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The use of RAPD genomic fingerprinting to study relatedness in strains of *Acidithiobacillus ferrooxidans*

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Abstract

Twelve strains of *Acidithiobacillus ferrooxidans* were recovered from acid mine drainage (AMD) sites from three different geographical locations: Copper Cliff, Ontario, Canada; Mineral City, OH, USA; and Cornwall, England. The spread-plate technique and various culture media were used to isolate and purify all strains. DNA was extracted from each purified culture and amplified using PCR and twenty, 10-mer primers. Amplification products were separated by gel electrophoresis and photographed under UV light. The RAPD (Randomly Amplified Polymorphic DNA) profiles were compared on the basis of the presence or absence of each DNA band and a data matrix was constructed. Strain diversity was calculated using the Jaccard's coefficient and UPGMA (Unweighted Pair-Group Arithmetic Average Clustering) cluster analysis.

The variations in the banding patterns indicated genomic variability among the twelve *A. ferrooxidans* strains tested. The primers used in this study grouped the twelve strains into five major groups. Similarities between the strains ranged from 5.49% to 85.14%. These results show that the strains have a high degree of genomic diversity and that the RAPD procedure is a powerful technique to assess strain variability in this bacterium.
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Keywords: RAPD; *Acidithiobacillus*; Molecular fingerprinting; Diversity

1. Introduction

Acidithiobacillus ferrooxidans (formerly *Thiobacillus ferrooxidans*) is the main bacterium of acid mine drainage environments and bioleaching systems (Leduc and Ferroni, 1994; Schippers et al., 1995). Various molecular methods such as rRNA analysis, G+C content, and DNA–DNA hybridization have been used to obtain a better understanding of the phylogenetic relationships among the many strains of *A. ferrooxidans* (Harrison, 1982; Lane et al., 1985, 1992). Studying various *A. ferrooxidans* strains at the

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molecular level is important in having a better understanding of phylogenetic relations and in establishing a possible correlation between genomic and phenotypic variability.

Welsh and McClelland (1990) used a sensitive and rapid DNA polymorphism assay referred to as RAPD (Random Amplified Polymorphic DNA) to detect strain variability. RAPD is a simple and fast method, which can be applied to any species for which DNA can be extracted. Also, little knowledge of the biochemistry or molecular biology of the species being studied is required. They concluded that this technique was sensitive enough to differentiate even the closely-related strains of the same species. Recently, the RAPD technique was used to determine genomic variability among strains of *A. ferrooxidans* (Novo et al., 1996). Novo et al. (1996) showed that genomic diversity was present in strains of *A. ferrooxidans* and that the RAPD method is a powerful technique to assess strain variability in this bacterium.

Selenska-Pobell et al. (1998) also investigated the use of molecular techniques in the study of strain diversity in *A. ferrooxidans*. In their study, ARDREA, RAPD and rep-APD were compared and found to be useful tools in discriminating closely-related strains of the bacterium. Indeed, the authors suggest that such molecular techniques can be used for the rapid and reliable classification and discrimination of strains of *A. ferrooxidans*. As well, molecular techniques can be used for the rapid screening of strains important in industrial environments, such as bioleaching operations.

In this investigation, RAPD genomic fingerprinting was used to study relatedness in strains of *A. ferrooxidans*, which were isolated or obtained from different geographic areas.

2. Materials and methods

2.1. Bacteria

This study employed various strains of *A. ferrooxidans,* which were recovered and isolated from acid mine drainage sites from three main geographical locations: Copper Cliff, Ontario, Canada; Cornwall and North Wales, United Kingdom; and, Tuscarawas and Carroll County, OH, USA. The strains used in this study are listed in Table 1.

A total of twelve strains of *A. ferrooxidans* were used in the study. Isolate Cc was recovered from a nickel/copper tailings area in Copper Cliff. Isolates Pa and Wh were recovered from AMD effluents from Parys Copper Mine, Anglesay, North Wales and Wheal Jane Tin Mine, Cornwall, England, respectively. Water samples from both mines were obtained from Dr. Barrie Johnson, University of Wales, UK. Isolates 15A, 15B, 16A, 31A, 33, and 33A were

Table 1
Strains of *A. ferrooxidans* and sources

Strain	Reference	Origin/source
ATCC 23270	American Type Culture Collection, Rockville, Maryland, USA	Bituminous coal mine effluent, USA
Cc	Danielle Waltenbury	Effluent of nickel and copper mine from Copper Cliff tailings A area, Sudbury, Canada
Pa	Dr. Barrie Johnson	Effluent from Parys copper mine, Anglesay, North Wales
Wh	Dr. Barrie Johnson	Effluent from Wheal Jane tin mine, Cornwall, England
A1	Dr. Marvin Silver	West Gore Antimony Mine, Hants County, Nova Scotia, Canada
W1	Dr. Marvin Silver	West Gore Antimony Mine, Hants County, Nova Scotia, Canada
15A	Mr. Jim Gue	Deep coal mine drainage mine from Carroll County, Ohio, USA
15B	Mr. Jim Gue	Mix deep coal mine and surface coal mine drainage from Carroll County, Ohio, USA
16A	Mr. Jim Gue	Deep coal mine drainage from Carroll County, Ohio, USA
31A	Mr. Jim Gue	Deep coal mine drainage from Tuscarawas County, Ohio, USA
33	Mr. Jim Gue	Mix of deep coal mine and surface coal mine drainage from Tuscarawas County, Ohio, USA
33A	Mr. Jim Gue	Deep coal mine drainage from Tuscarawas County, Ohio, USA

recovered from a mixture of deep coal mine drainage and surface coal mine drainage in and around Mineral City, OH, USA. Water samples were kindly provided by Mr. Jim Gue, scientist at ODNR (Ohio Department of Natural Resources). In addition, *A. ferrooxidans* ATCC 23270 was purchased from the American Type Culture Collection, Rockland, Maryland and strains A1 and W1 were originally obtained from Dr. Marvin Silver, Dalhousie University and subsequently described by Leduc et al. (1993).

2.2. Isolation and cultivation of bacteria

To recover strains Cc, Pa, Wh, 15A, 15B, 16A, 31A, 33, and 33A of *A. ferrooxidans*, 150 mL of acid mine drainage water was filtered through a sterile membrane filter (0.45 μm, pore size) and each membrane was placed into 100 mL of sterile TK medium, pH 2.1 (Tuovinen and Kelly, 1973) contained in 250-mL capacity Erlenmeyer flasks. Flasks were shaken at 200 rpm with aeration at room temperature for 3–5 days. Once growth was apparent (change in the colour of the medium from light blue-green to a deep red-brown), each isolate was purified by the spread-plate technique using a modification of the iron-salts purified (ISP) agarose medium of Manning (1975) containing 1% $FeSO_4$. To facilitate the spreading of the inoculum, 50 μL of Tween 80 were added to each inoculum and 0.2 mL were spread onto the surface of each plate. The inoculated plates were incubated in the dark at 22 °C for 2–3 weeks. Spread-plating was repeated three times to ensure the purity of the different isolates.

Since not all isolates grew well on ISP agarose, other solid media were used, including TK medium (pH 1.8) solidified with 0.6% agarose, FeTSB agarose using 25 mM and 33 mM $FeSO_4$ (Johnson, 1995), FeTSBo overlay agarose (Johnson, 1995), FeSo overlay agarose (Johnson, 1995), and TK overlay agarose using *Acidiphilium* strain SJH in the underlay.

2.3. DNA preparation

Pure cultures of each strain were grown in 600 mL of TK medium in 1-L Erlenmeyer flasks. The pH of the medium was poised at 1.8 to reduce jarosite deposits. All strains were grown with aeration at room temperature (20–22 °C) on a gyratory water bath shaker at 190 rpm. Following growth, the cultures were filtered to reduce the amount of jarosite, if any. The cells were pelleted in sterile 250-mL capacity Beckman screw-cap centrifuge bottles at 15,300 × g for 15 min at 4 °C. The supernatants were discarded and the cell pellets were washed with 10 mL of 0.01 N H_2SO_4. The cell suspensions were pooled and transferred to two sterile 60-mL capacity Teflon FEP (fluorinated ethylene propylene) centrifuge tubes. The cells were then harvested by centrifugation at 12,000 × g for 10 min at 4 °C. The cells were washed once again with 5 mL of 0.01 N H_2SO_4. The cell suspensions were pooled and transferred to a sterile 60-mL capacity Teflon FEP centrifuge tube. The cells were then centrifuged at 12,000 × g for 10 min at 4 °C and washed twice with STE (100 mM NaCl, 10 mM Tris–HCI, pH 8.0, 1 mM Na_2EDTA).

The cells were suspended in 10 mL of TGE (12.5 mM Tris–HCI, pH 8.0, 25 mM glucose, 5 mM Na_2EDTA). A volume of 1 mL of lysozyme (10 mg/mL) was added and the suspension was maintained for 10 min at room temperature. A small amount (625 μL) of 0.5 M Na_2EDTA, a chelating agent, was added to the mixture and incubated on ice for 10 min. Then, 1.25 mL of 10% SDS and 125 μL of pronase E were added, followed by digestion at 37 °C for 1 h.

The lysate was extracted once with 5 mL phenol–chloroform (1:1) by mixing thoroughly, but gently, for 5–10 min (i.e., no vortexing) and centrifuged at 12,000 × g for 15 min. The upper layer, containing the DNA was transferred to a sterile 60-mL Teflon FEP centrifuge tube. The extraction was repeated with chloroform. To the transferred upper layer, 0.1 volumes of 3 M sodium acetate (pH 5.2) and 0.6 volumes of isopropanol were added. The mixture was stored at 4 °C overnight to precipitate the DNA. The following morning, the preparation was centrifuged at 12,000 × g for 15 min at 4 °C and the supernatant was discarded. The DNA was then resuspended in 70% ethanol and centrifuged at 12,000 × g for 15 min at 4 °C. The supernatant was discarded. The DNA was resuspended in 500 μL of TE (10 mM Tris–HCI, pH 8.0, 1 mM Na_2EDTA) and transferred to a sterile microfuge tube.

To remove the RNA, 1.25 μL of RNase (20 mg/mL) were added and the mixture was incubated at room temperature (22 °C) for 0.5 h. Afterwards, 150 μL of phenol–chloroform (1:1) were added and the

preparation was mixed by inversion for 15 min (i.e. no vortexing) and centrifuged at 13,000 × g for 15 min at 4 °C. The upper layer was removed and transferred to a sterile microfuge tube. The procedure was repeated using a chloroform extraction. Then, the DNA was once again precipitated by the addition of 0.1 volumes of 3 M sodium acetate (pH 5.2) and 0.6 volumes of isopropanol. The precipitated DNA was then stored at −20 °C overnight. The DNA was centrifuged at 13, 000 × g for 15 min at 4 °C, the supernatant was discarded, and the DNA was washed with 70% ethanol. The mixture was centrifuged at 13, 000 × g for 15 min and the supernatant was discarded. The DNA was then resuspended in 500 μL of TE buffer. DNA concentrations and purity were determined by spectrophotometric readings. The DNA was stored at −70 °C until needed.

2.4. DNA amplification

Twenty 10-mer primers from Operon Technologies Inc. (Alameda, CA, USA) were used for the PCR reactions (Table 2). All amplification reactions were done by using 7 ng of DNA and controls were made by replacing DNA with water. The following components were added to a sterile 0.5-mL microcentrifuge tube. The reaction mixtures (24 μL) contained 0.5 μL (0.2 μmol) of the primer, 2.5 units (0.125 μL) of Platinum *Taq* DNA Polymerase (Life Technologies), 2.5 μL of 10 × supplied PCR buffer, 0.5 μL (0.2 mmol) of each dNTP, and 0.75 μL (1.5 mmol) of $MgCl_2$. Sterile, distilled water was added to make up the volume to 24 μL.

Table 2
Sequences of twenty oligonucleotides (Kit Operon) used for amplification with the PCR procedure

Primer	Sequence (5′ to 3′)
OPC-01	TTCGAGCCAG
OPC-02	GTGAGGCGTC
OPC-03	GGGGGTCTTT
OPC-04	CCGCATCTAC
OPC-05	GATGACCGCC
OPC-06	GAACGGACTC
OPC-07	GTCCCGACGA
OPC-08	TGGACCGGTG
OPC-09	CTCACCGTCC
OPC-10	TGTCTGGGTG
OPC-11	AAAGCTGCGG
OPC-12	TGTCATCCCC
OPC-13	AAGCCTCGTC
OPC-14	TGCGTGCTTG
OPC-15	GACGGATCAG
OPC-16	CACACTCCAG
OPC-17	TTCCCCCCAG
OPC-18	TGAGTGGGTG
OPC-19	GTTGCCAGCC
OPC-20	ACTTCGCCAC

A master mix, containing the sterilized double distilled water, buffer, DNTP, primers, $MgCl_2$ and the *Taq* polymerase, was prepared in order to minimize reagent loss and to enable accurate pipetting. The master mix was aliquotted (24 μL) to individual tubes. The DNA template (1 μL) was then added to each reaction tube, which made up the final volume to 25 μL. The contents of the tubes were mixed thoroughly and overlayed with 50 μL of mineral oil. The DNA amplifications were carried out in a Perkin-Elmer DNA Thermal Cycler. DNA denaturation was done at 94 °C for 5 min followed by 45-cycle amplification (94 °C, 1 min denaturation step; 35 °C, 1 min annealing step; 72 °C, 2 min extension step) and by a final DNA extension step at 72 °C for 7 min.

The reaction was maintained at 4 °C after cycling. The samples were stored at −70 °C until used. Each amplification was done in triplicate or more and results were reproducible when DNA from different extractions of the same strain were used.

All amplification products were separated by electrophoresis on 1.5% agarose gel using 1 × TAE buffer (40 mM Tris base, 20 mM sodium acetate, 1.8 mM EDTA, pH adjusted to 7.8 with glacial acetic acid) for 3 h at 40 V. Once completed, the gels were stained in 1 μg/mL ethidium bromide for 15 min and destained in double distilled water for the same period of time. Following the staining procedure, the gels were photographed under ultraviolet light (312 nm) in a Spectroline TX-312 transilluminator, with a Polaroid DS34 camera set at F5.6 and shutter speed of 1 s using Polaroid 667 black and white film.

2.5. Data analysis

The results were analyzed by comparing RAPD profiles on the basis of the presence (1) or absence (0) of each DNA band on the photographed agarose gels. An $n \times t$ data matrix was constructed, where t represents the OTU (operational taxonomic unit) to be grouped (the strains), and where n is the n unit

characters (number of bands). When a band was present, it was scored as 1, and when absent it was scored as 0. Afterwards, the Jaccard's coefficient (Sj) was used to determine similarity between each strain. The Jaccard's coefficient equation is as follows: $Sj = n11/n11 + n01 + n10$, where $n11$ is the number of characters coded 1 in both OTU, where $n01$ is the number of characters coded 0 for the first OTU and 1 for the other, and where $n10$ is the number of characters coded 1 for the first OTU and 0 for the other (Sneath and Sokal, 1973).

A dendrogram was then constructed using the UPGMA (Unweighted Pair-Group Arithmetic Average Clustering) cluster analysis and the cophenetic correlation was determined according to Sneath and Sokal (1973).

3. Results

The genomic DNA of each strain used in this study was amplified using 20 different primers (Table 2). A large number of reproducible bands was produced for most of the primers. However, some primers produced several non-reproducible bands. Primers OPC-01, OPC-03, OPC-04, OPC-12, OPC-13, OPC-16, and OPC-17 were excluded from data analysis due to the production of non-reproducible bands.

Thirteen primers tested produced specific band profiles revealing polymorphisms. The negative control tubes (in which the genomic DNA was replaced with double distilled water) did not show any DNA amplification. The primers used generated a total of 735 reproducible bands. The number of bands for each primer varied from 39 to 68 with an average of 56 bands per primer. The size of the amplified product ranged from 200 to 4000 bp. When the experiments were replicated, the number and location of all bands were found to be identical, and only minor variations in the intensities of some bands could be seen.

The RAPD profiles of the twelve *A. ferrooxidans* strains were compared, and variation in the band profiles was observed for each primer. Each strain, except strains 15A and 16A, had its own distinct banding pattern. Strains 15A and 16A had very similar banding patterns for all of the primers used. Fig. 1 shows a typical example of the banding pattern profiles obtained for several strains of *A. ferrooxidans* using the primer OPC-02. The variation found in the banding pattern in the amplification products indicates a genomic variability among most of the strains studied.

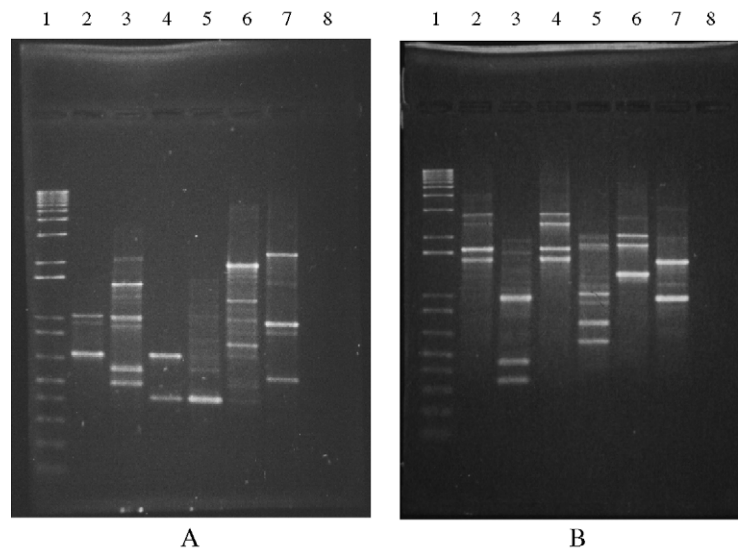

Fig. 1. Agarose gels showing RAPD products obtained by amplification of 7 ng of DNA using the primer OPC-02. (A) Lane 1, DNA molecular weight standard (1 Kb Plus DNA Ladder — Gibco-BRL); lanes 2–7, *A. ferrooxidans* isolates ATCC 23270, Cc, A1, W1, Wh, and Pa; lane 8, negative control. (B) Lane 1, DNA molecular weight standard (1 Kb Plus DNA Ladder — Gibco-BRL); lanes 2–7, *A. ferrooxidans* isolates 15A, 15B, 16A, 31A, 33, and 33A; lane 8, negative control.

Table 3
Jaccard's coefficient of similarity (%) of twelve *A. ferrooxidans* strains

	ATCC	Cc	A1	W1	Wh	Pa	15A	15B	16A	31A	33	33A
ATCC	100.00											
Cc	10.57	100.00										
A1	13.19	16.53	100.00									
W1	13.21	19.70	38.89	100.00								
Wh	11.21	33.60	18.58	17.05	100.00							
Pa	13.04	24.44	17.39	23.58	15.00	100.00						
15A	8.65	21.77	16.83	16.38	13.39	13.28	100.00					
15B	7.87	20.72	13.48	15.69	10.43	14.29	15.63	100.00				
16A	7.96	22.14	15.45	15.20	15.04	13.24	85.14	16.50	100.00			
31A	11.39	24.75	12.05	14.58	25.26	12.15	17.05	14.67	16.67	100.00		
33	5.49	22.94	8.60	11.32	23.30	14.29	15.63	11.90	16.50	28.36	100.00	
33A	5.68	18.02	11.36	16.16	12.72	12.61	24.14	12.35	21.88	13.70	12.35	100.00

The coefficients were calculated from the RAPD data of 735 polymorphic bands.

Amplified DNA fragments that were reproducible were scored as 0 and 1 in a data matrix and the relationships between the twelve strains were estimated using Jaccard's coefficient of similarity. Jaccard's coefficient was chosen because it excludes common negative data, i.e. where two strains lack the same band. Jaccard's coefficient between each pair of the *A. ferrooxidans* strains is indicated in the lower triangular matrix in Table 3.

Similarities between strains ranged from 5.49% (ATCC 23270 and 33) to 85.14% (15A and 16A). The resultant dendrogram obtained by UPGMA (Unweighted Pair-Group Arithmetic Average Clustering) cluster analysis is shown in Fig. 2. The dendrogram shows the differences among the *A. ferrooxidans* strains. Five groups were characterized: the first group formed by *A. ferrooxidans* strains Cc, Wh, 31A, and 33; the second group formed by *A. ferrooxidans* strain 15A, 16A, and 33A; the third group formed by *A. ferrooxidans* strains Pa, A1, and W1; the fourth and fifth group were formed by 15B and ATCC 23270, respectively. The ATCC 23270 strain was the most divergent. As shown in Fig. 2, the strains 15A and 16A were closely related (85.14%). These two strains (15A and 16A) were both isolated from

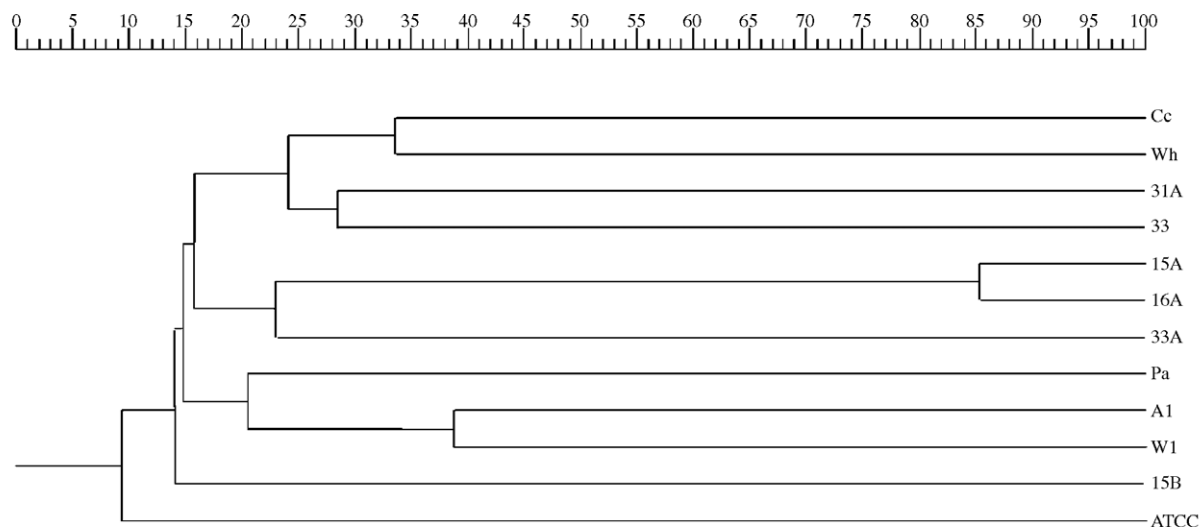

Fig. 2. Dendrogram representing the relationships between twelve *A. ferrooxidans* strains based on UPGMA cluster analysis of the RAPD profiles derived from 13 primers using the Jaccard's similarity coefficient (%) (cophenetic correlation was $r=0.961605$).

similar environments, deep coal mine drainage from Carroll County, OH, USA.

Other strains belonging to the same similarity group are either present in similar geographic areas or are present in distinct geographic areas. For example, the second similarity group, containing strains 15A, 16A, and 33A consisted of isolates from deep coal mine drainage.

4. Discussion

In this study, genomic DNA from each of the twelve *A. ferrooxidans* strains examined was amplified using 7 ng of DNA with a total of 13 primers. All primers tested produced distinctive DNA fragment patterns revealing polymorphisms. A total of 735 polymorphic bands were generated. The number of bands for each primer varied from 39 to 68 with an average of 56 bands per primer. These numbers are high when compared to the results of Novo et al. (1996). But according to Martin-Kearly et al. (1994), it is advantageous to produce such a high number of bands because it facilitates their alignment. The band profile obtained from each individual strain clearly shows their similarities as well as their differences.

Even though the amplification conditions used during the PCR procedure produced a large number of reproducible bands for most of the primers used, the primers OPC-01, OPC-03, OPC-04, OPC-12, OPC-13, OPC-16, and OPC-17 produced non-reproducible bands. This inconsistency in the banding pattern could have been due to variation in primer annealing between replicate PCR reactions (Bishop et al., 1993). Variation in primer annealing could occur because complex reactions take place during PCR experiments where a single primer competes for priming at many sites on the bacterial genome (Bishop et al., 1993). The production of non-reproducible bands can also arise when shorter primers (8- to 12-mers) are used (Versalovic et al., 1994). In this study, 10-mer primers were utilized and this may have been the cause of non-reproducibility. The annealing process is not as stringent when shorter primers are used. Janssen et al. (1996) also explain that DNA concentration, the type of DNA polymerase used during the PCR reaction and the DNA template's quality are crucial factors that affect the reproducibility of bands during RAPD–PCR fingerprinting methods. The use of lower annealing temperatures and degenerate primers can also cause variations in the banding pattern (Janssen et al., 1996). Because RAPD–PCR requires short primers with arbitrary nucleotide sequences to amplify polymorphic sequences under low stringency conditions, the primer adheres to the DNA template at many sites with reproducible but not perfect matches (Sakallah et al., 1995).

Once the RAPD profiles of the twelve *A. ferrooxidans* strains studied were compared, variations in the amplification products for each primer were evident. The variations in the banding patterns indicated genomic variability among the strains. Polymorphisms are most likely detected when arbitrary primers are used during the PCR procedure. These differences within the genome may often be located in protein coding genes or other unique sequences found in the DNA (Williams et al., 1990). The primers used during the PCR procedure grouped the twelve *A. ferrooxidans* strains into five major groups. Group 1 was comprised of strains Cc, Wh, 31A, and 33; Group 2 included strains 15A, 16A, and 33A; Group 3 consisted of strains Pa, A1, and W1; Group 4 included strain 15B only; and Group 5 comprised strain ATCC 23270 only. The primers also differentiated the strains genomes within each of the five groups. Strains within Group 1 showed high divergence.

The relationships between the twelve strains were estimated using Jaccard's coefficient of similarity. Similarities between strains from the five groups ranged from a low of 5.49% (ATCC 23270 and 33) to a high of 85.14% (15A and 16A). These results are in agreement with those of Novo et al. (1996) who reported similarity coefficients ranging from almost 0% to over 98%. The similarity among the strains of Group 1 was 22.94% to 33.60%. Among the strains within Group 2, the similarities ranged from 24.14% to 85.14%. The range in Group 3 was from 17.39% to 38.89% (Table 3). When looking at the dendrogram produced by cluster analysis, strain 33 was the most divergent *A. ferrooxidans* strain in the first group, strain 33A was the most divergent in the second group, and strain Pa was the most divergent in the third group.

Group 2 included strains with the highest degree of similarity. As indicated in Table 3 and Fig. 2, the

strains 15A and 16A were closely related with a similarity of 85.14%. The fact that RAPD–PCR analysis involves the whole bacterial genome might explain the similarity within the banding pattern of strain 15A to that of strain 16A. It can be speculated that the bands common for the various strains studied are derived from parts of the genome involved in essential cellular functions such as leaching (Selenska-Pobell et al., 1998).

Strains belonging to the same similarity group are either present in similar geographic areas or belong to distinct geographic areas. For example, the second similarity group containing strains 15A, 16A, and 33A were all isolated from deep coal mine drainage. However, the first similarity group, containing strains Cc, Wh, 31A, and 33 were isolated from an effluent of a nickel and copper mine (Cc), an effluent of a tin mine (Wh), deep coal mine drainage (31A), and a mix of deep coal mine and surface coal mine drainage (33). These results are in accordance with those of Novo et al. (1996) and Harrison (1982). Their studies reported no correlation between homology groups and geography. This suggests that strains found in the same similarity group prevailed in different geographic areas due to similar leaching microenvironments. Interestingly, strains 15B, 31A, and 33 were isolated from similar environments (coal mine drainage). *A. ferrooxidans* strains ATCC 23270, Cc, Pa, Wh, A1, and W1 were isolated from very different environments. The results show that DNA fingerprinting/RAPD analysis does not separate *A. ferrooxidans* strains on the basis of their geographical areas. It cannot be speculated that the different environments from which they were isolated could in part explain differences between *A. ferrooxidans* strains. Therefore, environmental and geographic areas do not seem to be involved in genetic variability among these strains.

RAPD analysis showed that the twelve *A. ferrooxidans* strains used in this study have a wide genomic diversity. Harrison (1982) also observed a high variability for other *A. ferrooxidans* strains. Such a high variability among the *A. ferrooxidans* strains can be explained by the fact that the RAPD method derives information from the whole bacterial genome where regions with higher variability are present. Other methods, such as ARDREA (Amplified Ribosomal DNA Restriction Enzyme Analysis), derive their information from the rRNA genes (Selenska-Pobell et al., 1998). Selenska-Pobell et al. (1998) reported that several culture-collection isolates were very closely related to the type strain ATCC 23270. Unlike these results, analysis by RAPD–PCR showed that none of the strains studied was closely related to the type strain, ATCC 23270. Low degree of similarity between the strain ATCC 23270 and the other strains was shown, ranging from 13.21% (ATCC 23270 and W1) to 5.49% (ATCC 23270 and 33). More research is needed to determine if the genomic differences imply different species status.

RAPD–PCR has been used as a genomic fingerprinting method that analyzes the entire genome. This method makes use of synthetic oligonucleotides that prime arbitrary segments of the genomic DNA, which in turn produces a pattern of amplified fragments that are characteristic of the specimen (Janssen et al., 1996). DNA fingerprinting and RAPD–PCR are powerful methods that are often used in taxonomic and epidemiological studies (Gomes et al., 1995). RAPD–PCR has been used to type a wide range of bacteria including *Klebsiella, Mycobacteria, Streptococcus, Neisseria*, and *Listeria* (Sakallah et al., 1995). RAPD fingerprinting has also been shown to be a powerful technique in detecting genetic polymorphisms among morphologically similar species of various fungal species (Caligiorne et al., 1999). Because this method is simple and sensitive, it can become an important tool for the characterization of strains of *A. ferrooxidans*. The sensitivity of RAPD–PCR can detect even slight differences between strains of the same species (Martin-Kearly et al., 1994). One of the advantages in using the RAPD technique over other genomic fingerprinting methods is that unlike ribotyping and RFLP (Restriction Fragment Length Polymorphism) analyses, RAPD uses the entire genome (Janssen et al., 1996). This study, as well as others, clearly indicates that the technique used may provide a way to clearly discriminate among strains that are otherwise indistinguishable by serological or biochemical analysis (Martin-Kearly et al., 1994).

The use of the RAPD method to assess genomic variability in *A. ferrooxidans* is still quite new. Because arbitrary primers are used and no knowledge of the genomic DNA is necessary, the RAPD technique is advantageous to provide a genomic fingerprint of any strain. A quick and reliable

alternative for differentiating *A. ferrooxidans* strains may consist in the use of RAPD markers in the isolation of DNA fragments related to characteristics such as metal resistance (Novo et al., 1996). *A. ferrooxidans* taxonomy is still in a state of flux and RAPD–PCR can become an important tool in the classification and identification of this bacterium. Further studies including a larger sample number need to be undertaken to better resolve *A. ferrooxidans* taxonomy.

In summary, twelve isolates of *A. ferrooxidans* were recovered from AMD environments from various geographical locations. The strains were isolated and purified using the spread-plate technique and specialized culture media. Once the DNA was extracted, it was subjected to PCR using 10-mer arbitrary primers. The RAPD profiles were compared on the basis of the presence or absence of each DNA band. Strain diversity was calculated using Jaccard's coefficient and UPGMA cluster analysis. The variations in the banding patterns indicated genomic variability among the strains. The primers used during the PCR procedure grouped the twelve *A. ferrooxidans* strains into five major groups. Similarities between the strains ranged from 5.49% (ATCC 23270 and 33) to 85.14% (15A and 16A). These results showed that the strains have a high degree of genomic diversity and that the RAPD method is a sensitive technique to assess strain variability in this bacterium.

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