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ENVIRONMENTAL BIOTECHNOLOGY

Bacterial biodiversity from anthropogenic extreme environments: a hyper-alkaline and hyper-saline industrial residue contaminated by chromium and iron

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Abstract Anthropogenic extreme environments are among the most interesting sites for the bioprospection of extremophiles since the selection pressures may favor the presence of microorganisms of great interest for taxonomical and astrobiological research as well as for bioremediation technologies and industrial applications. In this work, T-RFLP and 16S rRNA gene library analyses were carried out to describe the autochthonous bacterial populations from an industrial waste characterized as hyper-alkaline (pH between 9 and 14), hyper-saline (around 100 PSU) and highly contaminated with metals, mainly chromium (from 5 to 18 g kg^{-1}) and iron (from 2 to 108 g kg^{-1}). Due to matrix interference with DNA extraction, a protocol optimization step was required in order to carry out molecular analyses. The most abundant populations, as evaluated by both T-RFLP and 16S rRNA gene library analyses, were affiliated to *Bacillus* and *Lysobacter* genera. *Lysobacter* related sequences were present in the three samples: solid residue and lixiviate sediments from both dry and wet seasons. Sequences related to *Thiobacillus* were also found; although strains affiliated to this genus are known to have tolerance to metals, they have not previously been detected in alkaline environments. Together with *Bacillus* (already described as a metal reducer), such organisms could be of use in bioremediation technologies for reducing chromium, as well as for the prospection of enzymes of biotechnological interest.

Keywords Landfill · Industrial waste · DNA extraction optimization · Chromium hexavalent · T-RFLP

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Introduction

The industrial area of *León* and *Silao* cities (Guanajuato state, Mexico) has important leather industries and a car manufacturing plant. The chromium, necessary for tanning leather or for protecting metal parts from corrosion, is provided by an industry that processes chromite [$(Fe, Mg)Cr_2O_4$]. For more than 50 years, this industry has been producing large amounts of residues, which together with other anthropogenic and natural sources of chromium, has resulted in groundwater

pollution in the *Bajío* Valley (Armienta et al. 1993). The chromium extraction process requires the addition of large amounts of carbonates, producing salty alkaline wastes with high metal concentrations. The leaching of this residue generates hyper-alkaline and hyper-saline lixiviates. Chemically, this environment is characterized by a pH above 9, a salinity about 100 PSU and extremely high concentrations of iron and chromium (this paper), which makes this extreme environment a unique ecosystem. Microorganisms colonizing it may have developed physiological adaptations to survive under alkaline conditions and to resist to and/or transform toxic metals, especially chromium. Such features can be of interest for metal bioremediation technologies.

Among the different chemical species of chromium, hexavalent chromium [Cr(VI)] is the most toxic due to its high solubility in water (Losi et al. 1994; Katz and Salem 1993). For this reason, bacteria that reduce Cr(VI) to Cr(III) have been studied since the 80s and include *Pseudomonas* spp. (Bopp and Ehrlich 1988; Ishibashi et al. 1990), *Escherichia coli* (Shen and Wang 1994), and *Bacillus* spp. (Campos et al. 1995). However, all these organisms are usually described as moderately alkaliphilic and halotolerant.

The first step towards bioprospection consists in the biological and chemical characterization of a site. The chemical properties of extreme samples can inhibit DNA recovery, polymerase chain reaction (PCR) amplifications, and enzymatic restrictions (Hoshino and Matsumoro 2005; Whitehouse and Hotte 2007; Barton et al. 2006; Rajendhrana and Gunasekaran 2008). The consequence of such limitations can be a low recovered biomass or low efficiency in cell lysis due to a strong adsorption of cells to soil and sediment particles (Bruce et al. 1992; Tsai and Olson 1992; Zhou et al. 1996). They can also be related to the co-precipitation of metal ions and chemical impurities with metagenomic DNA, leading to the presence of salts and ions such as Ca^{2+} and in turn affecting the polymerase and other enzyme reactions (Fortin et al. 2004; Hinoue et al. 2004; Desai and Madamwar 2006; Herrera and Cockell 2007).

The aim of the present work is to describe the bacterial communities inhabiting this anthropogenic extreme site (i.e., residue and lixiviate from the chromite processing industry) by using terminal restriction fragment length polymorphism (T-RFLP) and 16S rRNA gene clone libraries analyses. We also present and discuss different approaches to decrease the interferences of the sample matrix with DNA extraction.

Materials and methods

Sampling site and chemical characterization

The samples originated from a chromite processing industry located in Guanajuato state, Mexico (21°02′32″N, 101°47′29″W), which over many years of production has generated a large amount of toxic waste (over than 300,000 t), deposited in a landfill. This landfill (around 25,000 m^2 and 5 m high) is isolated from the soil by a high density polyethylene sheet, and surrounded by lixiviation channels leading to a chemical treatment system. For the diversity study, several points of the residue and the lixiviated sediments were sampled, using a sterilized metal spoon. Measuring from the channel surrounding the landfill, the distance between residue (hereafter R) and lixiviate sediments (LD and LW, see below) is around 5 m. Samples were stored in sterile tubes (50 ml) and immediately transported to the laboratory and frozen (−20°C) until analysis. For chemical analyses, sampling was performed as described above but using plastic spoons, and sent to a reference laboratory (*Centro de Investigación en Materiales Avanzados en Chihuahua*, Chih, Mexico). Because of the Guanajuato climate, the lixiviation process can vary. In order to consider this parameter, samples from lixiviate sediments were collected in wet and dry seasons (respectively LW, September 2007 and LD, January 2007). R samples were taken in January 2007.

The pH measurements were performed in situ using a Conductronic PC 18 pH meter. The analysis of residue samples was performed on a suspension/slurry of 300 g to 1 L of distillated water. The salinity was measured using a salinometer ATAGO S-10 (Atago Co Ldt, Tokyo, Japan). Metal characterization was carried out by atomic absorption spectrometry analysis using the U.S. EPA standard method 3050B for solid waste samples (Environmental Protection Agency Methods 1996). Briefly, 1–2 g of sample (wet weight) was digested in nitric acid and hydrogen peroxide, followed by dilution with hydrochloric acid. This digest was filtered, the filter paper and residues rinsed with hot HCl, and the digest diluted to a final volume of 100 mL. Total metal concentrations were determined by FLAA.

Pre-treatments and DNA extraction

For DNA extraction, nine different approaches were tested and compared, combining different washing procedures, centrifugation steps and mechanical lyses, as well as a control with no pre-treatment. Briefly, the treatments were combinations of: washing in phosphate buffer, decreasing the pH to 7.1–7.3 (i.e., washing with phosphate buffer several times until a neutral pH is reached), freezing in liquid N_2 followed by crushing with mortar and pestle, centrifuging at 10,000×*g* for 15 min and thermal shock (60°C).

After the pre-treatments, 1 g of sample was used for DNA extraction (Torsvik et al. 1990; Tsai and Olson 1991). The samples were mixed with 400 μL Tris-Glucose-EDTA solution containing lysozyme and achromopeptidase (final concentrations 1 mg mL^{-1} and 1,500 U mL^{-1}) and incubated at

37°C for 10 min. Then, 2 μL of proteinase K (20 mg mL^{-1}) and 20 μL sodium dodecyl sulfate (SDS—10%) were added and incubated for 30 min at 37°C. A further 20 μL SDS 10% was added and incubated for 10 min at 60°C. After this cellular lysis, a deproteinization step with phenol:chloroform:isoamyl alcohol (24:24:1 *v*/*v*) (Ogram et al. 1987) was performed. DNA precipitation was carried out with 0.1 volume of sodium acetate (3 M; pH 5.2) and 2 volumes of 95% ethanol, followed by overnight incubation at −20°C. After a centrifugation step (14,000×*g*, 30 min) and washing with 70% ethanol (*v*/*v*), the DNA was resuspended in sterile water and incubated with 1 μL RNAse (1 mg mL^{-1}) at 37°C for 30 min. The DNA quality was verified by electrophoresis in 1% agarose gel in Tris-acetate-EDTA buffer (TAE). DNA solutions were stored at −20°C until further analysis.

Amplification of 16S rRNA gene sequences, T-RFLP, and cloning

The 16S rRNA gene was amplified by PCR as previously described (Paissé et al. 2008). The primers used were: 8F, 5′-AGAGTTTGATCCTGGCTCAG-3′ (Lane 1991); and 907R, 5′-GCCCCCGTCAATTCMTTTRAGTTT-3′ (Lane et al. 1985). The PCR products were purified using GFX PCR DNA purification kit (Amersham), following manufacturer's instructions.

For T-RFLP analysis, the amplification of 16S rRNA genes was performed using carboxyfluorescein (FAM) labeled 8F and unlabeled 907R primers. The purified PCR products were digested with 3 U of restriction enzymes *Hae*III, *Hin*P1I, or *Hpa*II at 37°C for 3 h. Approximately, 50 ng of digested DNA was mixed with 20 μL of deionized formamide and 0.5 μL of TAMRA® marker (Applied Biosystem®) and denatured (94°C for 5 min and chilled on ice). The length of the terminal restriction fragments (T-RFs) was determined by capillary electrophoresis on an ABI PRISM 310 genetic analyser (Applied Biosystem®). After an injection of 10 s, electrophoresis was carried out for 30 min, applying a voltage of 15 kV. The T-RFLP profiles were analyzed using GeneScan Software (Applied Biosystem®). Data sets were normalized and T-RFs representing less than 1% of total fluorescence were discarded (Hewson and Fuhrman 2006). Multivariate analyses of T-RFLP data were carried out with MVSP software (Multi-Variate Statistical Package 3.1, Kovach Computing Services).

The bacterial 16S rRNA gene fragments amplified by PCR (using unlabeled 8F and 907R primers) from total DNA were cloned using a pCR2.1 TOPO® TA cloning kit (Invitrogen®). The inserts were amplified by PCR as described above, using primers M13F and M13R (respectively, 5′-GTAAAACGACGGCCAG-3′ and 5′-CAGGAAACAGCTATGAC-3′). The reactions were cycled in a PTC 200 Thermo-cycler (MJ research) with an initial denaturation step at 94°C for 15 min followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 54°C for 45 s and extension at 72°C for 1 min, and then a final extension step at 72°C for 10 min. The PCR products were sequenced using a BigDye sequencing kit (Applied Biosystem®) following manufacturer's instructions.

The obtained sequences were compared to those of the NCBI database (National Center for Biotechnology Information http://www.ncbi.nlm.nih.gov) using BLAST and aligned with the clustalW® program. Phylogenetic trees were constructed with the MEGA® software using the neighbor-joining method. The confidence of the phylogenetic trees was estimated by bootstrapping to a value of 1,000. The library coverage was calculated (Good 1953) and the rarefaction curves were performed using the MOTHUR pipeline (http://www.mothur.org).

Clone sequences were digested in silico with *Hae*III, *Hin*P1I, and *Hpa*II to generate virtual T-RFLP fingerprints, which were subsequently compared to the real T-RFLP data (Caretta and Brito 2011).

Nucleotide sequence accession numbers

The sequences determined in this study have been submitted to the EMBL database and assigned accession no. FR687640–FR687744

Results

Chemical characterization

The samples presented high salinity, pH, and metal content (Table 1). The solid residue exhibited the highest chromium concentration. Zn and Cu were also detected in the samples at high concentrations. Fe and Ca concentrations were increased as a consequence of industrial processes. The pH varied between 9 and 14, the lixiviate samples being more alkaline. No determination of the salinity could be performed on residue samples; however, no differences in salinity could be determined between lixiviate samples. The Ca concentration can explain the high alkalinity and salinity values observed.

DNA extractions optimization

The nine trials of treatment prior to DNA extraction were compared to determine which produced the best DNA quality (Table 2). DNA quality was defined as (1) the capacity of the polymerase to amplify and (2) the highest diversity recovery, as determined by the number of Operational Taxonomic Units (OTUs) obtained by T-RFLP of 16S rRNA gene amplification. Although no DNA could be observed

Table 1 Chemical and physical characterization of the samples

	Lixiviate LD (dry season)	Lixiviate LW (wet season)	Solid residue R (dry season)	Similar environments (ref)
Ca (g kg^{-1})	43.8	3.7	20.3	
Cr (g kg^{-1})	7.0	5.1	18.3	10.7[a]
Cu (mg kg^{-1})	10	ND	20	
Fe (g kg^{-1})	19.6	2.7	10.9	
Zn (mg kg^{-1})	80	30	430	73.6[a]
Salinity (‰)	100	100	ND	
pH	14	12	9	

ND not detected

[a]Desai et al. 2009

after agarose gel electrophoresis, amplicons could be obtained (marked with "+" symbols in Table 2). Only pre-treatments B and F gave positive results for the three samples. The diversity observed in the pre-treated samples is summarized in Fig. 1. The highest diversity was obtained from DNA recovered after F pre-treatment (i.e., washing with phosphate buffer until the pH fell to 7, followed by crushing the sample in liquid N_2). The pre-treatment also produced the highest reproducibility between replicates (Bray Curtis similarity index around 80%, data not shown). Thus, F pre-treatment was chosen as a preliminary step before DNA extraction for all the analyses performed hereafter.

T-RFLP analysis

The LD sample showed the lowest diversity (a total of 38 T-RFs cumulating the results of the three enzymes, 10+15+13, respectively for *Hae*III, *Hin*P1I, and *Hpa*II), compared to LW and R samples (respectively, 55=19+18+18 and 59=18+16+25 T-RFs). The T-RFLP fingerprints are graphically represented in Fig. 1 (see also Caretta and Brito 2011 for a list of detected T-RFs). Thirteen T-RFs were common to the three samples (Fig. 2a) but showed different relative abundances. For example, T-RF 431 bp—*Hpa*II was dominant in the three samples (15% in R, 25% in LD and 50% in LW), while T-RF 203 bp—*Hae*III was dominant in LD sample (28%) but less important in LW and R samples (less than 5%, Fig. 1).

16S rRNA gene libraries and phylogenetic analyses

In order to identify the main populations observed by T-RFLP analysis, 16S rRNA gene clone libraries were constructed. The rarefaction curves (data not presented) showed that the plateau was reached only for the LD library. The coverage was 0.5 for R library and 0.8 for LW and LD libraries. A total of 25 phylotypes was observed: 17 for R (32 clones), 7 for LW (39 clones), and 6 for LD (28 clones) (Fig. 2).

The phylogenetic analysis of the 99 sequenced clones indicated the presence of eight bacterial classes (Fig. 3). Most of the sequences were related to Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria classes (15%, 14%, and 40%, respectively). Almost all the sequences related to Gammaproteobacteria were affiliated to *Lysobacter* sp. (37%). A second major group (20%) was related to the Bacilli class. Sequences related to the *Thiobacillus* genus were common to LW and R samples; however, most phylotypes found in R

Table 2 16S rRNA gene PCR amplification from extracted DNA after different pre-treatments tested to clean-up the matrices prior to DNA extraction: (−) no amplification observed, (+) positive amplification (the number of T-RFs obtained with the restriction enzyme *Hin*P1I is shown in round brackets)

	Treatments	Lixiviate LD (dry season)	Lixiviate LW (wet season)	Solid residue R (dry season)
A	No pre-treatment	+ (31)	−	+ (1)
B	Wash three times with phosphate buffer	+ (9)	+ (28)	+ (23)
C	Decrease the pH to 7.1–7.3 (wash with phosphate buffer several times until a neutral pH is reached)	−	−	+ (23)
D	Freeze in liquid N_2 and crush with mortar and pestle	+ (10)	−	−
E	B + D	+ (20)	−	+ (22)
F	C + D	+ (23)	+ (23)	+ (30)
G	C + centrifugation at 10,000×*g* (for 15 min)	−	−	+ (15)
H	G + D	−	−	−
I	H + thermal shock (60°C)	−	−	−

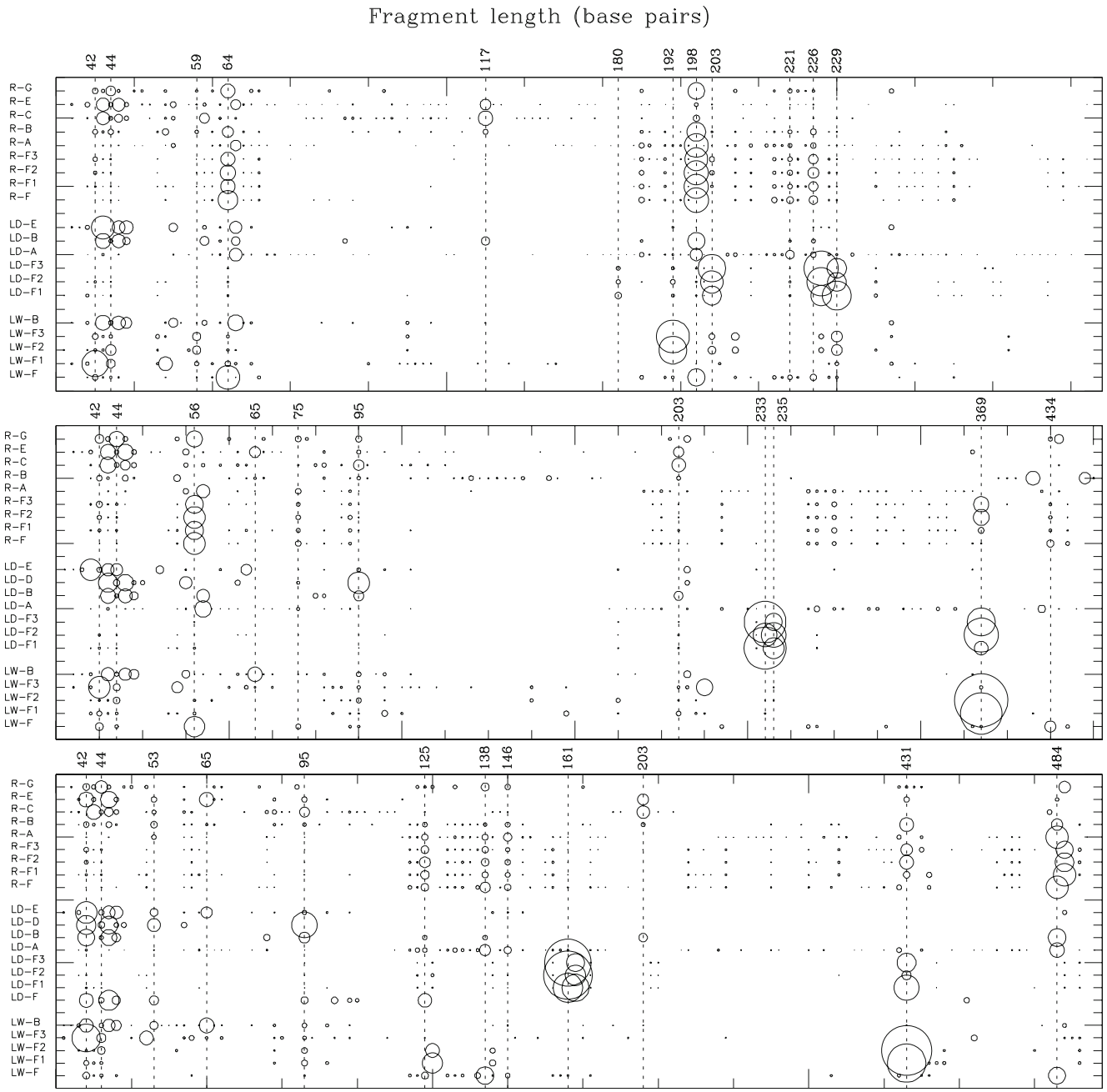

Fig. 1 Graphical representation of T-RFLP fingerprints of the bacterial communities found in the three samples submitted to the different pretreatments, digested with *Hae*III, *Hin*P1I, and *Hpa*II enzymes (respectively from top to bottom panels). F1, F2, and F3 are triplicates of F treatment. The size of the circles is proportional to the normalized area of the T-RFs present in each sample and treatment. The T-RFs of the dominant populations are marked with dashed vertical lines and labeled above the box

were exclusively found in that sample. Few sequences were affiliated to other classes: Acidobacteria (3), Deinococci (2), Sphingobacteria (1), and Actinobacteria (4).

In silico restriction

The in silico restriction analysis of the cloned sequences allowed the identification of most of the T-RFLP peaks, especially those related to the most abundant populations. *Lysobacter* sequences produced in silico T-RFs of 39, 371 and 436 bp for *Hae*III, *Hin*P1I, and *Hpa*II enzymes, respectively. 39 bp was out of the range of the T-RFLP fingerprints, but for *Hin*P1I and *Hpa*II, the expected T-RFs could match T-RFs 369 and 431 bp, the dominant omnipresent peaks in the fingerprints. The second most abundant population, belonging to the *Bacillus* genus, gave in silico T-RFs of 207 and 231 bp

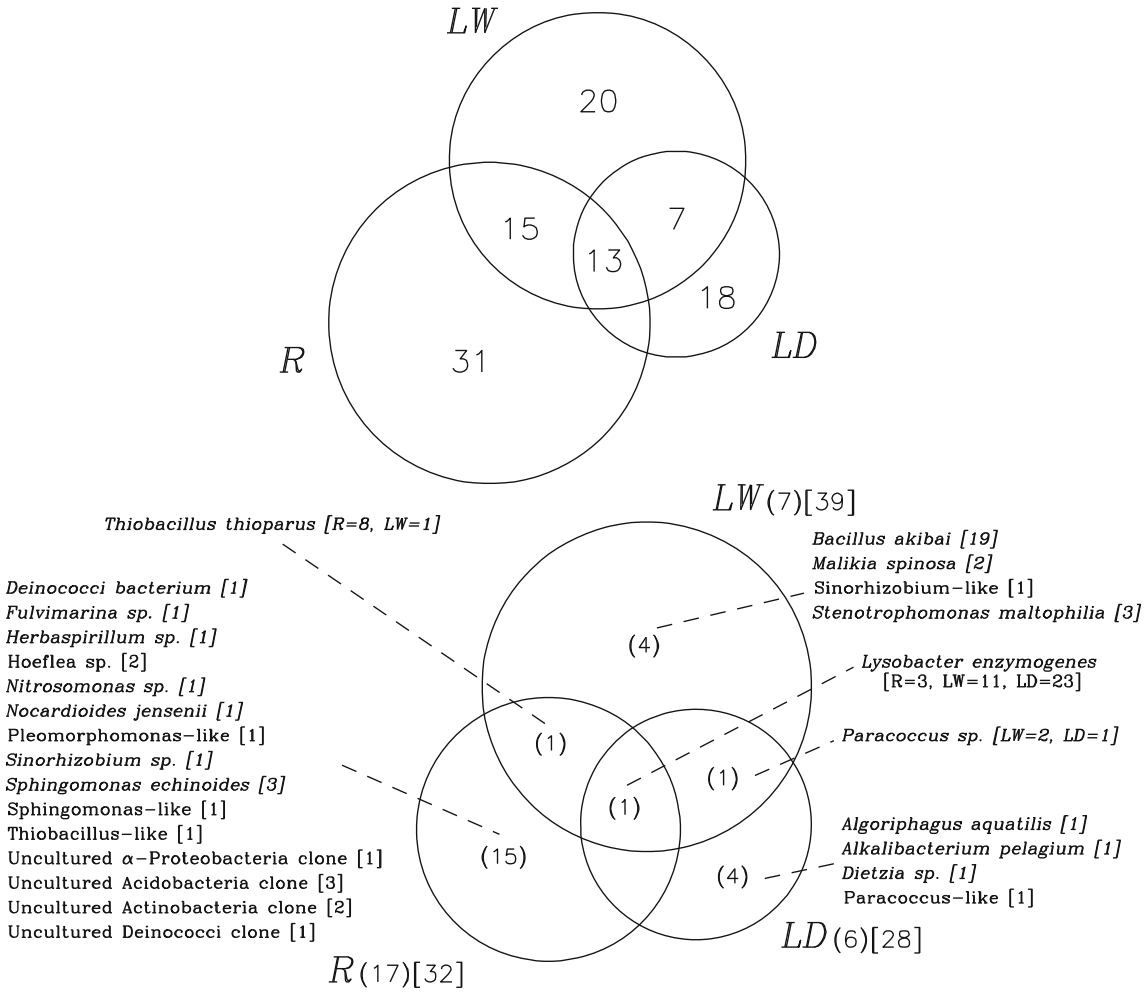

Fig. 2 Venn diagrams showing the bacterial diversity of lixiviate (LW and LD) and residue (R). *Top panel:* diversity assessed by T-RFLP analysis based on the 16S rRNA gene; the numbers in the *circles* indicates the T-RFs obtained with the three enzymes. *Bottom panel:* diversity assessed by cloning and sequencing the 16S rRNA gene; the *numbers in the round brackets* indicates the numbers of different phylotypes; the *numbers in the square brackets* after the OTUs specify the number of clone sequences. The size of the *circle* is proportional to the relative abundance of clones

with *Hae*III, 238 and 165 bp with *Hin*P1I and *Hpa*II, respectively, which corresponded to the observed T-RFs 206, 227–229; 233–235 and 161–162 for *Hae*III, *Hin*P1I, and *Hpa*II, respectively. The other detected T-RFs were consistent with in silico digestions of *Thiobacillus*, *Sinorhizobium*, *Paracoccus*, *Malikia*, and *Dietzia* phylotypes (see details on Table 4 of Caretta and Brito 2011).

Discussion

For many years, the process to extract of chromium from chromite has involved the addition of carbonate and heating at temperatures over 1,000°C. As a result, besides the desired product, large amounts of highly alkaline wastes (due the presence of Na^+, CO_3^{2-}, and Ca^{2+} ions) with high levels of metal (mainly Cr(VI) and Fe) have been generated. At the *Léon* extraction plant, this process was abandoned in the late 1990s; however, 300,000 t of residue remained stored in a tailing located above an isolation bed and surrounded by lixiviation channels to direct waters towards a chemical treatment system. The metal content of the sediments was consistently higher during the dry period, probably as a consequence of concentration by evaporation (dry period) as compared to dilution by flooding (wet period). The solid residue exhibited the highest Cr content (18.3 g kg^{-1}) compared to lixiviated samples (5.1–7 g kg^{-1}). These values are as high as those observed by Desai et al. (2009) on residual wastes contaminated by chromium in India, but consistently higher than the contamination observed from tannery effluent-contaminated soils (Alam et al. 2011). There are no international regulations for chromium disposal in soil, but due to the high water solubility and toxicity of Cr(VI), there are standards for the concentration of Cr(VI) in

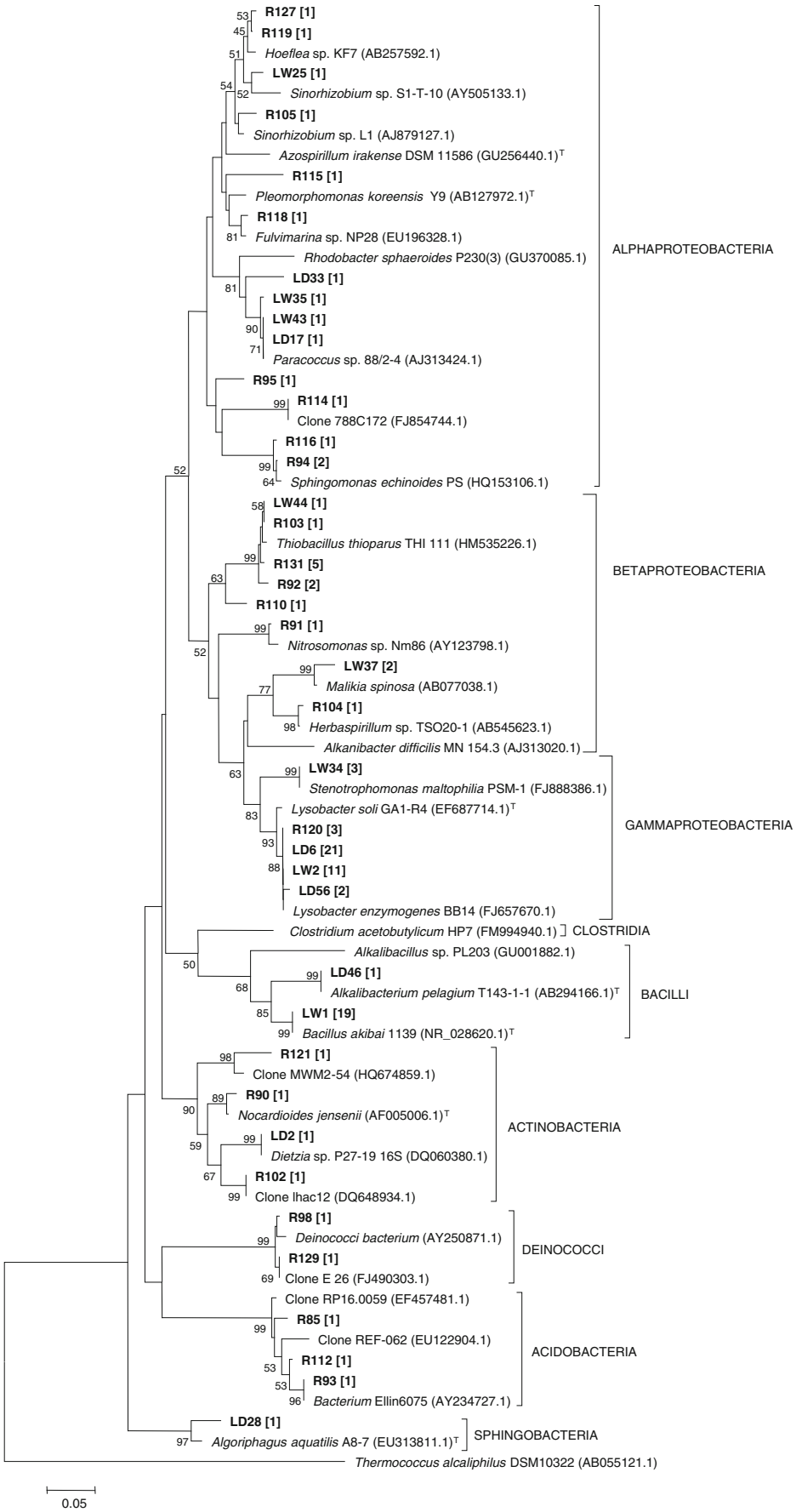

Fig. 3 Phylogenetic relationship between the 16S rRNA gene sequences obtained from sediment samples of lixiviate (LW and LD, wet and dry seasons, respectively) and residue waste sample (R). The tree was constructed using the neighbor-joining algorithm, with representatives of each phylotype (considering sequences of more than 97% identity to be the same phylotype) and with related sequences obtained from NCBI database. 196 nucleotides were considered in the sequence alignment. The *scale bar* represents the expected number of substitutions

wastewaters in almost all the industrialized countries. In Mexico, the Environmental Agency stipulated a limit of 5 mg L^{-1} of total Cr in industrial wastewater effluents (Official Mexican Standard 2006). The properties of the lixiviates generated by the chromate residue of *Léon* extraction plant, i.e., high pH and salinity and high heavy metal content are conditions rarely observed simultaneously in nature and confirm the status of this ecosystem as extreme for life.

In order to obtain total DNA from the wastes of *León* landfill, we tested different standard protocols previously described (Tsai and Olson 1991; Rochelle et al. 1992; van Elsas et al. 1997; Griffiths et al. 2000) as well as a commercial kit (Power Soil® DNA Isolation Kit from MoBio). Unfortunately, none of these protocols were successful (the DNA was neither visible after electrophoresis on agarose gels, nor were the 16S rRNA genes amplified by PCR). Thus, we considered the necessity to perform a pre-treatment step before DNA extraction in order to eliminate the matrix interferences. Few optimized DNA extraction protocols have previously reported the need to wash the sample prior to cell lysis (La Montagne et al. 2002). Different procedures were tested for cleaning up the samples in the present study (Table 2). The most efficient pre-treatment, F, consisted in decreasing the pH to around 7 and crushing the N_2-frozen sample with a mortar. The main problem was probably the presence of Ca^{2+} ions and metals, rather than the high pH. The presence of salts, especially those containing divalent cations that have a significant effect on the activity of the *Taq* polymerase, could explain the PCR inhibition (Mahony et al. 1998). The washing steps diluted Ca^{2+} and metals, reducing their interference in the subsequent steps. The treatment with mechanical disruption was efficient not only for the lysis of cells but also for the grinding of the sample matrix. This last process favored access to agglomerated cells or cells located within pores, increasing the contact surface of cells with the reagents and therefore facilitating cell lysis.

The dominant operational taxonomic units (OTUs) in the three samples were affiliated to *Lysobacter* and *Bacillus* genera. *Lysobacter*-related populations were omnipresent in the clone libraries and their corresponding T-RFs were found in all T-RFLP fingerprints. *Bacillus* populations were also omnipresent in the T-RFLP fingerprints but found only in LW library. The ability of *Bacillus* strains to grow on and transform metals is well known (Nies 2003), including Cr (VI) biotransformation (Garbisu et al. 1998; Pal and Paul 2004; Elangovan et al. 2006). In addition, *Bacillus* strains have been found in extremely haloalkaline environments (Sorokin et al. 2008). Indeed, alkaliphilic *Bacillus* species are the most characterized organisms among alkaliphiles and their cellular systems of adaptation to alkaline environments are well described (Fujinami and Fujisawa 2010). In contrast, few works have described *Lysobacter* spp. isolated from contaminated environments (Yassin et al. 2007; Mera and Iwasaki 2007), or their ability to degrade organic or organometallic compounds (Miller et al. 2004). Its resistance to heavy metals has recently been described. One study characterized *Lysobacter* strains from artificial microcosms containing $HgCl_2$ and suggested a possible mercury resistance (Mera and Iwasaki 2007). In addition, a *Lysobacter* strain was very recently described to be resistant to 200 μM of chromium and able to grow at pH 11 (Liu et al. 2011). Strains affiliated to this genus are usually associated with plants (Hayward et al. 2010). The omnipresence and the relative abundance of sequences related to this genus in all the samples suggested that this population may have specific metabolic properties conferring metal resistance, together with the capacity to live in alkaline and hypersaline conditions. This bacterial population may have interesting applications as a source of new enzymes, while, from an academic point of view, it has potential for the study of mechanisms involved in adaptations to extreme conditions such as those observed on the sampling site.

Sequences related to *Thiobacillus* genus were found in both R and LW clone libraries and were omnipresent in T-RFLP fingerprints. Representatives of this genus include chemolithoautotrophic sulfur-oxidizing bacteria (*Thiobacillus denitrificans*, Kelly and Wood 2000). This Betaproteobacterium can grow efficiently under several limiting conditions such as nutrient starvation and extremely acid pH (Justin and Kelly 1978). The tolerance of *Thiobacillus* species to metals (Cd, Zn, and Cu) has been observed (Touvinen et al. 1971; Butcher et al. 2000; Chen and Lin 2001), but exclusively under acidic conditions (Huber and Stetter 1990; Collmer et al. 1950; Sand et al.1992). The identification of *Thiobacillus* species in an alkaline environment (pH above 9) is a novelty. The only known alkaliphilic sulfur oxidizers, such as representatives of the genera *Thioalkalivibrio* or *Thioalkalimicrobium*, are Gammaproteobacteria (Sorokin et al. 2001), whereas the bacteria identified in the tailings and the lixiviates belong to the Betaproteobacteria. The isolation and investigation of *Thiobacillus* strains would improve our knowledge of their metabolic adaptations to alkaline environments, in addition to their obvious potential for bioremediation (bioleaching) of the site under study.

Although the sampling site was confirmed to have extreme characteristics, the observed microbial diversity was found to be relatively high, confirming its potential for bioprospection and, possibly, for bioremediation technologies (see also Piñon-Castillo et al. 2010). However, the study of this kind of environments presents some difficulties, and a protocol optimization step may be necessary to obtain DNA of sufficient quality. The molecular approaches used in this study unveiled the dominance of *Bacillus* and *Lysobacter* species, as well as less abundant but equally

interesting species, such as *Thiobacillus*. Extreme environments, as that under study here, may favor the growth of microorganisms that in normal conditions would be excluded by competition, or in such minority that they remain unnoticed. The natural continuity of this research is the challenge of isolating strains resistant to Cr(VI) using specifically developed media, such as *Thiobacillus* and *Lysobacter* spp., which may have a use in bioremediation and biotechnology.

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