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Diversity of chromium-resistant and -reducing bacteria in a chromium-contaminated activated sludge

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Aims: This study attempts to establish a relationship between the Cr(VI) resistance of the culturable microbial community and the Cr(VI) resistance and Cr(VI)-reducing ability of representative strains of each population, in order to assess whether these are exclusive characteristics of one microbial group or abilities shared among many groups.

Methods and Results: A group of 48 Cr(VI)-resistant isolates, with different colony types, was isolated from chromium-contaminated activated sludge. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis protein patterns and fatty acid methyl ester analysis identified six populations, representing 54% of the isolated bacteria, as belonging to the genera *Acinetobacter* and *Ochrobactrum*. The remaining populations included strains identified as species of the *β-Proteobacteria* and high G + C Gram-positive bacteria. The Cr(VI) resistance and reduction ability of the strains were tested. All but two isolates grew in the presence of 1 mmol l^{-1} Cr(VI). During enrichment, all isolates were able to survive to 2 mmol l^{-1} Cr(VI) and complete Cr(VI) reduction was achieved. Representative strains of each population were able to partially reduce (5·4–39·1%) the Cr(VI) present in the growth medium.

Conclusions: Most of the identified isolates have never been reported to be Cr(VI)-resistant and/or Cr(VI)-reducing strains. The mechanisms of Cr(VI) resistance and reduction may differ from group to group; therefore, it is evident that both Cr(VI) resistance and reduction are shared abilities and not an exclusive characteristic of a single group, possibly reflecting horizontal genetic transfer resulting from selective pressure in this contaminated environment.

Significance and Impact of the Study: To our knowledge, this is the first study of a microbial community under chronic chromate stress and, as the success of microbial-based metal remediation technologies requires a better understanding of the microbial community and the population response to metal stress, it may contribute to the implementation of a strategy of bioremediation of chromate-contaminated environments.

INTRODUCTION

The widespread use of chromium compounds by industries has led to large quantities of this element being released to the environment. Only hexavalent chromium (Cr(VI)) and trivalent chromium (Cr(III)) are ecologically important because they are the more stable oxidation states. Being mutagenic, carcinogenic and teratogenic, Cr(VI) is approx. 100-fold more toxic than Cr(III) (Shen and Wang 1995). However, Cr(III) is considered to be relatively innocuous because it is less soluble and does not permeate eukaryotic and prokaryotic membranes.

Serious concerns about the toxicity of Cr(VI) compounds necessitate the recovery and reuse of chromium from industrial wastes and it is essential to convert the unrecovered Cr(VI) to a less toxic form. Several bacteria have been described as being able to reduce Cr(VI) to Cr(III) under aerobic and anaerobic conditions and the biological transformation of the very toxic Cr(VI) to the less toxic and less

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mobilized Cr(III) has been reported as an alternative to the physical and chemical approaches (Benedict and Carlson 1971; Bopp and Ehrlich 1988; Wang *et al.* 1989; Ishibashi *et al.* 1990). The activated sludge process for the purification of wastewater is, in terms of total metabolized matter, today's most important biotechnological process. Activated sludge consortia can be regarded as mobilized biofilms cometabolizing diverse compounds. Past studies have shown that chronic metal stress affects the structure of microbial communities, resulting in decreased biomass, activity and microbial diversity. Despite toxic stress, micro-organisms that tolerate toxic stress conditions or more rapidly decompose pollutants are more likely to survive (Dean-Ross and Mills 1989; Roane and Kellogg 1996). Therefore, the success of microbial-based metal remediation technologies requires a better understanding of the microbial community and the population response to metal stress.

The objective of this study was to isolate and characterize the culturable microbial community of a chromium-contaminated activated sludge and to evaluate the Cr(VI) resistance and Cr(VI)-reducing ability of metal-stressed autochthonous populations, in order to evaluate whether these are exclusive characteristics of one microbial group or shared abilities. In metal-contaminated environments, the vitrification of the metal by adapted populations could be an efficient bioremediation process.

MATERIALS AND METHODS

Sampling and isolation of bacterial strains

Samples were collected from the activated sludge of a wastewater treatment plant receiving effluents from urban and industrial tannery areas of central Portugal. This plant has, as its main characteristics, a biologic tank capacity of 16 668 m^3, an F : M ratio of 0·18 (kg CBO_5 : kg MLVSS × d) (MLVSS = mixed liquor volatile suspended solids), a sludge age of 17 d and an returned activated sludge (RAS) control of 100%. The total chromium concentration in the activated sludge samples and along the wastewater treatment plant was determined (Anon. 1998). The total number of bacteria was determined as described by Tabor and Neihof (1982). The number of heterotrophic bacteria present in activated sludge, able to grow on Plate Count Agar (PCA; Difco) and on PCA with 0·5, 1, 1·5 and 2 mmol l^{-1} sodium chromate (Cr(VI)), was determined after 6 and 12 d incubation. Sludge samples (0·1 g) were enriched in the presence of 2 mmol l^{-1} Cr(VI) in 20 ml basal mineral medium: solution A, 100 ml l^{-1} ((in g l^{-1}): $CaCl_2.2H_2O$, 0·793; NaCl, 0·080; KNO_3, 1·030; $NaNO_3$, 6·980; $MgSO_4.7H_2O$, 1·000; nitrilotriacetic acid, 1·000); solution B, 10 ml l^{-1} ((in mg l^{-1}): $FeSO_4.7H_2O$, 200·00; $ZnSO_4.7H_2O$, 10·00; $MnSO_4.H_2O$, 3·00; H_3BO_3, 30·00; $CoSO_4.7H_2O$, 24·00; $CuSO_4.5H_2O$, 1·00; $NiCl_2.6H_2O$, 1·70; $NaMoO_4.2H_2O$, 3·00; EDTA, 500·00); NH_4SO_4, 0·5 g l^{-1}; glucose, 0·8 g l^{-1} and Cr(VI) 2 mmol l^{-1}, pH 7·6. During enrichment, the decrease in Cr(VI) concentration was followed using the diphenylcarbazide method (Anon. 1998). After 3 and 15 d, aliquots of 1 and 10 μl were taken from the enrichment flask, plated in duplicate on Nutrient Agar (NA; Difco) and incubated for 7 d at 30°C. All different colony types were isolated on NA. Subsequent purifications were performed on both NA and NA containing 1 mmol l^{-1} Cr(VI). A total of 48 isolates were obtained and maintained at −80°C in Nutrient Broth (NB; Difco) containing 15% glycerol. After cryopreservation, all of the bacteria were grown on NA containing 1 mmol l^{-1} Cr(VI).

Phenotypic characterization

The cellular morphology and Gram stain were visualized by optical microscopy. The API 20NE Test System (API System S.A., La-Balme Les Grottes, France) was used for biochemical characterization according to the manufacturer's instructions.

Fatty acid methyl ester analysis

All strains were grown in Tryptic Soy Broth Agar (BBL, Cockeysville, USA) at 28°C for 48 ± 2 h. The fatty acid methyl esters (FAMEs) were obtained and quantified using the protocol of the MIDI Identification System (MIDI, Microbial ID, Newark, DE, USA). The identification and quantification of FAMEs as well as the numerical analysis of the fatty acid profiles were performed using a software package (MIDI).

Polyacrylamide gel electrophoresis of whole cell proteins analysis

All strains were grown on NA for 48 h at 30°C. Extraction of whole cell proteins, storage and sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) of the extracts were performed as described by Morais *et al.* (1997).

Protein electrophoretic patterns were scanned on a laser densitometer (Ultrascan; LKB, Bromma, Sweden). The comparison and normalization of lanes on different gel slabs was achieved as described previously (Veríssimo *et al.* 1996). Densitometric analysis, normalization and interpolation of the protein profiles and construction of the database were accomplished using software package GelCompar W4.8 (Applied Maths, Kortrijk, Belgium). Computation of the similarity between all possible pairs of traces was performed using the Pearson correlation coefficient (r) and clustering was achieved by the unweighted pair group method with arithmetic averages (UPGMA) algorithm. Numerical

analysis was performed by using 20–300 of a total of 400 points for each interpolated trace.

Cr(VI) tolerance tests

Experiments were performed in NB containing Cr(VI) (1–4 mmol l^{-1}) and incubated at 30°C, with shaking, under aerobic conditions for 90 h. Experiments were also carried out under anaerobic conditions (Genbox anaer generators; bio-Merieux, La-Balme des grottes, France), in an anaerobic jar, under the same conditions. Overnight cultures were used as inocula, which were incubated in basal mineral medium (without Cr(VI)) containing glucose (1·0 g l^{-1}) at 30°C with shaking. The number of viable cells in the inoculum varied between $8{\cdot}3 \times 10^7$ and 5×10^8 bacteria ml^{-1} and 1 ml was used to inoculate the experiments. Growth was followed at 600 nm (Spectronic 601; Milton Roy, Stafford, UK).

Determination of Cr(VI)-reduction ability

Twenty-eight strains, representing at least 50% of each different population, were tested for their Cr(VI)-reduction ability in NB containing 1 mmol l^{-1} Cr(VI). Each strain was incubated, under aerobic conditions, at 30°C for 72 h with shaking and the growth followed at 600 nm. After 72 h, Cr(VI) reduction was determined as previously described (Anon. 1998). Overnight cultures were used as inocula, in NB, and incubated at 30°C with shaking. The number of viable cells varied between 9×10^8 and $3{\cdot}0 \times 10^{10}$ bacteria ml^{-1} and 1 ml was used to inoculate the experiments. The decrease in Cr(VI) concentration in the medium was determined by the diphenylcarbazide method (Anon. 1998). The Cr(VI)-reduction ability of each strain was expressed as a percentage of Cr(VI) disappearance from the medium.

RESULTS

Samples were collected from the activated sludge of a wastewater treatment plant. The total chromium concentration along the treatment plant ranged from 3·8 to 48×10^{-3} mmol l^{-1} and the pH varied from 8·1 to 8·5. The total chromium concentration in the activated sludge samples was 1·30 ± 0·03% (w/w).

The total number of micro-organisms in the sludge was estimated as $8{\cdot}0 \times 10^6 \pm 0{\cdot}85 \times 10^6$ bacteria g^{-1} sludge and a large number of clumps were visible. The number of culturable bacteria recovered from the sludge depended on the incubation time. Therefore, on PCA without Cr(VI), the number of culturable heterotrophic bacteria (cfu) after 6 d incubation was only $4{\cdot}6 \times 10^6 \pm 0{\cdot}71 \times 10^6$ bacteria g^{-1}, corresponding to 18% of the bacteria present after 12 d incubation (Table 1). A longer incubation time increased the number of bacteria recovered and allowed the recovery of populations resistant to 1·5 and 2 mmol l^{-1} Cr(VI). Increasing Cr(VI) concentrations led to a decrease in the number of bacteria recovered, e.g. after 12 d incubation in 2 mmol l^{-1} Cr(VI), the number of cfus able to grow was only 0·08% of the total cfu that grew on PCA in the absence of Cr(VI) (Table 1).

Activated sludge itself was able to decrease the Cr(VI) concentration to 80% within 8 d incubation in the enrichment culture. Complete Cr(VI) reduction was confirmed after 20 d.

A total of 48 bacteria were isolated, representing all of the different bacterial colony types present in the isolation medium. Based on numerical analysis of the protein electrophoregrams, the 48 bacteria isolated formed 10 clusters and three remained unclustered. All but two of the clusters were defined at a similarity level of more than 80% (Table 2). Fatty acid methyl ester analysis of the isolates revealed eight clusters and seven unclustered strains. The clusters formed after numerical analysis based on the FAME profiles were in agreement with those formed by protein SDS-PAGE (Table 2), except for FAME cluster E, which includes protein clusters VII and VIII, and protein clusters III, IX and X, which include FAME unclustered strains Un-a, Un-c, Un-d and Un-e, respectively (Table 2).

The identification suggestion for each population and unclustered strains, given by the MIDI Identification System, is illustrated in Table 2. Identification by API 20NE was in agreement with the MIDI identification for protein clusters I and II (FAME populations A and B), and

Table 1 Determination of the total number (using ethidium bromide) and heterotrophic plate counts (using plate count agar) of micro-organisms present in sludge

	Heterotrophic plate counts (g^{-1})				
	Chromium concentration in plate medium (mmol l^{-1})				
Incubation time (d)	0	0·5	1	1·5	2
6	$4{\cdot}6 \times 10^6 \pm 7{\cdot}1 \times 10^5$	$2{\cdot}6 \times 10^6 \pm 2{\cdot}8 \times 10^5$	$2{\cdot}4 \times 10^5 \pm 2{\cdot}8 \times 10^4$	0	0
12	$2{\cdot}5 \times 10^7 \pm 4{\cdot}7 \times 10^6$	$5{\cdot}8 \times 10^6 \pm 7{\cdot}1 \times 10^5$	$9{\cdot}2 \times 10^5 \pm 9{\cdot}9 \times 10^4$	$1{\cdot}8 \times 10^5 \pm 2{\cdot}8 \times 10^4$	$2{\cdot}0 \times 10^4 \pm 1{\cdot}4 \times 10^4$

Total number of cells (g^{-1}): $8{\cdot}0 \times 10^6 \pm 8{\cdot}5 \times 10^5$.

Table 2 Populations formed after numerical analysis of the protein electrophoregrams based on 80% similarity or higher (except for groups IV and X) and the fatty acid methyl ester (FAME) cluster correspondence

Protein cluster	No. of isolates	FAME cluster	MIDI identification	Isolates
I	6	A	*Ochrobactrum anthropi*	5-bvl-2b, 5-pte, 5-bvlme-b1, 5-bvl-2a, 5-bvl-1, 5-bvlme-a
II	2	B	*Ochrobactrum anthropi*	6-btpll, 12B-c2
III	4	F	*Aureobacterium esteroaromaticum*	12B-a1, 3′b-1, 3b-b1
		Un-a	*Corynebacterium mediolanum* or *Aureobacterium esteroaromaticum*	3′-a
IV	4	H	*Cellulomonas flavigena* or *Aureobacterium barkeri*	3b-a, 3′b-2a, 3ba, 3a
Un-b	1	Un-b	*Hydrogenophaga pseudoflava*	8
V	3	D	*Acinetobacter lwoffii*	3b-b2, 5-bvlme-b2, 3′b-2b
VI	4	C	*Acinetobacter lwoffii*	2, 1′-G, cas3-d, 12B-b
VII	4	E	Unknown	12B-d, 7, 6-bo-1, 6-abat
VIII	10	E	Unknown	4′-X, 4′-Xb, cas3-c1, cas3-c2, 12A-b, 4′-a2, 4, cas3-a1, cas3-b, 12A-a2
IX	2	D	*Acinetobacter* sp.	2′-P
		Un-c	Unknown	12B-c1
X	6	G	*Clavibacter michiganense* insidiosum	Bran3-a2, bran3-b, 9, 12A-a1
		Un-d	*Clavibacter michiganense* insidiosum	Bran3-a1
		Un-e	*Clavibacter michiganense* insidiosum	Cas3-a2
Un-f	1	Un-f	Unknown	6-bo-2
Un-g	1	Un-g	Unknown	1

Un, Unclustered.

for the unclustered strain Un-b. It also suggested that the strains of FAME cluster E belong to the species *Acinetobacter baumannii*. None of the isolates was able to ferment glucose and only strains from clusters I and II and unclustered strain Un-b reduced nitrate.

Under aerobic conditions, all strains except two (6-abat and 6-bo-1) grew in NB with 1 mmol l^{-1} Cr(VI). However, strains of only five populations (protein clusters I, III, IV, V and VII) were able to grow in 2 mmol l^{-1} Cr(VI) (Table 3). None of the strains were able to grow in the presence of 3 or 4 mmol l^{-1} Cr(VI). Under anaerobic conditions, only 8·3% of the strains (two strains of cluster I, one strain of cluster II and one strain of cluster VII) were able to grow in 1 mmol l^{-1} Cr(VI), but none grew in 2 mmol l^{-1} Cr(VI) (Table 3).

The Cr(VI)-reducing ability was studied by aerobically incubating 28 strains representative of each cluster and unclustered strains (Table 3). After 2 d, most of the bacteria achieved stationary phase and the remainder reached it in 3 d. After 3 d, the strains were able to reduce 13·2–46·4% of the Cr(VI) present (Table 3). Protein cluster IV (corresponding to FAME cluster H) included strains with different Cr(VI) resistances and Cr(VI)-reducing abilities. Strains with different Cr(VI) resistances and Cr(VI)-reducing abilities were also found in protein clusters V and IX (corresponding to cluster D by FAME analysis) and protein clusters VII and VIII (corresponding to cluster E by FAME analysis).

DISCUSSION

In this study we characterized the culturable microbial community of a treatment plant from a chromium-contaminated industrial tanning area. The Cr(VI) resistance of the culturable microbial community and the Cr(VI) resistance and Cr(VI)-reducing ability of representative strains of each population were evaluated to find whether the Cr(VI)

Table 3 Growth of bacterial isolates from sludge incubated in nutrient broth (NB) at 30°C with Cr(VI) under aerobic and anaerobic conditions and maximal reduction when incubated aerobically in NB at 30°C

	Cr(VI) reduction (1 mmol l^{-1})		Aerobic growth						Anaerobic growth	
			0 mmol l^{-1} Cr(VI)		1 mmol l^{-1} Cr(VI)		2 mmol l^{-1} Cr(VI)			
Protein cluster	Tested strains	Maximal reduction (%)	Max. O.D.	Growth rate (h^{-1})	Max. O.D.	Growth rate (h^{-1})	Max. O.D.	Growth rate (h^{-1})	0 mmol l^{-1} Cr(VI)	1 mmol l^{-1} Cr(VI)
I	5.bvl-1	25·6	1·55	0·3	1·52	0·19	1·21	0·08	+	–
	5-pte	28·3	1·49	0·2	1·34	0·10	1·22	0·06	+	–
	5-bvlme-b1	34·2	1·59	0·3	1·49	0·12	0·50	0·04	+	+
II	6-btpll	19·6	1·20	0·2	0·79	0·05	0	0	+	–
III	3b-b1	22·3	1·63	0·1	1·47	0·20	0·73	0·07	+	–
	3′-a	33·1	1·59	0·2	1·38	0·17	0	0	+	–
IV	3b-a	30·6	1·46	0·1	1·48	0·17	0	0	+	–
	3′b-2a	33·1	1·59	0·1	1·47	0·18	0	0	+	–
	3ba	31·1	1·60	0·2	1·44	0·18	0·30	0·05	+	–
	3a	46·4	1·68	0·1	1·19	0·22	0·09	0·02	+	–
Un-b	8	24·8	0·94	0·2	0·68	0·26	0	0	+	–
V	3b-b2	24·6	1·08	0·3	0·88	0·16	0·74	0·07	+	–
	5-bvlme-b2	36·9	1·15	0·3	0·62	0·11	0	0	+	–
VI	2	13·2	0·98	0·2	0·71	0·14	0	0	+	–
	12B-b	26·5	1·14	0·2	0·97	0·16	0	0	+	–
VII	12B-d	24·2	0·99	0·3	0·91	0·20	1·05	0·06	+	+
	7	24·6	1·15	0·3	0·93	ND	0	0	+	–
VIII	4′-Xb	21·9	1·01	0·3	0·86	0·30	0	0	+	–
	4′-X	18·4	1·05	0·3	1·13	0·24	0	0	+	–
	4	28·8	1·02	0·3	1·08	0·06	0	0	+	+
	Cas3-a1	19·8	1·09	0·2	1·01	0·07	0	0	+	–
	12A-b	28·5	1·10	0·2	0·95	0·07	0	0	+	–
IX	2′-P	24·7	1·00	0·2	0·58	0·27	0	0	+	–
X	Bran3-b	24·5	0·51	0·1	0·14	0·10	0	0	+	–
	9	21·0	0·26	0·1	0·28	0·14	0	0	+	–
	Bran3-a1	15·9	0·55	0·1	0·14	0·12	0	0	+	–
Un-f	6-bo-2	24·4	0·46	0·1	0·20	0·15	0	0	+	–
Un-g	1	24·0	0·43	0·1	0·11	0·10	0	0	+	–

+, Growth; –, no growth; ND, not determined; O.D., optical density.

resistance and Cr(VI)-reducing ability were exclusive characteristics of one microbial group or shared abilities.

Usually, the values reported for heterotrophic plate counts from activated sludge samples vary between 1 and 10% of the total number of cells present in the systems (Kämpfer *et al.* 1996; Snaidr *et al.* 1997). However, in our study with chromium-contaminated activated sludge, the total counts were lower than the number obtained by cultivation, probably as a result of the high number of aggregates counted as single cells, a problem that sometimes occurs during the evaluation of sludge samples (Wallner *et al.* 1995).

The number of heterotrophic plate counts was dependent on the incubation time and the Cr(VI) concentration present in the recovery medium. In our study, longer incubation times increased the number of heterotrophic bacteria recovered. This parameter was especially critical to the recovery of bacteria able to grow on a medium containing Cr(VI) concentrations higher than 1 mmol l^{-1}. The presence of increasing concentrations of Cr(VI) increased the

time needed for bacterial growth and probably selected the bacteria resistant to high Cr(VI) concentrations. In fact, the presence of 2 mmol l^{-1} Cr(VI) in the recovery medium reduced by 1000-fold the number of culturable bacteria.

During enrichment the bacterial populations present in the activated sludge sample were able to withstand the presence of 2 mmol l^{-1} Cr(VI) although, after isolation, only the strains of protein clusters I, II and VII and some strains of protein clusters III, IV and V were able to grow under these conditions. Most probably, some Cr(VI)-resistant members of the activated sludge bacterial community, able to grow in the presence of 2 mmol l^{-1} Cr(VI), were not isolated (unculturable bacteria) due to the cultivation methods used.

The different Cr(VI) resistances of the bacterial community in the activated sludge and each isolate may be explained by the existence of microenvironments in the sludge, containing different concentrations of Cr(VI). This will allow the bacteria to stabilize according to their metal resistance ability (J.S. Angle, T. Delorme and R.L. Chaney, unpublished data). Another possible explanation is the community metabolic structure, where the Cr(VI)-reduction ability of some populations allowed the survival of all bacteria as a result of the reduction in the concentration of the highly toxic Cr(VI) or a synergistic effect. However, we may not exclude the hypothesis of a loss of Cr(VI) resistance during cryopreservation, even though all of the isolates were subsequently able to grow in the presence of 1 mmol l^{-1} Cr(VI).

Ten clusters were defined by numerical analysis of the protein profiles of isolated strains. Three clusters, 33% of the total isolates, belonged to the high G + C Gram-positive bacteria. The isolates identified as *Aureobacterium esteroaromaticum*, *Clavibacter michiganensis* and *Cellulomonas flavigena* were able to resist the presence of Cr(VI) and also showed Cr(VI)-reduction ability. None of these species have been reported to be Cr(VI)-resistant or Cr(VI)-reducing strains. Their abundance, in a higher proportion than that usually found in activated sludge without chromium, by culturable methods (Kämpfer *et al.* 1996; Snaidr *et al.* 1997) may be due either to the Cr(VI) resistance and Cr(VI)-reducing ability of the strains and/or to the sludge heavy metal contamination, since shifts in the prevalence of bacterial groups in soil have been reported to be heavy metal concentration dependent (Sandaa *et al.* 1999).

Seventeen per cent of the isolates (protein clusters I and II) belonged to the α-subclass of the Proteobacteria. Although organisms belonging to this group are usually found in activated sludge, the presence of the species *Ochrobactrum anthropi* has not been previously reported. Its presence may be related to its ability to resist Cr(VI) and to grow within epithelial tissues, which are present in the tannery sewage (Velasco *et al.* 1998).

In our study, only one species of the β-subclass Proteobacteria (*Hydrogenophaga pseudoflava*) was isolated. This finding is in agreement with studies reporting that the β-subclass Proteobacteria are a minor group present in activated sludge when identified by culturable methods (Kämpfer *et al.* 1996).

In the present work, the major group of bacteria isolated belonged to the γ-Proteobacteria and only included strains from the genus *Acinetobacter*. Our results agree with findings reporting that the γ-Proteobacteria are usually the major group of bacteria isolated by cultivation methodologies (Kämpfer *et al.* 1996; Bond *et al.* 1999), suggesting that the presence of Cr(VI) had no effect on the viability of this species. In fact, we found that isolated strains from this genus were able to resist and reduce Cr(VI) present in the cultivation medium.

The clusters defined by protein analysis were broadly in agreement with those defined by FAME analysis, although the techniques usually resolved at different taxonomic levels (Vandamme *et al.* 1996). It was not possible to establish a relationship between the classification of the isolates and their Cr(VI) resistance and/or Cr(VI)-reducing abilities. In fact, our study revealed that, within the same protein and fatty acid cluster, there are differences in the Cr(VI) resistance and Cr(VI)-reducing ability of the strains.

Complete Cr(VI) reduction was achieved during enrichment in the presence of 2 mmol l^{-1} Cr(VI). However, in the reduction tests performed with each isolate, only partial Cr(VI) reduction was achieved. These results suggest either that the whole bacterial community acts co-operatively or that the bacterial populations able to reduce 2 mmol l^{-1} Cr(VI) were not isolated due to the cultivation methods used. However, the possibility of a Cr(VI)-reduction dependence on the composition of the medium used also cannot be excluded.

In this study, the ability of the populations to reduce Cr(VI) was not restricted to one species or one strain but was widespread in several Proteobacteria subclasses, as well as in Gram-positive high G + C bacteria. Although the mechanisms of Cr(VI) removal may differ from group to group, it is evident that both Cr(VI) resistance and Cr(VI) reduction are shared abilities and not an exclusive characteristic of a single group, possibly reflecting horizontal genetic transfer resulting from selective pressure in this contaminated environment (de la Cruz and Davies 2000).

In summary, this study was an attempt to establish a relationship between the Cr(VI) resistance of the culturable microbial community and the Cr(VI) resistance and Cr(VI)-reducing abilities of representative strains of each population and to determine whether these were exclusive characteristics of one microbial group. These are important prerequisites to the implementation of a microbial-based remediation strategy to clean up chromium-contaminated industrial wastewaters.

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