Cadmium Accumulation and DNA Homology with Metal Resistance Genes in Sulfate-Reducing Bacteria

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Cadmium resistance (0.1 to 1.0 mM) was studied in four pure and one mixed culture of sulfate-reducing bacteria (SRB). The growth of the bacteria was monitored with respect to carbon source (lactate) oxidation and sulfate reduction in the presence of various concentrations of cadmium chloride. Two strains Desulfovibrio desulfuricans DSM 1926 and Desulfovoccus multivorans DSM 2059 showed the highest resistance to cadmium (0.5 mM). Transmission electron microscopy of the two strains showed intracellular and periplasmic accumulation of cadmium. Dot blot DNA hybridization using the probes for the smtAB, cadAC, and cadB genes indicated the presence of similar genetic determinants of heavy metal resistance in the SRB tested. DNA sequencing of the amplified DNA showed strong nucleotide homology in all the SRB strains with the known smtAB genes encoding synechococcal metallothioneins. Protein homology with the known heavy metal-transporting ATPases was also detected in the cloned amplified DNA of Desulfomicrobium norvegicum II and Desulfovibrio desulfuricans DSM 1926, suggesting the presence of multiple genetic mechanisms of metal resistance in the two strains.

Many genetic systems are known in bacteria for maintaining intracellular homeostasis of essential metal ions and for acquiring resistance against toxic metals (44). Two well-studied genetic mechanisms of metal resistance in bacteria include heavy metal efflux systems (30) and the presence of metal binding proteins (32, 39).

Many operons of the efflux system are known, for example, the cadA operon (26), in which a P-type ATPase is involved in metal ion transport across the cell membranes. The cadA operon is composed of two genes designated cadA and cadC (44). CadA acts as a P-type ATPase, while cadC acts as a regulatory gene of cadA. The cadA operon has been reported to provide cadmium resistance in Bacillus subtilis (48), Staphylococcus aureus (31), Stenotrophomonas maltophilia (1), Pseudomonas putida (28), Listeria monocytogenes (26), and Helicobacter pylori (17). The cadA homolog smtA has been reported in Escherichia coli (5, 38), which acts as a Zn, Cd, and Pb translocating P-type ATPase pump. Another cadA homologue, ziaA, has been shown to confer Zn and Cd tolerance in Synechococcus sp. strain PCC 6803 (47).

A plasmid-mediated metal resistance mechanism in Staphylococcus aureus is governed by the cadB operon, with two genes designated cadB and cadX (35). It has been suggested that cadB provides protection by enabling cells to bind cadmium in their cell membranes (35). Chromosomal DNA mediated cadmium resistance gene cadD in Staphylococcus aureus (9) has shown sequence similarity with the cadB-like gene from Staphylococcus lagunensis (7).

Another mechanism of metal detoxification and homeostasis that involves metal-binding proteins is mediated by metallothionein-encoding genes (39). Metallothioneins are small, cysteine-rich proteins (15), synthesized under heavy metal stress conditions that have been found in both prokaryotes (32, 39) and eukaryotes (33). The only known bacterial metallothionein locus, designated smt, that has been cloned and structurally characterized is that in Synechococcus sp. strain PCC 6301 (39) and in Synechococcus sp. strain PCC 7942 (20). The smt locus consists of two divergently transcribed genes, smtA and smtB (20), and mediates resistance to zinc and cadmium in Synechococcus spp. (49).

Sulfate-reducing bacteria are dissimilatory anaerobes characterized by their ability to reduce sulfate with the simultaneous oxidation of the organic substrates (36). Sulfate reduction leads to production of sulfide, which can readily react with metals and form insoluble metal sulfides (12). Thus, SRB play an important role in the bioremediation of toxic wastes containing heavy metal ions (4). The use of SRB in bioremediation processes has been widely reported, for example, bioprecipitation of cadmium (53), copper (54), and selenium (19); reduction of chromium (45) and uranium (46); and biosorption of aluminum (16).

Genetic and molecular studies of SRB are limited and have been mostly carried out on hydrogen metabolism and electron transport systems (41, 51). In this study we demonstrate cadmium resistance and the presence of genetic determinants for metal resistance in SRB based on the strong DNA sequence homology with the known genes encoding bacterial metallothioneins and heavy metal-transporting ATPases.

MATERIALS AND METHODS

Microorganisms and plasmid DNA. The microorganisms used were a mixed culture of sulfate-reducing bacteria obtained from natural sediments (52), including Desulfomicrobium norvegicum II, a pure culture isolated from the mixed SRB culture (53); Desulfovibrio vulgaris DSM 644; Desulfovibrio desulfuricans
TABLE 1. Escherichia coli strains and plasmid DNA

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Characteristics</th>
<th>Source</th>
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<tr>
<td>LE392</td>
<td>pSI006 (Cd&lt;sup&gt;+&lt;/sup&gt; Ap&lt;sup&gt;R&lt;/sup&gt;) contained the cadD gene on a 1.8-kb HindIII fragment from <em>S. aureus</em> plasmid pRW001 cloned into pUC18</td>
<td>J. J. Iandolo (Oklahoma University)</td>
</tr>
<tr>
<td>XL1-Blue 1720</td>
<td>pMa39 (Ap&lt;sup&gt;R&lt;/sup&gt;) harbored a 3.1-kb EcoRI fragment with <em>cadA</em> and <em>cadC</em> from <em>L. monocytogenes</em> plasmid pLM74 cloned in <em>E. coli-Listeria</em> shuttle vector pMK4</td>
<td>P. Cossart (Institute Pasteur, France)</td>
</tr>
<tr>
<td>JM109</td>
<td>Ap&lt;sup&gt;R&lt;/sup&gt;</td>
<td>Promega (United Kingdom)</td>
</tr>
<tr>
<td>Plasmid pJHRN49</td>
<td>pJHRN49 contained a 1.8 kb HindIII/Sall fragment with smtA and smtB from <em>Synecococcus</em> sp. strain PCC 7942 cloned into pGEMM4Z</td>
<td>N. J. Robinson (University of Newcastle)</td>
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<sup>*</sup> Cd<sup>+</sup>, cadmium resistance; Ap<sup>R</sup>, ampicillin resistance.
was used as a positive control. Amplification was carried out by heating the mixture for 3 min at 94°C, followed by 40 cycles of 1 min at 94°C, 1 min at 48°C, 1 min at 72°C, and finally 1 cycle for 10 min at 72°C for extension.

DNA amplification was carried out using a thermal cycler (Hybaid OMN-E). The chromosomal DNA from SRB strains was used as the templates. Amplified products were purified using QIAquick PCR purification kit (QIAGEN, United Kingdom).

Southern blot hybridization. Following electrophoresis, the amplified DNA was transferred overnight to Hybond-N+ membrane (Amersham) by capillary blotting using 10% SSC (0.15 M sodium citrate, 1.5 M sodium chloride) as a transfer buffer. The membrane was prehybridized (at 40°C or 42°C as required) for 30 min in DIG Easy Hybridization buffer (DIG Random Prime Kit, Boehringer Mannheim). Labeled probe (25 ng/ml) was added and hybridization was carried out overnight at 40°C (cadA probe) and at 42°C (smtAB probe). Subsequent stringency washings were done as follows; twice for 5 min in 2× SSC, 0.1% SDS at room temperature and twice for 15 min in 0.5× SSC, 0.1% SDS at 55°C and at 58°C for the cadA and smtAB probes, respectively. Blocking, antibody incubation and washes, signal generation, and detection were carried out according to the instructions of the DIG Random Prime kit (Boehringer Mannheim).

Transformation and cloning of PCR products. DNA amplification was done under the same conditions as described above for cadA except for an extended 40-min cycle at 72°C. Amplified products were ligated into the pGEM-T easy vector (Promega), and the ligated vector was transformed into Escherichia coli JM109 high-efficiency competent cells (Promega) by the heat shock method. Transformation was detected on LB agar plates supplemented with ampicillin/ IPTG/X-Gal by screening the blue-white colonies. Selected colonies were grown in LB broth with ampicillin for plasmid preparations.

DNA sequencing and analysis. Amplified and cloned PCR products were sequenced using an ABI Prism 377 automated DNA sequencer. Amplified products were sequenced with the smt1 primer while cloned PCR products were sequenced with the M13 reverse primer. The sequences were analyzed using the sequence interpretation tools at GenomeNet Server (Kyoto Center), CLUSTAL W, and BCM Search Launcher available on the worldwide web.

Nucleotide sequence accession numbers. The nucleotide sequences reported in this paper have been deposited in GenBank under accession numbers AY587186, AY587187, AY587188, AY587189, AY587190, AY587191, and AY587192.

RESULTS

Cadmium resistance. Cadmium resistance was detected in all the SRB strains. Desulfovibrio vulgaris DSM 644 showed the least resistance to cadmium. The strain oxidized lactate and reduced sulfate in the presence of cadmium concentrations up to 0.1 mM cadmium (Fig. 1c) whereas Desulfovibrio microbium norvegicum DSM 11 did it at up to 0.4 mM cadmium (Fig. 1a), the mixed SRB culture at up to 0.3 mM cadmium (Fig. 1b), Desulfovibrio desulfuricans DSM 1926 at up to 0.5 mM cadmium (Fig. 1d) and Desulfooccus multivorans DSM 2059 at up to 0.5 mM cadmium (Fig. 1e). Lactate added to the growth medium (20 mM) was completely oxidized and approximately 30% sulfate was reduced in controls (cultures with no added cadmium) and in the presence of up to the respective subtoxic concentrations of cadmium. The data showed no significant difference ($P > 0.05$) among the strains with respect to lactate oxidation and sulfate reduction.

Cadmium accumulation. Transmission electron microscopic analysis of Desulfovibrio desulfuricans DSM 1926 and Desulfooccus multivorans DSM 2059 showed accumulation of cadmium inside the cells. Unstained micrographs of Desulfovibrio desulfuricans DSM 1926 (Fig. 2a) and Desulfooccus multivorans DSM 2059 (Fig. 2b) clearly showed intracellular and periplasmic accumulation of the metal. Stained micrographs (not shown) of the two strains also revealed cadmium accumulation inside the cells despite the background due to staining with uranyl acetate and lead citrate.

Homology of metal resistance determinants in SRB DNA. (i) Dot blot hybridization. A 1.8-kb fragment from pJHNR49; a 3.1-kb fragment from pMA39; and a 1.8-kb fragment from pS1006 were used as the smtAB, cadAC, and cadD probes, respectively, to screen the genomic DNA of SRB for the presence of similar metal resistance determinants.

The results showed strong hybridization signals of the smtAB probe with genomic DNA of all the SRB and plasmid pJHNR49 used as a positive control (Fig. 3a), indicating strong homology. The probe also showed weak hybridization signals with plasmids pS1006 and pMA39 (Fig. 3a), which were used as negative controls. This might suggest some sequence similarity between these two plasmids and the smtAB probe.

The cadAC probe showed strong hybridization signals with Desulfovibrio vulgaris DSM 644 and Desulfovibrio desulfuricans DSM 1926 and comparatively less hybridization with Desulfovibrio microbium norvegicum DSM 11, mixed SRB culture, and Desulfooccus multivorans DSM 2059 (Fig. 3b). This suggested more homology of the probe with the DNA of Desulfovibrio vulgaris DSM 644 and Desulfovibrio desulfuricans DSM 1926 than Desulfovibrio microbium norvegicum DSM 11, mixed SRB culture and Desulfooccus multivorans DSM 2059. A weak hybridization signal was also detected with pS1006 (Fig. 3b), used as a negative control, indicating the presence of some similar sequences in this plasmid DNA. However, hybridization was not observed with pJHNR49 (Fig. 3b), also used as a negative control. Strong hybridization was detected with the positive control pMA39 (Fig. 3b).

The cadD probe was found to hybridize with the DNA of all the SRB strains (Fig. 3c) but strong signals with Desulfooccus multivorans DSM 2059 showed more homology than Desulfovibrio desulfuricans DSM 1926, which showed comparatively less hybridization with the probe (Fig. 3c). Weak hybridization signals occurred with Desulfovibrio microbium norvegicum DSM 11, mixed SRB culture, and Desulfovibrio vulgaris DSM 644 (Fig. 3c). This suggested some DNA homology of the three strains with the probe. The probe also showed strong hybridization with the positive control pS1006 (Fig. 3c). Weak hybridization was observed with pMA39 (Fig. 3c), indicating some similarity with the plasmid. No hybridization was detected with pJHNR49 (Fig. 3c). Both pMA39 and pJHNR49 were used as negative controls.

(ii) Amplification of part of the smtAB coding regions. Primers smt1 and smt2 were designed to amplify an internal 507-bp region of smtAB genes using the known metallothionein sequence of Synechococcus sp. strain PCC 7942 (GenBank accession number X64585). The primers showed amplification of an approximately 500-bp product in all five SRB strains and the positive control pJHNR49 (Fig. 4a). However, amplification was not detected in the negative control PCR in which water was used as the template instead of DNA to rule out the possibility of primer contamination with the SRB DNA (Fig. 4a).

(iii) Southern hybridization and sequence analysis of smt1- and smt2-amplified DNA. Southern hybridization using the amplified product of pJHNR45 as the smtAB gene probe showed strong hybridization with the 500-bp amplified product of the SRB strains and positive control pJHNR45 (Fig. 4b). Hybridization was not detected with the negative control reaction (Fig. 4b). BLAST (2) search analysis showed 100%
nucleotide homology of the SRB amplified DNA with the smtAB gene sequences of *Synechococcus* sp. strain PCC 7942 (GenBank accession number X64585). The same search program also showed protein homology of *Desulfomicrobium norvegicum* 11 (87%), mixed SRB culture (87%), *Desulfovibrio vulgaris* DSM 644 (87%), *Desulfovibrio desulfuricans* DSM 1926 (84%), and *Desulfococcus multivorans* DSM 2059 (87%) amplified products with the SmtB protein sequence of *Synechococcus* sp. strain PCC 7942 (GenBank accession number X64585). The deduced amino acid sequences also showed the presence of a conserved signature sequence of bacterial regulatory Ars family proteins in all five SRB strains (Fig. 5). These results suggest that all the SRB strains possessed a DNA sequence similar to a region of the known smtAB genes.

(iv) Amplification of part of *cadA* coding region. Degenerate primers cad1 and cad2 were designed to amplify a 635-bp

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**FIG. 1.** Effect of cadmium on the growth of SRB strains (7 days incubation in the presence of 0 to 1.0 mM cadmium). The diamonds (◇) and squares (□) indicate residual lactate and sulfate concentrations, respectively. (a) *Desulfomicrobium norvegicum* 11; (b) mixed SRB culture; (c) *Desulfovibrio vulgaris* DSM 644; (d) *Desulfovibrio desulfuricans* DSM 1926; (e) *Desulfococcus multivorans* DSM 2059. Data are the means of five replicates. Error bars represent the standard errors of the means.
product using known CadA protein sequences of *Staphylococcus aureus* (GenBank accession number J04551), *Listeria monocytogenes* (GenBank accession number L28104), *Helicobacter pylori* (GenBank accession number L46864), and *Bacillus firmus* (GenBank accession number M90750). An amplified product of approximately 600 bp (Fig. 6a) was found in *Desulfomicrobium norvegicum* I1, *Desulfovibrio desulfuricans* DSM 1926, and pMa39 (used as a positive control). Amplification was not detected in the mixed SRB culture, *Desulfovibrio vulgaris* DSM 644, and *Desulfococcus multivorans* DSM 2059 (Fig. 6a).

**Southern hybridization, cloning, and sequence analysis of cad1- and cad2-amplified DNA.** Southern hybridization using the pMa39 amplified fragment as a labeled probe showed strong hybridization signals with the amplified products of pMa39, *Desulfomicrobium norvegicum* I1, and *Desulfovibrio desulfuricans* DSM 1926 (Fig. 6a).

**FIG. 2.** Transmission electron micrographs of *Desulfovibrio desulfuricans* DSM 1926 (a) and *Desulfococcus multivorans* DSM 2059 (b). Strains were grown in the presence of 0.5 mM cadmium (7 days incubation). Unstained micrographs show intracellular and periplasmic accumulation of the metal. Bars represent 100 nm (a) and 200 nm (b).

**FIG. 3.** Dot blot hybridization of SRB DNA with *smtAB* (a), *cadAC* (b), and *cadD* (c) gene probes. 1, *Desulfomicrobium norvegicum* I1; 2, mixed SRB culture; 3, *Desulfovibrio vulgaris* DSM 644; 4, *Desulfovibrio desulfuricans* DSM 1926; 5, *Desulfococcus multivorans* DSM 2059; 6, pS1006; 7, pMa39; 8, pJHNR49.

**FIG. 4.** PCR amplification using smt1/smt2 primer pair. (a) Gel electrophoresis of the amplified products; (b) Southern blotting and hybridization of the amplified DNA with DIG labeled *smtAB* probe Lanes: 1, 1-kb DNA ladder (MBI); 2 to 8, amplified products of: pJHNR49 (2), *Desulfomicrobium norvegicum* I1 (3), mixed SRB culture (4), *Desulfovibrio vulgaris* DSM 644 (5), *Desulfovibrio desulfuricans* DSM 1926 (6), *Desulfococcus multivorans* DSM 2059 (7), and control reaction without adding template DNA (8), respectively; 9, DNA ladder (Gibco).
desulfuricans DSM 1926 (Fig. 6b). Hybridization was not detected with the mixed SRB culture, Desulfovibrio vulgaris DSM 644, and Desulfococcus multivorans DSM 2059. The boldfaced and underlined amino acid sequences show the motif for bacterial regulatory proteins of the ArsR family.

Plasmid vector pGEM-T Easy was ligated with the amplified DNA fragments of Desulfomicrobium norvegicum I1 and Desulfovibrio desulfuricans DSM 1926 to obtain the recombinant plasmids pDnI1 and pDd1926, respectively. Escherichia coli JM109 was transformed with the recombinant plasmids and transformants were detected on X-Gal/IPTG/ampicillin plates by screening of blue-white colonies. Digestion of the cloned plasmids pDnI1 (not shown) and pDd1926 (Fig. 7) with EcoRI showed the presence of vector (3,015 bp) and insert DNA (600 bp). BLAST (2) sequence analysis of the pDnI1 cloned insert (GenBank accession number AY587191) showed protein homology with the heavy metal-transporting ATPases of Bacillus anthracis (65%; GenBank accession number AE017334), Bacillus cereus (65%; GenBank accession number AE017266), Bacillus thuringiensis (65%, GenBank accession number AE017355), Bacillus subtilis (58%; GenBank accession number Z99121), Thermoanaerobacter tengcongensis (56%; GenBank accession number AE013188), Pyrococcus abyssi (53%; GenBank accession number AE016999), Helicobacter felis (36%; GenBank accession number AF125316), and Escherichia coli K-12 (35%; GenBank accession number U82664). The deduced protein sequence of the pDd1926 insert also showed the presence of part of a conserved DKTGT(L/I)T signature sequence associated with the phosphorylation of bacterial P-type ATPases.

**DISCUSSION**

The metabolic properties of sulfate-reducing bacteria enable them to play an important role in the bioremediation of harmful pollutants (25). For effective bioremediation purposes, it is...
important to determine the subtoxic concentrations of pollutants (12). During this study, SRB strains showed growth in the presence of cadmium. However, determination of the subtoxic concentration showed variations among the strains with respect to cadmium resistance. Desulfovibrio vulgaris DSM 644 was the most sensitive while Desulfovibrio desulfuricans DSM 1926 and Desulfococcus multivorans DSM 2059 showed the highest resistance towards cadmium. Desulfomicrobium norvegicum I1 showed more resistance to cadmium than the parental mixed SRB culture. The reason could be that the other bacterial strains present in the mixed SRB culture were more sensitive to cadmium and thus, the mixed SRB culture on the whole showed less resistance to cadmium than the purified strain of Desulfomicrobium norvegicum I1.

Mixed microbial cultures are generally considered more advantageous than pure cultures for environmental biotechnology (52), however, the present study showed more potential of the pure culture of Desulfomicrobium norvegicum I1 compared to the parental mixed SRB culture for bioremediation of the toxic metal ions. A number of studies have been carried out regarding heavy metal resistance in SRB (16, 54). Several reports have also established the toxicity of heavy metal ions, for example nickel, copper, lead, and zinc, for growing SRB (37, 43).

In the present study, the effect of cadmium on the growth of five SRB strains was monitored in terms of changes in their lactate-oxidizing and sulfate-reducing activity. The strains showed the same lactate-oxidizing and sulfate-reducing activities whether in the absence of cadmium or in the presence of subtoxic cadmium concentrations. After 7 days incubation, all the lactate added to the medium (20 mM) was oxidized and approximately 30% sulfate was reduced in controls and at subtoxic concentrations of cadmium.

Many bacteria that can accumulate metal species have been described (50). In this study, transmission electron microscopy of Desulfovibrio desulfuricans DSM 1926 and Desulfococcus multivorans DSM 2059 (Fig. 2) demonstrated intracellular and periplasmic accumulation of cadmium. The two strains showed more resistance to cadmium and could grow in the presence of higher concentration of the metal than the other SRB strains tested. A high metal concentration may lead to intracellular precipitation of the metal (21). Additionally, cadmium transport via the Mn2+ transport system has been reported in many bacteria (25, 35) and may also contribute to cadmium transport in SRB.

In the present work, intracellular and periplasmic cadmium accumulation in Desulfovibrio desulfuricans DSM 1926 and Desulfococcus multivorans DSM 2059 suggested the presence of metal-binding and/or efflux mechanisms inside the cells mediating resistance against metal toxicity. Cytoplasmic (57) and periplasmic (34) accumulation of heavy metal ions as a result of metallothioneins expression has been reported in Escherichia coli. In the present study, the dot blot and DNA hybridization results showed homology of the SRB DNA with gene probes of smtAB encoding bacterial metallothionein (20), cadAC encoding P-type ATPase (26), and cadD (9) encoding metal-binding protein. DNA hybridization has been used to detect the hydrogenase and cytochrome genes in SRB (10, 51). The technique has also been widely used for the detection of homologous metal resistance determinants in many other species of bacteria (20, 26, 56).

Another strategy for detecting SRB DNA homology with well-known metal resistance determinants is PCR amplification. PCR has become a powerful technique for gene analysis, cloning, and characterization of different regions of DNA without screening entire genomic libraries (42). In this study, the technique was used to amplify a 507-bp coding region of the known smtAB genes. The primers designed for this purpose caused the amplification of a product of approximately the same size in all the SRB strains and the positive control pJHNR49. Southern blotting and strong hybridization of the amplified SRB DNA with the pJHNR49 amplified smtAB probe also confirmed the amplification of part of the smtAB genes in the SRB strains. The analysis of nucleotide sequences of the amplified fragments showed significant homology with the smtAB genes and SmtB protein of Synechococcus sp. strain PCC 7942 (GenBank accession number X64585). The deduced protein sequence of the amplified SRB DNA also showed the presence of conserved motif C-x(2)-D-[LIVM]-x(6)-[ST]-x(4)-S-[HYR]-[HQ] for bacterial regulatory proteins of the ArsR family. Other members of the ArsR family of regulatory proteins are CadC (11), ArsR (40), and SmtB (29).

It is possible to obtain specific DNA amplification using degenerate primers designed from known protein sequences (14, 27). Thus, degenerate primers were designed using known protein sequences of CadA ATPases for the amplification of a 635-bp region of the cadA gene in the SRB strains. The results showed such amplification in Desulfomicrobium norvegicum I1, Desulfovibrio desulfuricans DSM 1926, and pMa39. The amplification was further confirmed by Southern blot analysis of the amplified products using pMa39 amplified DNA as a probe for the cadA gene. Hybridization of the probe with the amplified products was detected, indicating the presence of sequence similarity.

Amplification of the smtA gene in Synechococcus sp. strain PCC 6803 with degenerate primers designed using known protein sequence of Synechococcus metallothionein has been reported (39). A PCR-amplified region of the smtA gene from Synechococcus sp. strain PCC 6301 (39) has been used to screen a Synechococcus sp. strain PCC 7942 genomic library for the presence of sequence homology (20). The technique has also been used to amplify cadAC genes in Listeria monocytogenes (26) and the cadC gene (11) and cadD gene (9) in Staphylococcus aureus.

Reports are also available regarding the use of PCR amplification for the mapping of genes encoding flavodoxin (24) and rubredoxin (23) in Desulfovibrio vulgaris DSM 644. In the present study, sequence analysis of the cloned plasmids pDn11 and pDd1926 showed homology with the known protein sequences encoding heavy metal-transporting ATPases. The deduced protein sequence of pDd1926 insert also showed the presence of part of the signature sequence of ATPase phosphorylation.

The amplification and hybridization studies in the present work strongly suggest the existence of genetic determinants encoding bacterial metallothioneins in all the SRB strains. The occurrence of metallothioneins in eukaryotes is a common phenomenon (15, 33). However, in prokaryotes, the presence of metallothioneins has been reported only in Pseudomonas.
putida (18) and Synechococcus spp. (20, 32, 39). In Synechococcus spp., genes encoding metallothioneins have been found that enable resistance to toxic metal ions such as zinc and cadmium (49). Strong homology of the SRB DNA with cyanobacterial metallothionein genes suggests genome homology between the two diverse groups of bacteria, both having some unique metabolic capabilities.

Many SRB are known for their ability to gain energy by coupling the oxidation of organic substrates with the reduction of sulfate to sulfide (36). On the other hand, cyanobacteria are unusual in that they contain thylakoid membranes and perform oxygenic photosynthesis using photosystems similar to those in plant chloroplasts (22). In the present study, the cloned insert of Desulfovibrio norvegicus DSM 1926 showed homology with the protein sequences of known genes encoding heavy metal-translocating ATPases. The two strains thus showed the presence of more than one genetic determinant for metal resistance. Multiple mechanisms of metal resistance have also been found in Synechococcus sp. strain PCC 6803, including genes encoding a P-type ATPase and bacterial metallothionein (13, 49), which again revealed genome homology between SRB and cyanobacteria.

Intracellular bioaccumulation processes involving metallothioneins and P-type ATPases can be used as an alternative for the removal and recovery of heavy metals such as cadmium from contaminated wastes. Genetically engineered Escherichia coli strains have been constructed to simultaneously express a mercury transport system and a cloned yeast metallothionein gene to accumulate the metal transported by a metal transport system (8). SRB are of great significance for the carbon and sulfur cycles in sediment ecosystems. Metal resistance in SRB is thought to be due mainly to sulfide produced by them. However, this study demonstrated the presence of DNA sequences showing homology with known metal resistance genes. Therefore, it is suggested that chromosomal genetic determinants might also be involved in cadmium resistance in SRB.

This study provides useful knowledge regarding the metabolic and genetic abilities of SRB to decontaminate toxic heavy metal ions.

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REFERENCES

23. Lee, S. W., E. Glickmann, and D. A. Cooksey. 1996. Cloning and expression of the gene encoding flavodoxin from Staphylococcus aureus from contaminated wastes. Genetically engineered Escherichia coli strains have been constructed to simultaneously express a mercury transport system and a cloned yeast metallothionein gene to accumulate the metal transported by a metal transport system (8). SRB are of great significance for the carbon and sulfur cycles in sediment ecosystems. Metal resistance in SRB is thought to be due mainly to sulfide produced by them. However, this study demonstrated the presence of DNA sequences showing homology with known metal resistance genes. Therefore, it is suggested that chromosomal genetic determinants might also be involved in cadmium resistance in SRB.

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