remained vital although with altered appearance; further exposure to 100 ppm caused a stressed appearance after 2 days, and after 7–10 days all barley plants died. Toxic symptoms produced by Cr(VI) are stronger than those caused by Cr(III), and occur earlier and at lower concentrations [74].

A decrease in protein content and in nitrate reductase activity analyzed in vitro has been reported [2]. Cr also elicits the synthesis of polyamines in barley; Cr(VI) was a faster and more efficient inducer of putrescine synthesis than Cr(III). Shortly after putrescine induction, decrease in growth, chlorosis, induction of leaf chitinase activity and later reduction of shoot growth and lowered water content in leaves were observed [74].

Micronuclei formation and chromosome aberrations have been observed in *Vicia faba* and *Allium cepa* root tips exposed to heavy metals [75,76]. Although micronuclei formation correlated with Cr levels detected in contaminated soils [77], the levels of other heavy metals present in the soil samples were not measured, and hence the possibility that the nuclear aberrations observed could be due to the presence of other heavy metals remains.

4. Resistance to chromium compounds

4.1. Bacterial plasmid-mediated resistance

A variety of chromate-resistant bacterial isolates has been reported, including strains from environmental and

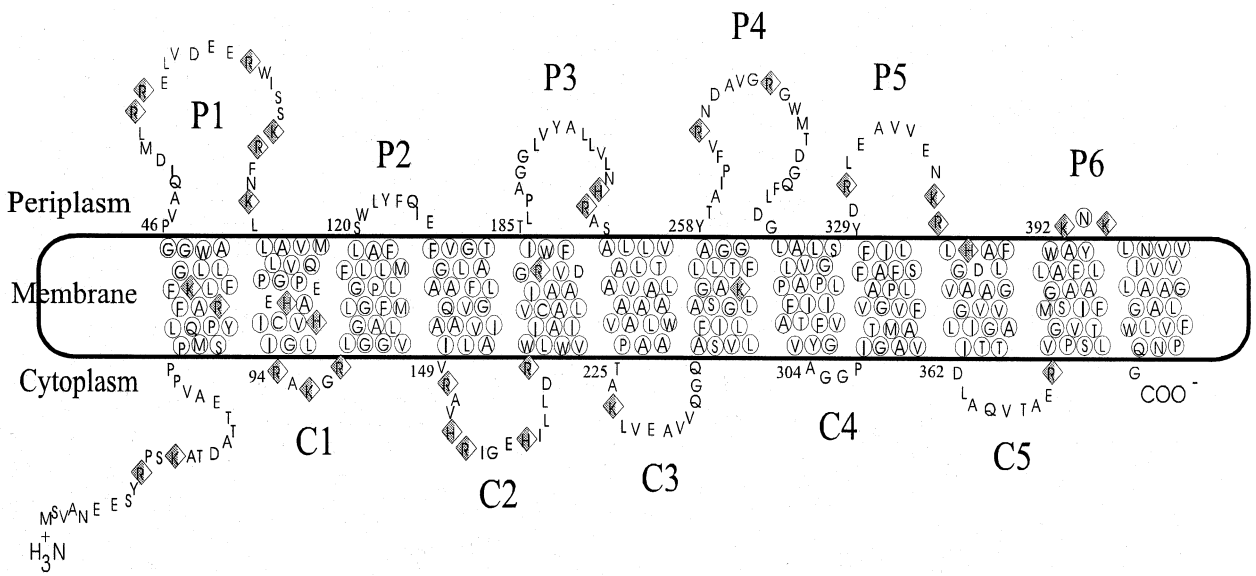

Fig. 2. Topological model of the ChrA chromate resistance protein from *Pseudomonas aeruginosa*. The scheme shows the amino acid sequence of ChrA (416 aa) and its probable location within the inner membrane (according to hydropathy profiles obtained with the DNAstar software). The model proposes 12 transmembrane spans (circled residues). Cytoplasmic (C) and periplasmic (P) numbered loops and the NH_3^+ and COO^- ends are also shown. Basic residues (◇), probably involved in chromate binding, are highlighted.

clinical settings; in these natural isolates chromate tolerance is usually associated with plasmids [78]. The involvement of sulfate transport in plasmid-conferred chromate resistance has been ruled out in both *P. fluorescens* [23] and *A. eutrophus* [24]: resistant strains with plasmids transported sulfate with a similar kinetics to that shown by plasmid-less chromate-sensitive strains. Bopp and Ehrlich [79] also showed that Cr(VI) reduction is independent of the chromate resistance mechanism conferred by plasmid pLHB1 of *P. fluorescens*. Dhakephalkar et al. [80] reported that plasmid pARI180, from a *Pseudomonas mendocina* strain, determined both chromate resistance and reduction. The mechanism of resistance has not been elucidated yet.

Two plasmid-associated chromate resistance determinants have been analyzed to the molecular level: those from plasmids pUM505 of *P. aeruginosa* [81] and pMOL28 of *A. eutrophus* [82]. The chromate resistance *chrA* genes were cloned and sequenced. From the nucleotide sequence it was deduced that pUM505 and pMOL28 encode hydrophobic proteins, named ChrA, of 416 and 401 amino acid residues, respectively [81,82]. The ChrA polypeptides shared 29% of identical residues (Table 1). ChrA from both *Pseudomonas* and *Alcaligenes* were expressed in *E. coli* but they did not confer Cr resistance in this host [81,82]. A second gene, *chrB*, was found in the *Alcaligenes* determinant but not in that of *Pseudomonas* and was assigned a role in the regulation (induction) by chromate of the resistance phenotype [82].

Based on their amino acid composition and hydropathic profiles, ChrA from both *Pseudomonas* and *Alcaligenes* are considered as membrane proteins probably having 12 transmembrane spans (Fig. 2) [78], as has been shown for other inner membrane transport proteins [83]; Nies et al. [84] suggested that the ChrA proteins have only 10 transmembrane spans. Further ChrA homologs have been found from protein sequence databases, including cyanobacterial and Archaeal outputs (Table 1). The role of these putative ChrA homologs in chromate resistance has not been established. Sulfur-regulated protein SrpC from a

Table 1
Similarity of ChrA protein homologs

Protein (bacteria)	Size (aa)	Identical amino acids (%)[a]	Hydrophobic amino acids (%)	Reference
ChrA (*P. aeruginosa*)	416	–	63	[81]
ChrA (*A. eutrophus*)	401	29	63	[82]
SrpC (*Synechococcus*)	393	23[b]	58	[85]
X* (*Synechocystis*)	399	30	60	[131]
MJ0718 (*Methanococcus*)	402	26	60	[132]
ArsB (*E. coli*)[c]	429	20	62	[133]

[a]As compared with *P. aeruginosa* ChrA protein.
[b]Shares 62% identity with *A. eutrophus* ChrA protein.
[c]Membrane protein from the arsenic-resistance operon of *E. coli* plasmid R773.

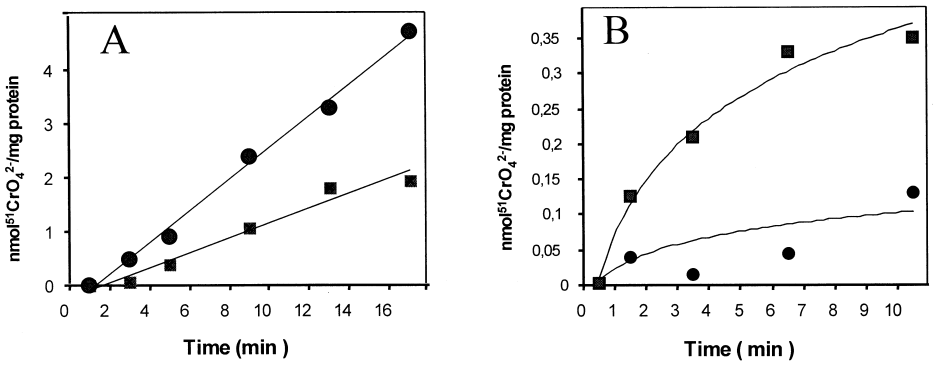

Fig. 3. Chromate uptake by *P. aeruginosa*. Intact cells (A) or everted membrane vesicles (B) of chromate-sensitive PAO1 strain (●) and chromate-resistant PAO1(pCRO616) strain (■) were tested. A: Bacterial suspensions of exponential phase cultures were incubated at 37°C with 50 μM $Na_2^{51}CrO_4$ and the radioactivity incorporated to cells was measured after filtration. B: Everted membrane vesicles were incubated with 10 μM $Na_2^{51}CrO_4$ and processed as in (A). Alvarez et al., unpublished data.

plasmid of the cyanobacterium *Synechococcus* also showed similarity to ChrA (Table 1); SrpC seems to be involved in sulfate transport, but not in chromate resistance [85]. Amino acid composition and hydropathic profiles of ChrA are similar to those from ArsB, the inner membrane protein of the arsenic resistance operons which extrudes arsenite in several bacterial species [86] (Table 1). ArsB also possess 12 transmembrane segments [86]. Thus, a chromate efflux system was postulated as the mechanism of chromate resistance by ChrA [78].

In agreement with its apparent membrane location, ChrA caused a diminished chromate uptake in *Pseudomonas*-resistant strains [81,87] (Fig. 3A). Similar results were also found in *Alcaligenes* [82]. A reduced chromate uptake has also been observed in plasmid-containing cells of *P. fluorescens* [23] and *Enterobacter cloacae* [88]. Nies et al. [84] suggested that ChrA proteins may function as chromate/sulfate antiporters although no experimental evidence has been obtained in that direction.

Using everted membrane vesicles it was recently demonstrated that resistance to chromate conferred by ChrA in *P. aeruginosa* is based on an efflux system that extrudes chromate from the cytoplasm [87]. Vesicles obtained from the resistant strain accumulated significantly more chromate than those from the sensitive one (Fig. 3B). Cr accumulation followed a Michaelis–Menten kinetics with a K_m of 0.12 mM chromate; interestingly, this K_m value was similar to that of 0.14 mM reported for the efflux of arsenite by ArsB [89]. It was also shown that chromate efflux is dependent on NADH addition and it was abolished by respiratory-chain inhibitors and uncouplers, suggesting that chromate efflux depends on the membrane potential [87]. Chromate efflux by membrane vesicles was also inhibited by sulfate, suggesting that ChrA binds sulfate.

4.2. Bacterial Cr(VI) reduction

Reduction of Cr(VI) to less toxic Cr(III) has been suggested as an additional chromosome-encoded chromate-resistance mechanism, in addition to the plasmid-encoded tolerance (reviewed by Cervantes and Silver [78] and Ohtake and Silver [90]). *E. cloacae* HO1 strain, described as chromate-resistant [91], is the most studied example of Cr(VI)-reducing bacteria [90]. *Bacillus* sp. QC1-2, a strain isolated from a Cr-polluted zone, was also selected by its ability to both tolerate chromate and reduce Cr(VI) to Cr(III) (Fig. 4) [92]. A Cr(VI)-reducing activity was found in both soluble and membrane fractions of *Desulfovibrio vulgaris* [93]. Cytochrome *c3* was reported to catalyze Cr(VI) and uranium [U(VI)] reduction in *D. vulgaris* [94], suggesting that this cytochrome may function as both U(VI) and Cr(VI) reductase.

4.3. Chromium resistance in algae

Only a few reports on Cr resistance in algae have been

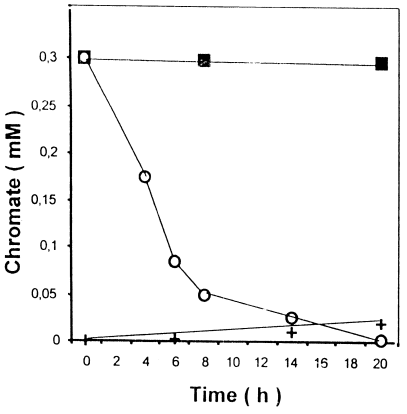

Fig. 4. Chromate reduction by *Bacillus* sp. QC1-2. Cell suspensions (ca. 1×10^9 cells ml^{-1}) containing 0.3 mM chromate were incubated at 30°C for the indicated times. After centrifugation, Cr(VI) was quantified in the supernatant (○) and in the pellet (+). Total chromium was also measured in the supernatant (■). Total chromium and Cr(VI) were measured by atomic absorption spectrophotometry and by a spectrophotometric method using diphenylcarbazide, respectively; Cr(III) was obtained by the difference between total chromium and Cr(VI). Adapted from Campos et al. (1995) with permission.

published and no details on tolerance mechanisms are known. *S. acutus* develops tolerance to Cr(VI) after being treated with chromate, leading to a Cr-tolerant progeny by sexual reproduction; with chromate, asexual reproduction does not occur [95]. Induction of plastid-deprived gametes is favored in the presence of Cr [66]. Such interference of Cr with plastidial metabolism has been described in *Scenedesmus* [96], *Chlorella* [97], and *Euglena* [64]. Interestingly, tolerance to Cr(VI) in *Scenedesmus* is not related to a decreased metal uptake [98]. The Cr-tolerant strain is less sensitive to copper but not to zinc [99]. *S. acutus* cultured in the presence of Cr(VI) forms aggregates; when the aggregates break up, biflagellated cells are released, allowing the cell to survive [95].

Analysis of metal pollution in a river, receiving waste from a paper mill, revealed that Cr reached toxic values of 20–80 ppm at the point of discharge and 1 km away [9]. Several algae showed tolerance to these Cr levels, which promoted increased populations, particularly of *Oscillatoria*, *Phormidium*, *Scenedesmus* and *Pandorina* [9].

4.4. Chromium resistance in fungi

Chromium resistance has been described in filamentous fungi and yeasts, either by mutagenic induction of laboratory strains [33,100] or through the isolation of Cr-resistant organisms from Cr-contaminated environments [35,101–103]. In the filamentous fungus *N. crassa*, chromate-resistant mutants induced by UV treatment were defective in sulfate transport [33]. Previous observations linked a defect in sulfate uptake of *S. cerevisiae* to an increased resistance to chromate and selenate, ions that share the same permeases [104–106]. It was proposed that this defect caused *S. cerevisiae* to down-regulate its sulfate uptake system; this lowered available sulfur led to the replacement of non-essential sulfur amino acids in proteins, resulting in a significantly lower cysteine content compared to other eukaryotes [107].

In *Schizosaccharomyces pombe*, UV- and nitrosoguanidine-mutagenesis led to the isolation of chromate-sensitive and -tolerant mutants [100]. Growing cells of the sensitive mutant exhibited high accumulation of total Cr, whereas those of the resistant mutant showed a significantly lower accumulation of the metal. These observations suggested that chromate resistance was due to reduced uptake of the metal [100]. The analysis of chromate-sensitive mutants of *S. cerevisiae* showed the involvement of at least six loci in the maintenance of wild-type levels of resistance to chromate [108]. One of these loci was identified as *LYS7* [108], which is the structural gene for homocitric dehydratase, an enzyme involved in lysine biosynthesis; however, the role of this enzyme in Cr detoxification is unclear.

Mutants of *S. cerevisiae* devoid of vacuolar structures or deficient in specific protein subunits of the vacuolar (V)-H(+) ATPase showed increased sensitivity to chromate and tellurite; such sensitivity was associated to an increased accumulation of Cr and Te [109]. The accumulation of both Cr and Te occurred mainly in the cytosol, whereas detoxification was influenced by the presence of a functionally active vacuole [109].

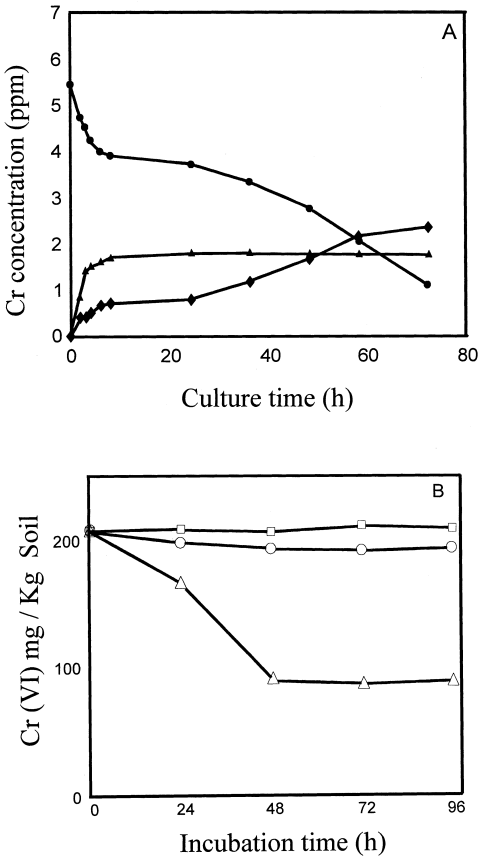

Fig. 5. Interactions of *Candida* cells with chromium. A: Distribution of chromium during the growth of *Candida* sp. strain RR1. (●), Cr(VI) or (◆), Cr(III) remaining in the spent medium; (▲), total Cr in the biomass. B: Decrease of Cr(VI) in Cr-contaminated soil inoculated with strain RR1. (○), Non-inoculated control; (△), inoculated soil; (□), inoculated soil with 2 mM sodium azide. Chromium was measured as described in Fig. 4. Ramírez-Ramírez et al., unpublished results.

Yeast strains isolated from Cr-contaminated environments include the genus *Candida* [35,101–103] and to a minor extent *Rhodosporidium* [35]. These yeasts are resistant to high concentrations of chromate as compared to the most common *S. cerevisiae*, which is more sensitive [35,101–103]. In some species of *Candida* and *Rhodosporidium*, the general mechanism of chromate resistance is related to reduced ion uptake, rather than to biological reduction of Cr(VI) to Cr(III), since Cr accumulation was much higher in chromate-sensitive yeasts *S. cerevisiae* and *C. famata* [35,101].

Strain RR1 of *Candida* sp., isolated from a leather tanning plant, was able to grow in the presence of 2 mM chromate. RR1 caused the removal of about 60% of total Cr(VI) initially present in the medium after 14 h incubation [103] (Fig. 5A). Laboratory strains of the zygomycete fungus *Mucor rouxii* and the yeasts *Candida albicans*, *S. cerevisiae* and *Yarrowia lipolytica* were unable to grow

under these conditions and showed no effect on the concentration of chromate in the medium [103]. When *Candida* strain RR1 was cultured for longer periods with chromate, there was a further increase in ion removal that correlated with the appearance of Cr(III) in the medium (Fig. 5A). These observations indicated that in strain RR1 exists a causal correlation between Cr(VI) reduction and Cr(VI) resistance.

4.5. Chromium resistance in plants

The mechanisms of Cr resistance in plants have not been elucidated. The observation that members of the Brassicaceae family are high Cr accumulators makes these plants suitable models for the analysis of Cr resistance mechanisms [15]. Natural resistance to heavy metals has been observed in liverworts growing in contaminated soils and in aquatic macrophytes growing in polluted waters [44,110–112]. From these studies, a correlation between contents of Cr in soil and the presence of the macrophyte *Comocephalum conicum* has been established, suggesting that this plant may be useful in monitoring soil contamination by metals [111].

Plants may also be able to reduce Cr(VI) to Cr(III), a detoxification reaction that very likely occurs in roots, and that may be catalyzed by Cr reductases similar to those found in bacteria. However, no such enzymes have been identified in plants [45].

5. Bioremediation of chromium pollution

Chromium, with its great economic importance in industrial grounds, is a major metal pollutant of the environment [113]. To our knowledge, no large-scale processes to bioremediate Cr pollution have been implemented. Thus, we will describe some laboratory demonstrations of Cr decontamination with microorganisms and plants which might be applicable to Cr bioremediation. Biological reduction of Cr(VI) to Cr(III) is a potentially useful mechanism to detoxify Cr pollution and to bioremediate contaminated wastes [90,114]. However, as Cr(III) is also harmful to several organisms, the reduction of Cr(VI) together with Cr(III) compartmentation could be a valuable strategy to control Cr dispersion. Subcellular compartmentation may be carried out more efficiently by plants.

5.1. Bioremediation with bacteria

The Cr(VI)-reducing ability found in some bacteria (Table 2) has raised the possibility of using these microorganisms as a biotechnological tool for remediation of chromate-polluted zones [90,114–116]. The main advantages of using bacterial Cr(VI) reduction are that it does not require high energy input nor toxic chemical reagents and the possibility of using native, non-hazardous strains. Several microbiological strategies to remove chromate involving Cr(VI) reduction have been extensively reviewed by Ohtake and Silver [90] and will not be detailed here.

A distinct type of bacterial interaction with Cr is extracellular precipitation. Since insoluble metallic complexes are usually less toxic than ionic forms, precipitation is considered a detoxification mechanism [117]. Precipitation of Cr has been reported in anaerobic *Clostridium* [118] and by sulfate-reducing bacteria [119].

Engineering of bacterial heavy-metal resistance genes have resulted in the generation of biosensors for several toxic metals [120]. Peitzsch et al. [121] reported a chromate sensor consisting of the transcriptional fusion of *chr* chromate-resistance genes and *lux* reporter genes. These fusions, expressed in *A. eutrophus*, responded to micromolar concentrations of chromate and dichromate ions but were 10 times less sensitive to Cr(III) compounds; moreover, no signal was observed with other oxyanions. The authors proposed that this biosensor may be used to monitor chromate levels in industrial sewage waters [121].

5.2. Bioremediation with algae

Adsorption of Cr(III) by heat-dried biomass of the cyanobacterium *Phormidium laminosum* has been reported [122]. Binding of Cr(III) by microalgae was enhanced at lower pH values (from 3.5 to 5.5) than that of Cr(VI), which was in turn favored at pH 2.0 or lower [123]. These results demonstrate that optimal pH for bioremoval also depends on the oxidation state of Cr. Reduction of Cr(VI) to Cr(III) was increased from 25 to 55°C [123].

The filamentous alga *Cladophora* accumulated several

Table 2
Properties of bacterial Cr(VI) reductases

Bacteria	Reduction conditions	Enzyme location	Electron donor	Molecular mass (kDa)	Reference
E. cloacae	Ana	M	NADH	ND	[91]
Pseudomonas ambigua	Aer	S	NAD(P)H	65	[134]
Bacillus sp.	Aer and Ana	S	NADH	44	[135]
P. fluorescens	Aer and Ana	M	ND	ND	[79]
P. putida	Aer	S	NAD(P)H	ND	[136]
E. coli	Aer and Ana	S	ND	ND	[137]
D. vulgaris	Ana	S and M	H_2	ND	[93]

Abbreviations: Ana, anaerobic; Aer, aerobic; M, membrane; S, soluble; ND, non-determined.

heavy metals, but Cr was the metal with the higher accumulation and the faster uptake rate (72% after 15 min) [124]. *Selenastrum* removed 39% of the Cr in solution from samples of a post-anaerobic digester in a tannery effluent [62]. Cr(III) was effectively removed (83–99%) in laboratory tests by *Scenedesmus*, *Selenastrum* and *Chlorella*, whereas Cr(VI) was removed in a surprisingly lower degree (18–22%) [62]. The lower Cr removal from wastewater in comparison to artificial solutions may be due to competition with chelating compounds including organic matter. Using immobilized cells in columns with kappa-carragenan (fluidized bed) or polyurethane foam (packed bed), *C. vulgaris* removed 48 and 34% of Cr, respectively, whereas *S. acutus* removed 36 and 31% [61]. Since the Cr concentration used was well above the level of industrial effluents, a possible use of immobilized algae in wastewater treatment seems viable.

The removal of Cu, Ni, Al and Cr by the red alga *Cyanidium caldarium* from acidic mine wastes occurs by cell surface precipitation of metal-sulfide microcrystals [125]. Thus, this alga may be a suitable organism for removal or recovery of metals from heavy-metal-polluted wastewaters.

5.3. Bioremediation with fungi

Yeasts and filamentous fungi also offer a viable alternative for the bioremediation of waters and soils polluted by Cr. However, no practical use of fungal cells in Cr bioremediation has been reported. Many fungi contain chitin, the homopolymer of *N*-acetylglucosamine, and chitosan, a heteropolymer of *N*-acetylglucosamine and glucosamine, as an integral part of their cell wall structure. The deacetylated amino groups of glucosamine may act as binding sites for metals [113]. In addition, a new siderophore type, named rhizoferrin, which shows increased Cr(III) biosorption, has been identified in Mucorales. Rhizoferrin is a polycarboxylate siderophore that is able to bind Fe(III), Cr(III), and Al(III). Trivalent Cr and Al should not be reduced under physiological conditions. These metals remain bound to the siderophore and may be accumulated by the cells in a further stage; for some microorganisms this can be a mechanism of resistance to toxic metals [113].

Chemically treated mycelia from *Mucor mucedo* and *Rhizomucor miehei* efficiently bind Cr [126]. The biomasses obtained from *Rhizomucor arrhizu*, *Candida tropicalis* and *Penicillium chrysogenum* were excellent biosorbents of Cr [26]. *Aspergillus carbonarius* NRC401121 adsorbs Cr from solution and the amount of adsorbed metal per biomass unit increased with a decrease in the biomass concentration [127]. *Mucor hiemalis* MP/92/3/4 was also able to accumulate substantial amounts of Cr. Binding of Cr(III) by *M. hiemalis* seemed to be mainly a passive biosorption to the cell wall, whereas for the uptake of Cr(VI) intracellular accumulation as well as biosorption are possible [113]. *S. cerevisiae* and *Candida utilis* have the ability to sorb Cr(VI) and the sorption capacity of dehydrated cells is considerably higher than that of intact cells [128].

Although in most cases Cr accumulation in the chromate-resistant fungi was lower than in chromate-sensitive strains, the biosorption and bioaccumulation processes were similar [100]. In the context of bioremediation, the biosorption ability of chromate-resistant mutants could be combined with their ability to reduce chromate. Chromate-resistant strains of *Aspergillus* spp. [129] and *Candida* spp. [103], isolated from Cr-polluted environments, have shown Cr(VI)-reducing activity. As previously mentioned, the latter strain caused a decrease in Cr(VI) from the culture medium which was accompanied by the appearance of Cr(III) (Fig. 5A). This *Candida* spp. strain also decreased by 40% the amount of Cr(VI) when incubated in Cr-contaminated soil (Fig. 5B). This observation suggests that Cr-resistant fungi with Cr(VI)-reducing activity have a good potential for bioremediation of Cr-polluted soils.

5.4. Bioremediation with plants

Although the use of plants to remediate contaminated soils (phytoremediation) is in its initial stages of research and development, it is a possibility for the detoxification of heavy-metal-polluted areas. This technology is based on the ability of the plant to absorb, sequester, and/or transform a contaminant [130]. Plants, because of their size, can be easily managed, their distribution can be controlled, and they can be readily processed for recovering valuable metals. As mentioned above, the macrophyte *C. conicum* has been proposed as a tool for monitoring soil contamination by Cr [111].

In contrast to cadmium and mercury, in which several strategies have been successfully applied to generate plants able to accumulate or transform these metals [130], there are only a few reports on Cr phytoremediation. Recent works provide evidence that some members of the Brassicaceae family [15,46], as well as water hyacinth [45], could be high Cr accumulators. Although these plants do not fulfill the hyperaccumulator criteria, since they do not accumulate higher levels of Cr in their shoots, they seem currently the best option available as Cr phytoremediators. The reduction of Cr(VI) to Cr(III) by these plants [15] will obviously not terminate the problem of Cr contamination, since Cr(III) is also detrimental for plant growth; however, their use will produce a less harmful and more manageable compound.

6. Concluding remarks

Microbes and plants display diverse interactions with Cr in the environment. Bacterial plasmid-encoded chromate resistance genes have been cloned and sequenced. The mechanism of tolerance is based on the efflux of chromate

ions. Cr(VI) reduction by bacteria has been largely postulated as a good candidate for bioremediation. In the case of fungi, most of the reports link chromate resistance to modifications in ion uptake systems. A few studies describe Cr(VI)-reducing activities in fungi and plants (but not in algae yet) and the possible relationship of this process with chromate resistance and bioremediation. Algal and fungal biomasses have proven useful in biosorption studies for the removal of Cr from contaminated sources. Although still poorly understood, plant Cr bioaccumulators seem promising as Cr bioremediation tools.

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References

[1] McGrath, S.P. and Smith, S. (1990) Chromium and nickel. In: Heavy Metals in Soils (Alloway, B.J., Ed.), pp. 125–150. Wiley, New York.
[2] Vajpayee, P., Sharma, S.C., Tripathi, R.D., Rai, U.N. and Yunus, M. (1999) Bioaccumulation of chromium and toxicity to photosynthetic pigments, nitrate reductase activity and protein content of *Nelumbo nucifera* Gaertn. Chemosphere 39, 2159–2169.
[3] Shewry, P.R. and Peterson, P.J. (1976) Distribution of chromium and nickel in plants and soil from serpentine and other sites. J. Ecol. 64, 195–212.
[4] Stern, R.M. (1982) Chromium compounds: production and occupational exposure. In: Biological and Environmental Aspects of Chromium (Langard, S., Ed.), pp. 5–47. Elsevier, Amsterdam.
[5] Papp, J.F. (1985) Chromium. In: Mineral Facts and Problems. Bureau of Mines bulletin 675 (Knoerr, A.W., Ed.), pp. 139–155. U.S. Government Printing Office, Washington, D.C.
[6] Khasim, D.I., Kumar, N.V. and Hussain, R.C. (1989) Environmental contamination of chromium in agricultural and animal products near a chromate industry. Bull. Environ. Contam. Toxicol. 43, 742–746.
[7] Armienta-Hernández, M.A. and Rodríguez-Castillo, R. (1995) Environmental exposure to chromium compounds in the valley of León, México. Environ. Health Perspect. 103, 47–51.
[8] De Filippis, L.F. and Pallaghy, C.K. (1994) Heavy metals: sources and biological effects. In: Advances in Limnology Series: Algae and Water Pollution (Rai, L.C., Gaur, J.P. and Soeder, C. J., Eds.), pp. 31–77. E. Scheizerbartsche Press, Stuttgart.
[9] Sudhakar, G., Jyothi, B. and Venkateswarlu, V. (1991) Metal pollution and its impact on algae in flowing waters in India. Arch. Environ. Contam. Toxicol. 21, 556–566.
[10] Dreyfuss, J. (1964) Characterization of a sulfate and thiosulfate transporting system in *Salmonella typhimurium*. J. Biol. Chem. 239, 2292–2297.
[11] Wiegand, H.J., Ottenweilder, H. and Bolt, H.M. (1985) Fast uptake kinetics in vitro of ^{51}Cr(VI) by red blood cells of man and rat. Arch. Toxicol. 57, 31–34.
[12] Alexander, J. and Aashet, J. (1995) Uptake of chromate in human red blood cells and isolated rat liver cells: the role of the anion carrier. Analyst 120, 931–933.
[13] Arslan, P., Beltrame, M. and Tomasi, A. (1987) Intracellular chromium reduction. Biochim. Biophys. Acta 931, 10–15.
[14] Liu, K.J., Jiang, J., Shi, X., Gabrys, H., Walczak, T. and Swartz, M. (1995) Low-frequency EPR study of chromium (V) formation from chromium (VI) in living plants. Biochem. Biophys. Res. Commun. 206, 829–834.
[15] Zayed, A., Lytle, C.M., Qian, J.H. and Terry, N. (1998) Chromium accumulation, translocation and chemical speciation in vegetable crops. Planta 206, 293–299.
[16] Cary, E.E. (1982) Chromium in air, soil and natural waters. In: Biological and Environmental Aspects of Chromium (Langard, S., Ed.), pp. 48–64. Elsevier, Amsterdam.
[17] Levis, A.G. and Bianchi, V. (1982) Mutagenic and cytogenetic effects of chromium compounds. In: Biological and Environmental Aspects of Chromium (Langard, S., Ed.), pp. 171–208. Elsevier, Amsterdam.
[18] Alcedo, J. and Wetterhahn, K.E. (1990) Chromium toxicity and carcinogenesis. Int. Rev. Exp. Path. 31, 85–107.
[19] Pardee, A.B., Prestidge, L.S., Whipple, M.B. and Dreyfuss, J. (1966) A binding site for sulfate and its relation to sulfate transport into *Salmonella typhimurium*. J. Biol. Chem. 241, 3962–3969.
[20] Hryniewicz, M., Sirko, A., Palucha, A., Böck, A. and Hulanicka, D. (1990) Sulfate and thiosulfate transport in *Escherichia coli* K-12: identification of a gene encoding a novel protein involved in thiosulfate binding. J. Bacteriol. 172, 3358–3366.
[21] Karbonowska, H., Wiater, A. and Hulanicka, D. (1977) Sulphate permease of *Escherichia coli* K12. Acta Biochim. Pol. 24, 329–334.
[22] Sirko, A., Hryniewicz, M., Hulanicka, D. and Böck, A. (1990) Sulfate and thiosulfate transport in *Escherichia coli* K-12: nucleotide sequence and expression of the *cysTWAM* gene cluster. J. Bacteriol. 172, 3351–3357.
[23] Ohtake, H., Cervantes, C. and Silver, S. (1987) Decreased chromate uptake in *Pseudomonas fluorescens* carrying a chromate resistance plasmid. J. Bacteriol. 169, 3853–3856.
[24] Nies, A., Nies, D.H. and Silver, S. (1989) Cloning and expression of plasmid genes encoding resistance to chromate and cobalt in *Alcaligenes eutrophus*. J. Bacteriol. 171, 5065–5070.
[25] Rai, L.C., Dubey, S.K. and Mallick, N. (1992) Influence of chromium on some physiological variables of *Anabaena doliolum*: interaction with metabolic inhibitors. BioMetals 5, 13–16.
[26] Volesky, B. and Holan, Z.R. (1995) Biosorption of heavy metals. Biotechnol. Prog. 11, 235–250.
[27] Snyder, S.L., Walker, R.I., MacVittie, T.J. and Sheil, J.M. (1978) Biologic properties of bacterial lipopolysaccharides treated with chromium chloride. Can. J. Microbiol. 24, 495–501.
[28] Flemming, C.A., Ferris, F.G., Beveridge, T.J. and Bailey, G.W. (1990) Remobilization of toxic heavy metals adsorbed to bacterial cell wall-clay composites. Appl. Environ. Microbiol. 56, 3191–3203.
[29] McLean, R.J.C., Beauchemin, D., Claphan, L. and Beveridge, T.J. (1990) Metal-binding characteristics of the γ-glutamyl capsular polymer of *Bacillus licheniformis* ATCC 9945. Appl. Environ. Microbiol. 56, 3671–3677.
[30] Schroll, H. (1978) Determination of the absorption of Cr^{6+} and Cr^{6+} in an algal culture of *Chlorella pyrenoidosa* using ^{51}Cr. Bull. Environ. Contam. Toxicol. 20, 271–274.
[31] Sims, I.R. and Reynolds, P.J. (1999) Effects of atmospheric pollution on a lichenophagous lepidopteran. Ecotoxicol. Environ. Saf. 42, 30–34.
[32] Borst-Pauwels, G.W.F.H. (1981) Ion transport in yeast. Biochim. Biophys. Acta 650, 88–127.
[33] Marzluf, G.A. (1970) Genetic and metabolic controls for sulfate metabolism in *Neurospora crassa*: isolation and study of chromate-resistant and sulfate transport-negative mutants. J. Bacteriol. 102, 716–721.

[34] Ramana, V.V. and Sastry, K.S. (1994) Chromium toxicity in *Neurospora crassa*. J. Inorg. Biochem. 56, 87–95.
[35] Pepi, M. and Baldi, F. (1992) Modulation of chromium(VI) toxicity by organic and inorganic sulfur species in yeasts from industrial wastes. BioMetals 5, 179–185.
[36] Huffman Jr., E.W.D. and Allaway, W.H. (1973) Chromium in plants: distribution in tissues, organelles and extracts and availability of bean leaf Cr to animals. J. Agric. Food Chem. 21, 982–986.
[37] Srivastava, S., Nigam, R., Prakash, S. and Srivastava, M.M. (1999) Mobilization of trivalent chromium in presence of organic acids: a hydroponic study of wheat plant (*Triticum vulgare*). Bull. Environ. Contam. Toxicol. 63, 524–530.
[38] Skeffington, R.A., Shewry, P.R. and Peterson, P.J. (1976) Chromium uptake and transport in barley seedlings (*Hordeum vulgare* L.). Planta 132, 209–214.
[39] Ramachandran, V., D'Souza, R.J. and Mistry, K.B. (1980) Uptake and transport of chromium in plants. J. Nuclear Agric. Biol. 9, 126–128.
[40] Shewry, P.R. and Peterson, P.J. (1974) The uptake and transport of chromium by barley seedlings (*Hordeum vulgare* L.). J. Exp. Bot. 25, 785–797.
[41] Mishra, S., Singh, V., Srivastava, S., Srivastava, R., Srivastava, M.M., Dass, S., Satsangi, G.P. and Prakash, S. (1995) Studies on uptake of trivalent and hexavalent chromium by maize (*Zea mays*). Food Chem. Toxicol. 33, 393–397.
[42] Kleiman, I.D. and Cogliatti, D.H. (1998) Chromium removal from aqueous solutions by different plant species. Environ. Technol. 19, 1127–1132.
[43] Srivastava, S., Shanker, K., Srivastava, S., Shirvastav, R., Das, S., Prakash, S. and Srivastava, M.M. (1998) Effect of selenium supplementation on the uptake and translocation of chromium by spinach (*Spinacea oleracea*). Bull. Environ. Contam. Toxicol. 60, 750–758.
[44] Shallari, S., Schwartz, C., Hasko, A. and Morel, J.L. (1998) Heavy metals in soils and plants of serpentine and industrial sites of Albania. Sci. Total Environ. 209, 133–142.
[45] Lytle, C.M., Lytle, F.W., Yang, N., Qian, J.H., Hansen, D., Zayed, A. and Terry, N. (1998) Reduction of Cr(VI) to Cr(III) by wetland plants: potential for in situ heavy metal detoxification. Environ. Sci. Technol. 32, 3087–3093.
[46] Kumar, P., Dushenkov, V., Motto, H. and Raskin, I. (1995) Phytoextraction: the use of plants to remove heavy metals from soils. Environ. Sci. Technol. 29, 1232–1238.
[47] Wong, P.T. and Trevors, J.T. (1988) Chromium toxicity to algae and bacteria. In: Chromium in the Natural and Human Environments (Nriagu, J.O. and Nieboer, E., Eds.), pp. 305–315. Wiley, New York.
[48] Katz, S.A. and Salem, H. (1993) The toxicology of chromium with respect to its chemical speciation: a review. J. Appl. Toxicol. 13, 217–224.
[49] Kawanishi, S., Inoue, S. and Sano, S. (1986) Mechanism of DNA cleavage induced by sodium chromate (VI) in the presence of hydrogen peroxide. J. Biol. Chem. 261, 5952–5958.
[50] Kadiiska, M.B., Xiang, Q.H. and Mason, R.P. (1994) In vivo free radical generation by chromium (VI): an electron resonance spin-trapping investigation. Chem. Res. Toxicol. 7, 800–805.
[51] Shi, X. and Dalal, N.S. (1990) On the hydroxyl radical formation in the reaction between hydrogen peroxide and biologically generated chromium (V) species. Arch. Biochem. Biophys. 277, 342–350.
[52] Shi, X.L. and Dalal, N.S. (1990) NADPH-dependent flavoenzymes catalyze one electron reduction of metal ions and molecular oxygen and generate hydroxyl radicals. FEBS Lett. 276, 189–191.
[53] Nishio, A. and Uyeki, E.M. (1985) Inhibition of DNA synthesis by chromium compounds. J. Toxicol. Environ. Health 15, 237–244.
[54] Costa, M. (1991) DNA–protein complexes induced by chromate and other carcinogens. Environ. Health Perspect. 92, 45–52.
[55] Kortenkamp, A., O'Brien, P. and Beyersmann, D. (1991) The reduction of chromate is a prerequisite of chromium binding to cell nuclei. Carcinogenesis 12, 1143–1144.
[56] Bridgewater, L.C., Manning, F.C., Woo, E.S. and Patierno, S.R. (1994) DNA polymerase arrest by aducted trivalent chromium. Mol. Carcinog. 9, 122–133.
[57] Aiyar, J., Berkovits, H.J., Floyd, R.A. and Wetterhahn, K.E. (1991) Reaction of chromium(VI) with glutathione or with hydrogen peroxide: Identification of reactive intermediates and their role in chromium(VI)-induced DNA damage. Environ. Health Perspect. 92, 53–62.
[58] Itoh, M., Nakamura, M., Suzuki, T., Kawai, K., Horitsu, H. and Takamizawa, K. (1995) Mechanism of chromium(VI) toxicity in *Escherichia coli*: is hydrogen peroxide essential in Cr(VI) toxicity? J. Biochem. 117, 780–786.
[59] Luo, H., Lu, Y., Shi, X., Mao, Y. and Delal, N.S. (1996) Chromium (IV)-mediated Fenton-like reaction causes DNA damage: implication to genotoxicity of chromate. Ann. Clin. Lab. Sci. 26, 185–191.
[60] Snow, E.T. (1994) Effects of chromium on DNA replication in vitro. Environ. Health Perspect. 3, 41–44.
[61] Travieso, L., Cañizarez, R.O., Borja, R., Benítez, F., Domínguez, A.R., Dupeyrón, R. and Valiente, V. (1999) Heavy metal removal by microalgae. Bull. Environ. Contam. Toxicol. 62, 144–151.
[62] Brady, D., Letebele, B., Duncan, J.R. and Rose, P.D. (1994) Bioaccumulation of metals by *Scenedesmus, Selenastrum* and *Chlorella* algae. Water SA 20, 213–218.
[63] Brochiero, E., Bonaly, J. and Mestre, J.C. (1984) Toxic action of hexavalent chromium on *Euglena gracilis* strain Z grown under heterotrophic conditions. Arch. Environ. Contam. Toxicol. 13, 603–608.
[64] Fasulo, M.P., Bassi, M. and Donini, A. (1983) Cytotoxic effects of hexavalent chromium in *Euglena gracilis*. II. Physiological and ultrastructural studies. Protoplasma 114, 35–43.
[65] Bassi, M. and Donini, A. (1984) Phallotoxin visualization of F-actin in normal and chromium-poisoned *Euglena* cells. Cell Biol. Int. Rep. 8, 867–871.
[66] Corradi, M.G., Gorbi, G., Ricci, A., Torelli, A. and Bassi, A.M. (1995) Chromium-induced sexual reproduction gives rise to a Cr-tolerant progeny in *Scenedesmus acutus*. Ecotoxicol. Environ. Saf. 32, 12–18.
[67] Frey, B.E., Riedl, G.F., Bass, A.E. and Small, L.F. (1983) Sensitivity of estuarine phytoplancton to hexavalent chromium. Estuar. Coast Shelf Sci. 17, 181–187.
[68] Lasheen, M.R., Shiehata, S.A. and Ali, G.H. (1990) Effect of cadmium, copper and chromium (VI) on the growth of the Nile (Egypt) water algae. Water Air Soil Pollut. 50, 19–30.
[69] Henderson, G. (1989) A comparison of the effects of chromate, molybdate and cadmium oxide on respiration in the yeast *Saccharomyces cerevisiae*. Biol. Metals 2, 83–88.
[70] Kharab, P. and Singh, I. (1987) Induction of respiratory deficiency in yeast by salts of chromium, arsenic, cobalt and lead. Ind. J. Exp. Biol. 25, 141–142.
[71] Kharab, P. and Singh, I. (1985) Genotoxic effects of potassium dichromate, sodium arsenite, cobalt chloride and lead nitrate in diploid yeast. Mutat. Res. 155, 117–120.
[72] Galli, A., Boccardo, P., Del Carratore, R., Cundari, E. and Bronzetti, G. (1985) Conditions that influence the genetic activity of potassium dichromate and chromium chloride in *Saccharomyces cerevisiae*. Mutat. Res. 144, 165–169.
[73] Cheng, L., Liu, S. and Dixon, K. (1998) Analysis of repair and mutagenesis of chromium-induced DNA damage in yeast, mammalian cells, and transgenic mice. Environ. Health Perspect. 106, 1027–1032.
[74] Hauschild, M.Z. (1993) Putrescine (1,4-diaminobutane) as an indicator of pollution-induced stress in higher plants: barley and rape stressed with Cr(III) or Cr(VI). Ecotoxicol. Environ. Saf. 26, 228–247.
[75] Minissi, S., Caccese, D., Passafiume, F., Grella, A., Eleanora, C. and Rissoni, M. (1998) Mutagenicity (micronucleus test in *Vicia faba* root tips), polycyclic aromatic hydrocarbons and heavy metal content of

sediments collected in Tiber river and its tributaries within the urban area of Rome. Mutat. Res. 420, 77–84.
[76] Rank, J. and Nielsen, M.H. (1998) Genotoxicity testing of wastewater sludge using the *Allium cepa* anaphase-telpohase chromosome aberration assay. Mutat. Res. 418, 113–119.
[77] Wang, H. (1999) Clastogenicity of chromium contaminated soil samples evaluated by *Vicia* root-micronucleus assay. Mutat. Res. 426, 147–149.
[78] Cervantes, C. and Silver, S. (1992) Plasmid chromate resistance and chromate reduction. Plasmid 27, 65–71.
[79] Bopp, L.H. and Ehrlich, H.L. (1988) Chromate resistance and reduction in *Pseudomonas fluorescens* LB300. Arch. Microbiol. 150, 426–431.
[80] Dhakephalkar, P.K., Bhide, J.V. and Paknikar, K.M. (1996) Plasmid mediated chromate resistance and reduction in *Pseudomonas mendocina* MCM B-180. Biotechnol. Lett. 18, 1119–1122.
[81] Cervantes, C., Ohtake, H., Chu, L., Misra, T. and Silver, S. (1990) Cloning, nucleotide sequence, and expression of the chromate resistance determinant of *Pseudomonas aeruginosa* plasmid pUM505. J. Bacteriol. 172, 287–291.
[82] Nies, A., Nies, D.H. and Silver, S. (1990) Nucleotide sequence and expression of a plasmid-encoded chromate resistance determinant from *Alcaligenes eutrophus*. J. Biol. Chem. 265, 5648–5653.
[83] Nikaido, H. and Saier Jr., M.H. (1992) Transport proteins in bacteria: common themes in their design. Science 258, 936–941.
[84] Nies, D.H., Koch, S., Wachi, S., Peitzsch, N. and Saier Jr., M.H. (1998) CHR, a novel family of prokaryotic proton motive force-driven transporters probably containing chromate/sulfate antiporters. J. Bacteriol. 180, 5799–5802.
[85] Nicholson, M.L. and Laudenbach, D.E. (1995) Genes encoded on a cyanobacterial plasmid are transcriptionally regulated by sulfur availability and CysR. J. Bacteriol. 177, 2143–2150.
[86] Wu, J., Tisa, L.S. and Rosen, B.P. (1992) Membrane topology of the ArsB protein, the membrane subunit of an anion-translocating ATPase. J. Biol. Chem. 267, 12570–12576.
[87] Alvarez, A.H., Moreno-Sánchez, R. and Cervantes, C. (1999) Chromate efflux by means of the ChrA chromate resistance protein from *Pseudomonas aeruginosa*. J. Bacteriol. 181, 7398–7400.
[88] Ohtake, H., Komori, K., Cervantes, C. and Toda, K. (1990) Chromate-resistance in a chromate-reducing strain of *Enterobacter cloacae*. FEMS Microbiol. Lett. 67, 85–88.
[89] Kuroda, M., Dey, S., Sanders, O.I. and Rosen, B.P. (1997) Alternate energy coupling of ArsB, the membrane subunit of the ArsA anion-translocating ATPase. J. Biol. Chem. 272, 326–331.
[90] Ohtake, H. and Silver, S. (1994) Bacterial detoxification of toxic chromate. In: Biological Degradation and Bioremediation of Toxic Chemicals (Chaudhry, G.R., Ed.), pp. 403–415. Dioscorides, Portland, OR.
[91] Wang, P., Mori, T., Komori, K., Sasatsu, K., Toda, K. and Ohtake, H. (1989) Isolation and characterization of an *Enterobacter cloacae* strain that reduces hexavalent chromium under anaerobic conditions. Appl. Environ. Microbiol. 55, 1665–1669.
[92] Campos, J., Martínez-Pacheco, M. and Cervantes, C. (1995) Hexavalent chromium reduction by a chromate-resistant *Bacillus* sp. strain. Antonie van Leeuwenhoek 68, 203–208.
[93] Lovley, D.R. and Phillips, E.J.P. (1994) Reduction of chromate by *Desulfovibrio vulgaris* and its *c3* cytochrome. Appl. Environ. Microbiol. 60, 726–728.
[94] Lovley, D.R., Widman, P.K., Woodward, J.C. and Phillips, J.P. (1993) Reduction of uranium by cytochrome *c3* of *Desulfovibrio vulgaris*. Appl. Environ. Microbiol. 59, 3572–3576.
[95] Corradi, M.G., Gorbi, G. and Bassi, M. (1995) Hexavalent chromium induces gametogenesis in the freshwater algae *Scenedesmus acutus*. Ecotoxicol. Environ. Saf. 30, 106–110.
[96] Corradi, M.G. and Gorbi, G. (1993) Chromium toxicity on two linked trophic levels. II Morphophysiological effects on *Scenedesmus acutus*. Ecotoxicol. Environ. Saf. 25, 72–78.
[97] Wong, P.K. and Chang, L. (1991) Effects of copper, chromium and nickel on growth, photosynthesis and chlorophyll a synthesis of *Chlorella pyrenoidosa* 251. Environ. Pollut. 72, 127–139.
[98] Gorbi, G., Corradi, M.G., Torelli, A. and Bassi, M. (1996) Comparison between a normal and a Cr-tolerant strain of *Scenedesmus acutus* as a source food to *Daphnia magna*. Ecotoxicol. Environ. Saf. 35, 109–111.
[99] Abd-El-Monem, M., Corradi, M.G. and Gorbi, G. (1998) Toxicity of copper and zinc to two strains of *Scenedesmus acutus* having different sensitivity to chromium. Environ. Exp. Bot. 40, 59–66.
[100] Czakó-Vér, K., Batié, M., Raspor, P., Sipiczki, M. and Pesti, M. (1999) Hexavalent chromium uptake by sensitive and tolerant mutants of *Schizosaccharomyces pombe*. FEMS Microbiol. Lett. 178, 109–115.
[101] Baldi, F., Vaughan, A.M. and Olson, G.J. (1990) Chromium(VI)-resistant yeast isolated from a sewage treatment plant receiving tannery wastes. Appl. Environ. Microbiol. 56, 913–918.
[102] Baldi, F. and Pepi, M. (1991) Study of metal and non-metal resistances in chromate-resistant yeasts isolated from industrial wastes. In: Proc. Int. Symp. on Environmental Biotechnology, Vol. 1 (Verachtert, H. and Verstraete, W., Eds.), pp. 255–258. Ostend.
[103] Ramírez-Ramírez, R., Calvo-Méndez, C., Avila-Rodríguez, M. and Gutiérrez-Corona, J.F. (2000) Chromate resistance and reduction in a yeast strain isolated from industrial waste discharges. In: Environmental Engineering and Health Sciences, Section 4: Environmental Engineering Application (Raynal, J.A., Nuckols, J.R., Reyes, R. and Ward M., Eds.), pp. 437–445. Water Resources Publication, LCC, Englewood, CO, USA.
[104] Breton, A. and Surdin-Kerjan, Y. (1977) Sulfate uptake in *Saccharomyces cerevisiae*: biochemical and genetic study. J. Bacteriol. 132, 224–232.
[105] Smith, F.W., Hawkesford, M.J., Prosser, I.M. and Clarkson, D.T. (1995) Isolation of a cDNA from *Saccharomyces cerevisiae* that encodes a high affinity sulfate transporter at the plasma membrane. Mol. Gen. Genet. 247, 709–715.
[106] Lachance, M.-A. and Pang, W.-M. (1997) Predacious yeasts. Yeast 13, 225–232.
[107] Scheibel, T., Bell, S. and Walke, S. (1997) *S. cerevisiae* and sulfur: a unique way to deal with the environment. FASEB J. 11, 917–921.
[108] Ono, B. and Weng, M. (1982) Chromium sensitive mutants of the yeast *Saccharomyces cerevisiae*. Curr. Genet. 5, 215–220.
[109] Gharieb, M.M. and Gadd, G.M. (1998) Evidence for the involvement of vacuolar activity in metal(loid) tolerance: vacuolar-lacking and -defective mutants of *Saccharomyces cerevisiae* display higher sensitivity to chromate, tellurite and selenite. BioMetals 11, 101–106.
[110] Samecka-Cymerman, A. and Kempers, A.J. (1996) Bioaccumulation of heavy metals by aquatic macrophytes around Wroclaw, Poland. Ecotoxicol. Environ. Saf. 35, 242–247.
[111] Samecka-Cymerman, A., Marczonek, A. and Kempers, A.J. (1997) Bioindication of heavy metals in soil by liverworts. Arch. Environ. Contam. Toxicol. 33, 162–171.
[112] Mallick, N., Shardendu, P. and Rai, L.C. (1996) Removal of heavy metals by two free floating aquatic macrophytes. Biomed. Environ. Sci. 9, 399–407.
[113] Pillichshammer, M., Pumpel, T., Poder, R., Eller, K., Klima, J. and Schinner, F. (1995) Biosorption of chromium to fungi. BioMetals 8, 117–121.
[114] Wakatsuki, T. (1995) Metal oxidoreduction by microbial cells. J. Ind. Microbiol. 14, 169–177.
[115] Silver, S. and Williams, J.W. (1984) Bacterial resistance and detoxification of heavy metals. Enzyme Microb. Technol. 6, 531–537.
[116] Lovley, D.R. (1993) Dissimilatory metal reduction. Annu. Rev. Microbiol. 47, 263–290.
[117] Unz, R.F. and Shuttleworth, K.L. (1996) Microbial mobilization and immobilization of heavy metals. Curr. Opin. Biotechnol. 7, 307–310.

[118] Francis, A.J. and Dodge, C.J. (1990) Anaerobic microbial remobilization of toxic metals coprecipitated with iron oxide. Environ. Sci. Technol. 24, 373–378.
[119] Dvorak, D.H., Hedin, R.S., Edeborn, H.M. and McIntire, P.E. (1992) Treatment of metal-contaminated water using bacterial sulfate reduction. Results from pilot-scale reactors. Biotechnol. Bioeng. 40, 609–616.
[120] Ramanathan, S., Ensor, M. and Daunert, S. (1997) Bacterial biosensors for monitoring toxic metals. Trends Biotechnol. 15, 501–506.
[121] Peitzsch, N., Eberz, G. and Nies, D.H. (1998) *Alcaligenes eutrophus* as a bacterial chromate sensor. Appl. Environ. Microbiol. 64, 453–458.
[122] Sampedro, M.A., Blanco, A., Llama, M.J. and Serra, J.L. (1995) Sorption of heavy metals to *Phormidium laminosum* biomass. Biotechnol. Appl. Biochem. 22, 355–366.
[123] Wilde, E. and Benemann, J.R. (1993) Bioremoval of heavy metals by the use of microalgae. Biotechnol. Adv. 11, 781–812.
[124] Vymazal, J. (1990) Uptake of lead, chromium, cadmium and cobalt by *Cladophora glomerata*. Bull. Environ. Contam. Toxicol. 44, 468–472.
[125] Wood, J.M. and Wang, H.-K. (1983) Microbial resistance to heavy metals. Environ. Sci. Technol. 17, 582–590.
[126] Wales, D.S. and Sagar, B.F. (1990) Recovery of metal ions by microfungal filters. J. Chem. Technol. Biotechnol. 49, 345–355.
[127] Al-Asheh, S. and Duvnjak, Z. (1995) Adsorption of copper and chromium by *Aspergillus carbonarius*. Biotechnol. Prog. 11, 638–642.
[128] Rapoport, A.I. and Muter, O.A. (1995) Biosorption of hexavalent chromium by yeast. Process. Biochem. 30, 145–149.
[129] Paknikar, K.M. and Bhide, J.V. (1993) Aerobic reduction and biosorption of chromium by a chromate resistant *Aspergillus* sp. In: Biohydrometallurgical Technologies (Torma, A.E., Apel, M.L. and Brierley, C.L., Eds.), pp. 237–244. The Minerals, Metals and Materials Society, Warrendale, PA.
[130] Cunningham, S.D. and Ow, D. (1996) Promises and prospects of phytoremediation. Plant Physiol. 110, 715–719.
[131] Kaneko, T., Sato, S., Kotani, H., Tanaka, A., Asamizu, E., Nakamura, Y., Miyajima, N., Hirosawa, M., Sugiura, M., Sasamoto, S., Kimura, T., Hosouchi, T., Matsuno, A., Muraki, A., Nakazaki, N., Naruo, K., Okumura, S., Shimpo, S., Takeuchi, C., Wada, T., Watanabe, A., Yamada, M., Yasuda, M. and Tabata, S. (1996) Sequence analysis of the genome of the unicellular cyanobacterium *Synechocystis* sp. strain PCC6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions. DNA Res. 3, 109–136.
[132] Bult, C.J., White, O., Olsen, G.J., Zhou, L., Fleischmann, R.D., Sutton, G.G., Blake, J.A., Fitzgerald, L.M., Clayton, R.A., Gocayne, J.D., Kerlavage, A.R., Dougherty, B.A., Tomb, J.-F., Adams, M.D., Reich, C.I., Overbeek, R., Kirkness, E.F., Weinstock, K.G., Merrick, J.M., Glodek, A., Scott, J.L., Geoghagen, N.S.M., Weidman, J.F., Fuhrmann, J.L., Nguyen, D., Utterback, T.R., Kelley, J.M., Peterson, J.D., Sadow, P.W., Hanna, M.C., Cotton, M.D., Roberts, K.M., Hurst, M.A., Kaine, B.P., Borodovsky, M., Klenk, H.-P., Fraser, C.M., Smith, H.O., Woese, C.R. and Venter, J.C. (1996) Complete genome sequence of the methanogenic archeon, *Methanococcus jannaschi*. Science 273, 1058–1073.
[133] Chen, C.-M., Misra, T.K., Silver, S. and Rosen, B.P. (1986) Nucleotide sequence of the structural genes for an anion pump. The plasmid-encoded arsenical resistance operon. J. Biol. Chem. 261, 15030–15038.
[134] Suzuki, T., Miyata, N., Horitsu, H., Kawai, K., Takamizawa, K., Tai, Y. and Okazaki, M. (1992) NAD(P)H-dependent chromium (VI) reductase of *Pseudomonas ambigua* G-1: a Cr(V) intermediate is formed during the reduction of Cr(VI) to Cr(III). J. Bacteriol. 174, 5340–5345.
[135] Campos-García, J., Martínez-Cadena, G., Alvarez-González, R. and Cervantes, C. (1997) Purification and partial characterization of a chromate reductase from *Bacillus*. Rev. Lat. Am. Microbiol. 39, 73–81.
[136] Ishibashi, Y., Cervantes, C. and Silver, S. (1990) Chromium reduction in *Pseudomonas putida*. Appl. Environ. Microbiol. 56, 2268–2270.
[137] Shen, H. and Wang, Y. (1993) Characterization of enzymatic reduction of hexavalent chromium by *Escherichia coli* ATCC33456. Appl. Environ. Microbiol. 59, 3771–3777.