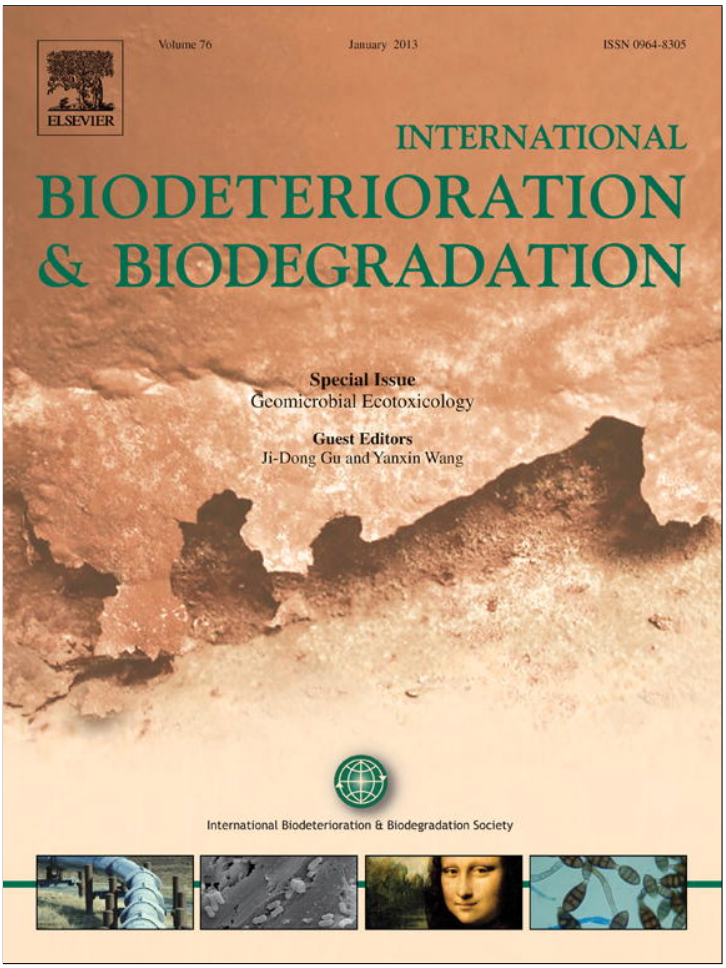

International Biodeterioration & Biodegradation 76 (2013) 76–80

Contents lists available at SciVerse ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Phylogenetic and genome analyses of antimony-oxidizing bacteria isolated from antimony mined soil

Jie Li, Qian Wang, Shengzhe Zhang, Dong Qin, Gejiao Wang*

State Key Laboratory of Agricultural Microbiology, College of Life Science and Technology, Huazhong Agricultural University, Wuhan 430070, PR China

Keywords:
Antimony
Antimony mined soil
Sb(III)-resistant bacteria
Sb(III)-oxidizing bacteria
Comamonas

A B S T R A C T

Antimony-oxidizing bacteria play an important role in the biogeochemical cycling of antimony, converting the more toxic Sb(III) to the less toxic Sb(V). Little is known about the bacterial species and mechanisms involved in mediating Sb(III) oxidation. In this study, 25 Sb(III)-resistant bacteria were isolated from Sb mined soil. Among these strains, 6 were Sb(III)-oxidizing bacteria including *Acinetobacter* sp. JL7, *Comamonas* sp. JL25, *Comamonas* sp. JL40, *Comamonas* sp. S44, *Stenotrophomonas* sp. JL9 and *Variovorax* sp. JL23. Strain S44 showed the highest Sb(III) oxidation rate which could aerobically oxidize 50 μM Sb(III) to Sb(V) in 3 d. Whole genome sequencing of strain S44 revealed a number of genes encoding putative metal(loid) resistance proteins including 2 putative As(III)/Sb(III) efflux pump proteins ArsB. Based on our study, the ability of Sb(III) resistance and oxidation was found among diverse bacterial lineages. Microbiological oxidation of Sb(III) may therefore be widely distributed across different bacterial lineages mediating detoxification of Sb at high concentrations in the environment.

1. Introduction

As a very toxic metal, antimony (Sb) occurs widely in soil and aquatic systems. Sb is located in Group 15 of the Periodic Table, which lies directly below arsenic (As) and shares some similar chemical and toxicological properties (Nies, 1999). Although antimonials have been used for the treatment of leishmaniasis for over 60 years (Goyeneche-Patino et al., 2008; Liarte and Murta, 2010), great attention has been paid to the damage of heart and liver, the carcinogenesis (Cooper and Harrison, 2009), and the environmental pollution (Wilson et al., 2003). The maximum concentration of Sb in drinking water has been set by the World Health Organization at 5 μg L^{-1}. For this standard, the antimony pollution is quite severe in China, especially in the area of the world largest Xikuangshan antimony mine in Hunan province (Liu et al., 2010).

Antimony mainly exists as two valence states, Sb(III) and Sb(V). The mechanisms of microbial Sb resistances have been investigated by some researchers. Considering the similarity between As and Sb, *arsB* gene, encoding the As(III)/Sb(III) efflux pump protein, a member of *arsRDABC* operon, has been shown to confer both As(III) and Sb(III) resistances (Wang et al., 2004; Branco et al., 2008). The *arsB* encodes a 12α helix transmembrane protein which often associates with regulatory protein ArsR, As(V) reductase ArsC, ATPase ArsA and transcriptional repressor ArsD to extrude As(III) and Sb(III) out of cells (Parvatiyar et al., 2005; Li and Krumholz, 2007; Rosen and Liu, 2009). The *ars* operons responsible for As/Sb resistance were located either on plasmid or on chromosome of the microorganisms (Silver et al., 2001). Mukhopadhyay et al. (2009) characterized a As(V)/Sb(V) reductase LmACR2 which showed lower homology with the As(V) reductase ArsC. In addition, glycerol transporters were also found to associate with Sb(III) resistance in *Escherichia coli* (GlpF, Sanders et al., 1997) and yeast (Fpslp, Ghosh et al., 1999; Wysocki et al., 2001). In one case, microbial methylation was also found (Bentley and Chasteen, 2002).

Biotransformation of metals and metalloids by microorganisms is very applicable for bioremediation (White et al., 1995; Hong and Gu, 2009). Sb(III) oxidation converts the more toxic Sb(III) to the less toxic Sb(V), which is very important for environmental Sb bioremediation. Such oxidation is extremely slow under environment conditions using O_2 as the electron acceptor (Leuz and Johnson, 2005). Sb(III)-oxidizing bacteria impact environmental Sb cycles as their important life activities in nature (Nies, 1999). Nowadays, the research on microbial Sb(III) oxidation is limited and little is known about the bacterial species and molecular mechanisms mediating Sb(III) oxidation. To our knowledge, only 3 Sb(III)-oxidizing bacteria have been reported so far, including *Stibiobacter senarmontii* (Lialikova, 1974), *Thiobacillus ferrooxidans* (Torma and Gabra, 1977) and *Agrobacterium tumefaciens* strain 5A (Lehr et al., 2007). The *A. tumefaciens* 5A could oxidize both As(III) and Sb(III), however, a mutant strain of As(III) oxidase encoding genes *aioBA*

* Corresponding author. Tel.: +86 27 87281261; fax: +86 27 87280670.
E-mail address: gejiaow@yahoo.com.cn (G. Wang).

0964-8305/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ibiod.2012.06.009

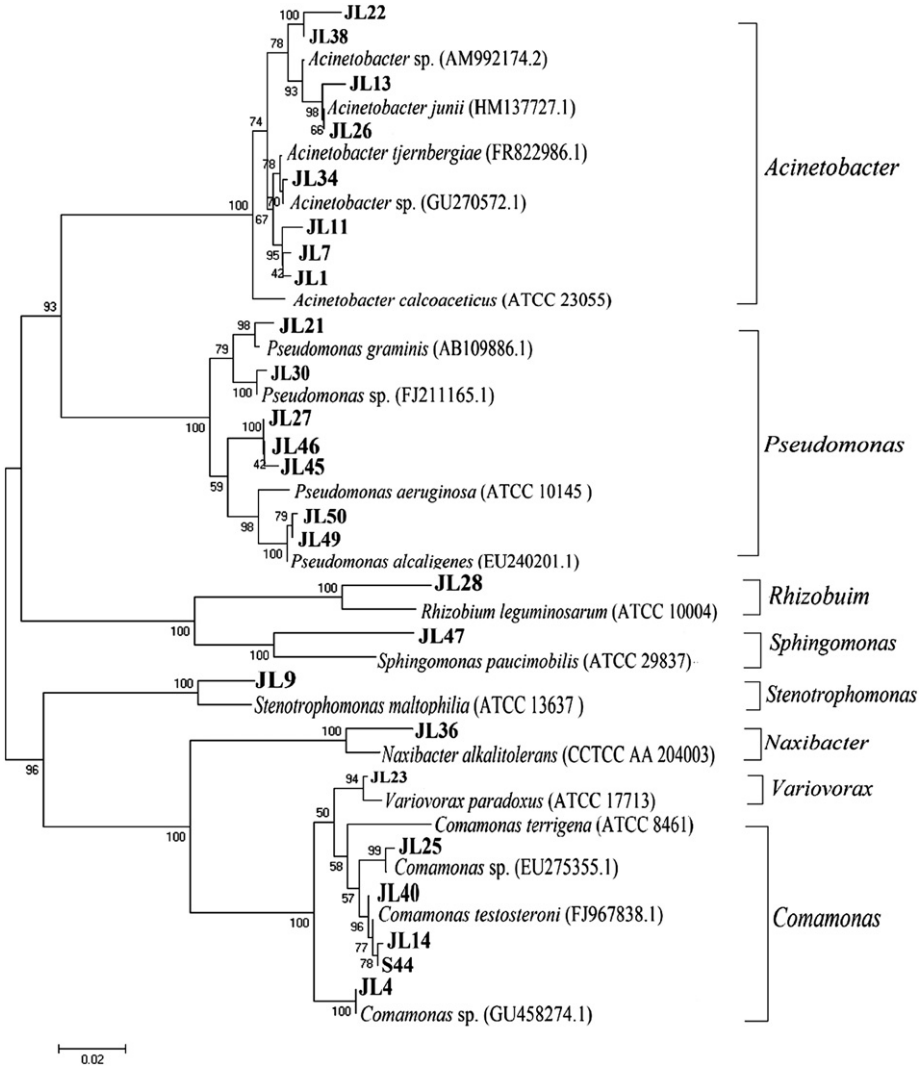

Fig. 1. A neighbor-joining 16S rRNA gene phylogenetic tree showing the genetic relationship of the Sb-mined soil bacteria (bold). The others are reference 16S rRNA sequences from the GenBank. Scale bar 0.02 = 2% difference among nucleotide sequences.

responsible for the oxidation of As(III) showed a Sb(III) oxidation activity. This indicated that As(III) oxidase could not catalyze Sb(III) oxidation by strain 5A (Lehr et al., 2007).

The objectives of this study were: (1) study the diversity of Sb(III)-resistant and Sb(III)-oxidizing bacterial strains isolated from antimony mined soil; and (2) genome analysis of a representative Sb(III)-oxidizing *Comamomas* strain. The knowledge of bacterial species and genes associated with Sb resistance and oxidization in Sb-contaminated sites will provide the basis for understanding of microbial antimony metabolism and for potential bioremediation applications.

2. Materials and methods

2.1. Isolation and identification of Sb(III)-resistant bacteria

Soil sample was collected from the world largest Xikuangshan antimony mine in Lengshuijiang City, situated between 27°45–46′N and 111°28–29′E in Hunan province, central-south of China. This location has been smelted Sb for more than 200 years (Liu et al., 2010).

One-hundred grams soil were amended with antimony potassium tartrate [$C_8H_4K_2O_{12}Sb_2 \cdot 3(H_2O)$] to a final concentration of 10 mg kg^{-1} and incubated at 28 °C. After one week, 1 g of the enrichments (triplicates) were added into 9 ml 0.85% sterilized NaCl solution, shaken at 180 r min^{-1} for 30 min, plated with serial dilution on chemically defined medium CDM-A plates [CDM (Weeger et al., 1999), without Fe_2SO_4 and $NaHCO_3$] containing 50 μM $C_8H_4K_2O_{12}Sb_2 \cdot 3(H_2O)$, and incubated at 28 °C for another week. Single colonies were obtained and each single colony of Sb(III)-resistant bacteria were streaked repeatedly for pure isolates (Cai et al., 2009).

The DNA of each strain was purified (Cai et al., 2009) and amplified by PCR using 16S rRNA universe primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (Wilson et al., 1990). DNA sequencing and phylogenetic analysis were performed as described before (Cai et al., 2009).

2.2. Identification of Sb(III)-oxidizing bacteria and determination of their resistances for Sb(III) and As(III)

For the Sb(III) oxidation test, each Sb(III)-resistant strain was inoculated into 10 ml liquid CDM-A medium containing 50 μM $C_8H_4K_2O_{12}Sb_2 \cdot 3(H_2O)$, incubated at 28 °C with 180 r min^{-1} shaking for 10 days and detected the quantity of Sb(V) and Sb(III) using an equipment combining HPLC with a hydride-generation atomic

fluorescence spectroscopy (HPLC-HG-AFS) (Beijing Titan Instruments Co., Ltd., China). Later, 6 strains showing Sb(III) oxidation abilities were each inoculated into 100 mL liquid CDM-A medium with 50 μM $C_8H_4K_2O_{12}Sb_2 \cdot 3(H_2O)$ (for strains S44 and JL40) or with 10 μM $C_8H_4K_2O_{12}Sb_2 \cdot 3(H_2O)$ (for strains JL7, JL9, JL23 and JL25), respectively, incubated at 28 °C and shaken with 180 r min^{-1}. Every 4 h, 4.5 mL cultures were taken and analyzed for OD_{600} value and the growth of the strains. At the same time, 0.5 mL cultures were centrifuged, filtered through 0.2-μm-filter membranes, diluted 100 times with sterilize dd H_2O and measured the concentration of Sb(III) or Sb(V) using the HPLC-HG-AFS. Bacterial As(III) oxidation tests were observed using a qualitative $KMnO_4$ method (Salmassi et al., 2002).

Physiological and biochemical tests of the 6 Sb(III)-oxidizing bacteria were performed using API 20NE system (bioMérieux, France). For Sb(III) and As(III) resistance tests, the minimum inhibitory concentrations (MICs) of Sb(III) or As(III) for completely inhibition of the bacterial growth in CDM-A medium, were determined with increasing concentrations of $C_8H_4K_2O_{12}Sb_2 \cdot 3(H_2O)$ or $NaAsO_2$, respectively, as described in Cai et al. (2009).

2.3. Genome analysis and nucleotide accession numbers

High-molecular-mass genomic DNA of strain S44 was isolated using the Blood and Cell Culture DNA Mini Kit (Qiagen, Maryland, USA). Whole genome shotgun sequencing was performed at the University of Arizona Genetics Core Facility, using with a Roche 454 Genome Sequencer FLX instrument. Genome analysis was performed as described by He et al. (2010). The GenBank number of the

Table 1
Physiological/biochemical characters, Sb(III)/As(III) oxidation rates and resistant levels (MIC) of the 6 Sb(III)-oxidizing strains.

Characters	JL7	JL9	JL23	JL25	JL40	S44
Reduction of nitrate	+	+	+	+	+	+
Indole production	−	−	−	−	−	−
Acidification of glucose	−	−	−	−	−	−
Urease	−	+	+	−	−	−
Arginine dihydrolase	−	+	+	+	−	−
Hydrolysis of:						
Asculin	+	+	+	+	−	+
Gelatin	−	−	−	−	−	−
Assimilation of:						
Glucose	−	−	+	+	−	−
Arabinose	−	−	+	+	−	−
Mannose	−	+	+	+	−	−
Mannitol	−	−	+	+	−	−
N-acetyl-glucosamine	+	+	+	+	−	−
Maltose	+	+	+	+	−	−
Gluconate	+	−	+	+	+	+
Caprate	+	−	−	+	+	+
Adipate	+	−	+	+	+	+
Malate	+	+	+	+	+	+
Citrate	−	+	−	+	−	−
Phenyl-acetate	−	−	−	−	−	−
Sb(III) oxidation rate (μM d^{-1})	1.25	1.89	6.67	<1.00	10.00	16.67
Capability of As(III) oxidation	−	−	−	−	−	−
MIC for Sb(III) (mM)	2	0.1	1	0.4	5	0.3
MIC for As(III) (mM)	20	12	13	10	33	18

+, positive; −, negative.

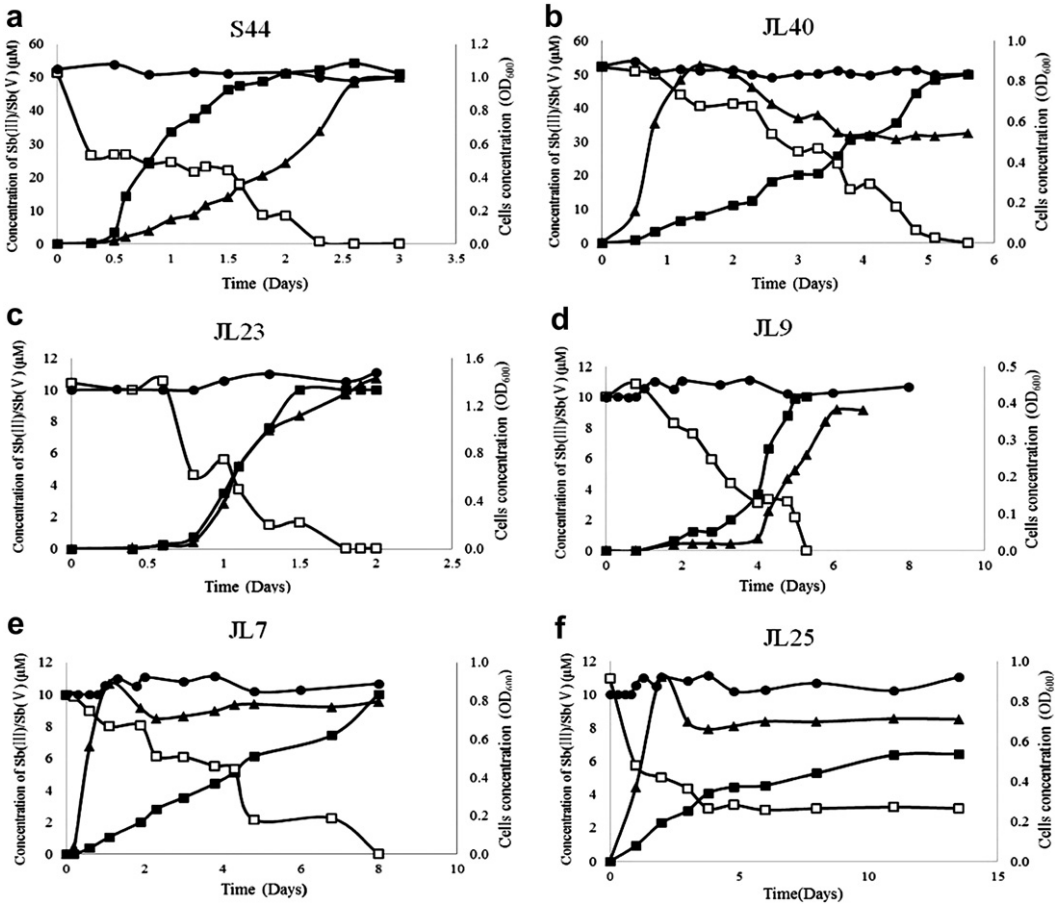

Fig. 2. The Sb(III) oxidation and growth curves of the Sb(III)-oxidizing strains, S44 (a), JL40 (b), JL23 (c), JL9 (d), JL7 (e) and JL25 (f). ■, concentration of Sb(V). □, concentration of Sb(III). ▲, cell concentration (OD_{600}). ●, concentration of Sb(III) in the controls without bacterial inoculation.

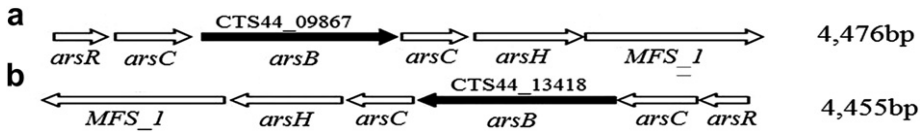

Fig. 3. The physical maps of two gene clusters containing the putative *arsB* and the flanking genes in the genome of strain S44. (a), *arsB* gene locus CTS44_09867. (b), *arsB* gene locus CTS44_13418.

whole genome sequence of strain S44 is ADVQ00000000 (Xiong et al., 2011). The 16S rRNA gene sequences were posted in the NCBI GenBank database with accession numbers of FJ210285 (for strain S44) and JF740032-JF740055 for the other 24 Sb(III)-resistant bacterial strains.

3. Results and discussion

3.1. Identification of Sb(III)-resistant bacteria from antimony mined soil

The Sb concentration of the soil sample was 186.6 μg g^{-1} with 160.5 and 26.1 μg g^{-1} Sb(III) and Sb(V), respectively, indicating that the Sb concentration was very high and mainly existed as Sb(III) form in the Sb mined soil. A total of 25 Sb(III)-resistant bacteria were isolated. Phylogenetic analysis using the nearly full-length 16S rRNA gene sequences affiliated them with *Acinetobacter* (8 strains, maximum identities ranged 97–99%), *Comamonas* (5 strains, 98–99%), *Pseudomonas* (7 strains, 98–99%), *Naxibacter* (96%), *Sphingomonas* (99%), *Stenotrophomonas* (99%), *Rhizobium* (99%) and *Variovorax* (99%) (Fig. 1). Twenty strains were grouped into *Acinetobacter*, *Pseudomonas* and *Comamonas*, suggesting they were the 3 main genera of Sb(III)-resistant bacteria in this Sb mined soil (Fig. 1).

3.2. The Sb(III) oxidation efficiencies of the Sb(III)-oxidizing bacteria and their resistances for Sb(III) and As(III)

A total of 6 strains showed Sb(III) oxidation abilities among the tested 25 Sb(III)-resistant strains, including 3 strains of *Comamonas* (spp. JL25, JL40 and S44), *Acinetobacter* sp. JL7, *Stenotrophomonas* sp. JL9 and *Variovorax* sp. JL23. The physiological and biochemical characteristics of them are shown in Table 1. Strain S44 showed the highest Sb(III) oxidation efficiency which could aerobically oxidize 50 μM Sb(III) in 3 d (16.67 μM d^{-1}). Other strains showed different Sb(III) oxidation efficiencies with the decreasing order of JL40, JL23, JL9, JL7 and JL25 (Fig. 2 and Table 1). Strains JL7, JL9 and JL23 were able to oxidize 10 μM Sb(III) completely (Fig. 2c, d and e), but could not oxidize 50 μM Sb(III) even with extended time of incubation (data not shown). *Comamonas* sp. JL25 showed the lowest oxidation efficiency which could not completely oxidize 10 μM Sb(III) in 14 d (Fig. 2f and Table 1). No obvious Sb(III) oxidation was observed in the controls without bacterial inoculation (Fig. 2). None of the 6 Sb(III) oxidizers were able to oxidize As(III) (data not shown).

The 6 Sb(III) oxidizers showed different levels for Sb(III) and As(III) resistances (Table 1). The MICs for As(III) were generally higher than those for Sb(III) indicating that Sb was much more toxic for the bacterial strains. Among the strains, *Comamonas* JL40 showed the highest resistances for both Sb(III) and As(III) and a high Sb(III) oxidation rate (10 μM d^{-1}, Table 1). It appeared that there was a positive correlation between the Sb(III) oxidation efficiency and Sb(III) resistant level in strain JL40. However, the MIC for Sb(III) of strain S44 was lower (0.3 mM) than the other Sb(III) oxidizers (JL7, JL23, JL25 and JL40), while it showed the highest Sb(III) oxidation efficiency.

3.3. Genome analysis of strain S44

The genome size of strain S44 was about 5.5 Mb with 5,218 open reading frames predicted. Two gene clusters containing the putative *arsB* genes (CTS44_09867 and CTS44_13418) encoding As(III)/Sb(III) efflux pump proteins, were found in two different contigs on the genome. The two gene clusters showed similar gene arrangements. Both of the *arsBs* were 1,289 bp long, sharing 97% amino acid (aa) identities and flanked with 2 putative *arsC* genes and each of the *arsR*, *arsH* and *MFS_1* (Fig. 3, Table 2). No other putative Sb resistant genes, i.e. genes encoding glycerol transporters or As(V)/Sb(V) reductase LmACR2, were found in the genome of strain S44. A great number of genes encoding putative metal(loid) resistant proteins were also detected, including four Pb/Zn/Cd transporting ATPase ZntA (CTS44_03693, 05983, 21952 and 02095), two Cr transporters ChrA (CTS44_04204 and 21140), three Cu resistance proteins CopD (CTS44_05998, 26023 and 26098), one Hg reductase MerA (CTS44_25963) and five heavy metal efflux proteins CzcC (CTS44_00284, 03648, 10337, 19067 and 24473). These metal(loid)

Table 2
Gene annotations of the two *arsBs* (CTS44_09867 and CTS44_13418, Fig. 3) and their flanking genes in the genome of strain S44.

Gene name	Gene locus	Amino acid (aa) number	Putative protein function	Reference strains (% of aa identity)
(a) The *arsB* (CTS44_09867) and its flanking genes				
arsR	CTS44_09857	117	Transcriptional regulator	*Comamonas testosteroni* CNB-2 (95%)
arsC	CTS44_09862	168	As(V) reductase	*Ramlibacter tataouinensis* TTB310 (72%)
arsB	CTS44_09867	429	As(III)/Sb(III) efflux pump protein	*Comamonas testosteroni* CNB-2 (98%)
arsC	CTS44_09872	143	As(V) reductase	*Comamonas testosteroni* KF-1 (95%)
arsH	CTS44_09877	238	As resistance protein	*Comamonas testosteroni* KF-1 (99%)
MFS_1	CTS44_09882	393	Major facilitator superfamily MFS_1	*Comamonas testosteroni* CNB-2 (96%)
(b) The *arsB* (CTS44_13418) and its flanking genes				
arsR	CTS44_13428	107	Transcriptional regulator	*Comamonas testosteroni* CNB-2 (99%)
arsC	CTS44_13423	168	As(V) reductase	*Methyloversatilis universalis* FAM5 (64%)
arsB	CTS44_13418	429	As(III)/Sb(III) efflux pump protein	*Comamonas testosteroni* CNB-2 (98%)
arsC	CTS44_13413	143	As(V) reductase	*Comamonas testosteroni* CNB-2 (96%)
arsH	CTS44_13408	238	As resistance protein	*Comamonas testosteroni* KF-1 (99%)
MFS_1	CTS44_13403	396	Major facilitator superfamily MFS_1	*Comamonas testosteroni* CNB-2 (98%)

resistant proteins may associate with the adaptation of this strain in the mining environment.

Compared to the other Sb(III)-oxidizing bacteria, the resistant level of strain S44 was high for As(III) and was low for Sb(III), which demonstrated that mechanisms devoted to As and Sb resistances may be quite different in strain S44. Four *arsC* genes encoding As(V) reductase proteins were found in the genome of strain S44, which was most probably associated with the highly As(III) resistant level. Although As and Sb share similar physicochemical properties, none of the 6 Sb(III)-oxidizing bacteria showed As(III) oxidation abilities. This is different from the case of *A. tumefaciens* 5A, in which both As(III) and Sb(III) oxidation abilities were found in a single strain (Lehr et al., 2007). In addition, the whole genome of strain S44 did not contain the putative As(III) oxidase genes *aioBA*, which indicated that there were different genes coupling with bacterial oxidations of Sb(III) and As(III).

4. Conclusion

In this study, 25 Sb(III)-resistant bacterial strains were identified from antimony mined soil. Among them, 6 were Sb(III)-oxidizing strains and *Comamonas* species were the dominant Sb(III)-oxidizing bacteria. Several novel Sb(III)-oxidizing bacterial strains belonging to *Acinetobacter*, *Comamonas*, *Stenotrophomonas* and *Variovorax* were found in this study. *Comamonas* sp. JL40 showed the highest Sb(III) resistance and *Comamonas* sp. S44 showed the fastest Sb(III) oxidation rate. Our results showed that strains with the abilities of Sb(III) resistance and oxidation belonged to diverse bacterial lineages. In addition, the bacterial species and the mechanisms for As(III) and Sb(III) oxidations appeared to be quite different. We are now performing transposon mutagenesis study of strain S44 in order to gain gene informations for Sb resistance and oxidation. Our results indicated that microbiological oxidation of Sb(III) may therefore be widely distributed across bacterial lineages and mediate detoxification of Sb(III) where it exists in high concentrations in the environment.

Acknowledgments

This work was supported by a Major International Joint Research Project, National Natural Science Foundation of China (31010103903) and the National Fundamental Fund of Personnel Training of China (J1103510).

References

Bentley, R., Chasteen, T.G., 2002. Microbial methylation of metalloids: arsenic, antimony and bismuth. Microbiology and Molecular Biology Reviews 66, 250–271.

Branco, R., Chung, A.P., Morais, P.V., 2008. Sequencing and expression of two arsenic resistance operons with different functions in the highly arsenic-resistant strain *Ochrobactrum tritici* SCII24T. BMC Microbiology 8, 95.

Cai, L., Liu, G.H., Rensing, C., Wang, G.J., 2009. Genes involved in arsenic transformation and resistance associated with different levels of arsenic-contaminated soils. BMC Microbiology 9, 4.

Cooper, R.J., Harrison, A.P., 2009. The exposure to and health effects of antimony. Indian Journal of Occupational and Environmental Medicine 13, 3–10.

Ghosh, M., Shen, J., Rosen, B.P., 1999. Pathways of As(III) detoxification in *Saccharomyces cerevisiae*. Cell Biology 96, 5001–5006.

Goyeneche-Patino, D.A., Valderrama, L., Walker, J., Saravia, N.G., 2008. Antimony resistance and trypanothione in experimentally selected and clinical strains of *Leishmania panamensis*. Antimicrobial Agents and Chemotherapy 52, 4503–4506.

He, M.Y., Li, X.Y., Guo, L., Miller, S.J., Rensing, C., Wang, G.J., 2010. Characterization and genomic analysis of chromate resistant and reducing *Bacillus cereus* strain SJ1. BMC Microbiology 10, 221–230.

Hong, Y.G., Gu, J.-D., 2009. Bacterial anaerobic respiration and electron transfer relevant to the biotransformation of pollutants. International Biodeterioration & Biodegradation 63, 973–980.

Lehr, C.R., Kashyap, D.R., McDermott, T.R., 2007. New insights into microbial oxidation of antimony and arsenic. Applied and Environmental Microbiology 73, 2386–2389.

Leuz, A.K., Johnson, C.A., 2005. Oxidation of Sb(III) to Sb(V) by O_2 and H_2O_2 in aqueous solutions. Geochimica et Cosmochimica Acta 69, 1165–1172.

Li, X.K., Krumholz, L.R., 2007. Regulation of arsenate resistance in *Desulfovibrio desulfuricans* G20 by an *arsRBCC* operon and an *arsC* Gene. Journal of Bacteriology 189, 3705–3711.

Lialikova, N.N., 1974. *Stibiobacter senarmontii*: a new microorganism oxidizing antimony. Mikrobiologiia 43, 941–943.

Liarte, D.B., Murta, S.M.F., 2010. Selection and phenotype characterization of potassium antimony tartrate-resistant populations of four New World Leishmania species. Parasitology Research 107, 205–211.

Liu, F., Le, X.C., McKnight-Whitford, A., Xia, Y.L., Wu, F.C., Elswick, E., Johnson, C.C., Zhu, C., 2010. Antimony speciation and contamination of waters in the Xikuangshan antimony mining and smelting area, China. Environmental Geochemistry and Health.

Mukhopadhyay, R., Bisacchi, D., Zhou, Y., Armirotti, A., Bordo, D., 2009. Structural characterization of the As/Sb reductase LmACR2 from *Leishmania major*. Journal of Molecular Biology 386, 229–239.

Nies, D.H., 1999. Microbial heavy-metal resistance. Applied Microbiology and Biotechnology 51, 730–750.

Parvatiyar, K., Alsabbagh, E.M., Ochsner, U.A., Stegemeyer, M.A., Smulian, A.G., Hwang, S.H., Jackson, C.R., McDermott, T.R., Hassett, D.J., 2005. Global analysis of cellular factors and responses involved in *Pseudomonas aeruginosa* resistance to arsenite. Journal of Bacteriology 187, 4853–4864.

Rosen, B.P., Liu, Z.J., 2009. Transport pathways for arsenic and selenium: a minireview. Environment International 35, 512–515.

Salmassi, T.M., Venkateswaren, K., Nealson, K.H., Newman, D.K., Hering, J.G., 2002. Oxidation of arsenite by *Agrobacterium albertimagni*, AOL15, sp. nov., isolated from Hot Creek, California. Geomicrobiology Journal 19, 53–66.

Sanders, O.I., Rensing, C., Kuroda, M., Mitra, B., Rosen, B.P., 1997. Antimonite is accumulated by the glycerol facilitator GlpF in *Escherichia coli*. Journal of Bacteriology 179, 3365–3367.

Silver, S., Schottel, J., Weiss, A., 2001. Bacterial resistance to toxic metals determined by extrachromosomal R factors. International Biodeterioration & Biodegradation 48, 263–281.

Torma, A.E., Gabra, G.G., 1977. Oxidation of stibnite by *Thiobacillus ferrooxidans*. Antonie van Leeuwenhoek 43, 1–6.

Wang, G.J., Kennedy, S.P., Fasiludeen, S., Rensing, C., DasSarma, S., 2004. Arsenic resistance in *Halobacterium* sp. strain NRC-1 examined by using an improved gene knockout system. Journal of Bacteriology 186, 3187–3194.

Weeger, W., Li`evremont, D., Perret, M., Lagarde, F., Hubert, J.C., Leroy, M., Lett, M.C., 1999. Oxidation of arsenite to arsenate by a bacterium isolated from an aquatic environment. Biometals 12, 141–149.

White, C., Wilkinson, S.C., Gadd, G.M., 1995. The role of microorganisms in biosorption of toxic metals and radionuclides. International Biodeterioration & Biodegradation 35, 17–40.

Wilson, K.H., Blitchington, R.B., Greene, R.C., 1990. Amplification of bacterial 16S ribosomal DNA with polymerase chain reaction. Journal of Clinical Microbiology 28, 1942–1946.

Wilson, N.J., Craw, D., Hunter, K., 2003. Antimony distribution and environmental mobility at an historic antimony smelter site, New Zealand. Environmental Pollution 129, 257–266.

Wysocki, R., Chéry, C.C., Wawrzycka, D., Hulle, M.V., Cornelis, R., Thevelein, J.M., Tamás, M.J., 2001. The glycerol channel Fps1p mediates the uptake of arsenite and antimonite in *Saccharomyces cerevisiae*. Molecular Microbiology 40, 1391–1401.

Xiong, J.B., Li, D.M., Li, H., He, M.Y., Miller, S.J., Yu, L., Rensing, C., Wang, G.J., 2011. Genome analysis and characterization of zinc efflux systems of a highly zinc-resistant bacterium, *Comamonas testosteroni* S44. Research in Microbiology 162, 671–679.