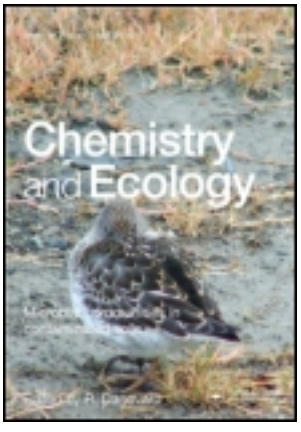

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gche20

Phylogenetic analysis on the arsenic-resistant bacteria isolated from three different freshwater environments

Domenico Davolos [a] & Biancamaria Pietrangeli [a]

[a] Department of Productive Plants and Interaction with the Environment (DIPIA), National Institute for Occupational Safety and Prevention (ISPESL), Rome, Italy

Available online: 18 Feb 2011

To cite this article: Domenico Davolos & Biancamaria Pietrangeli (2011): Phylogenetic analysis on the arsenic-resistant bacteria isolated from three different freshwater environments, Chemistry and Ecology, 27:S1, 79-87

To link to this article: http://dx.doi.org/10.1080/02757540.2010.536157

Chemistry and Ecology
Vol. 27, Supplement, February 2011, 79–87

Taylor & Francis
Taylor & Francis Group

Phylogenetic analysis on the arsenic-resistant bacteria isolated from three different freshwater environments

Domenico Davolos* and Biancamaria Pietrangeli

Department of Productive Plants and Interaction with the Environment (DIPIA), National Institute for Occupational Safety and Prevention (ISPESL), Rome, Italy

(*Received 15 January 2010; final version received 27 October 2010*)

Several aquatic environments are arsenic (As)-contaminated by geochemical sources and by residues derived from industrial and agricultural activities. In the current study, arsenic-resistant bacterial strains were isolated from Lake Albano, the Tiber River and a freshwater well (Bassano Romano), three sites located in the Latium region, central Italy, in which volcanic formations and aquifers containing As contribute to water As contamination. Bacteria capable of either oxidising As(III) or reducing As(V) belonged, on the basis of the 16*S* rRNA gene sequences, to the Proteobacteria, Firmicutes and Bacteroidetes. Moreover, phylogenetic analysis conducted on the gene codifying the ArsB, an As(III) efflux membrane protein pump related to the arsenic resistance, suggested the occurrence of horizontal *arsB* transfer events for some of the examined environmental strains, even among taxa belonging to taxonomically distant bacteria. Overall, these results showed that in each of the environments investigated, bacteria related to the redox of As coexisted, confirming important roles of microbial populations in the speciation of As and increasing the knowledge in view of the bioremoving of As compounds.

Keywords: 16*S* rDNA; *arsB*; phylogeny; Proteobacteria; Firmicutes; Bacteroidetes

1. Introduction

The metalloid arsenic (As), released from geological formations and from residues derived from anthropogenic activities (industry, agriculture, etc.), contaminated several aquatic sites. The most common arsenate and arsenite, oxidation states As(V) and As(III), respectively, are recognised as toxic ions. As(III) can covalently bind protein sulphydryl groups and is more toxic and mobile than As(V), which shows high similarity to phosphate anions. However, phylogenetically diverse bacteria related to the redox of As have been isolated from various aquatic environments containing natural and man-made As [1]. Moreover it has been shown that the bacterial metabolism of arsenical species in the environment can be critical in the biogeochemical As cycle [2,3].

In bacteria, a common arsenic resistance mechanism is based on the presence of the *ars* operon (typically *arsRBC* but in some cases *arsRDABC* or *arsRBCH*; see [4]), that involves As(V)

*Corresponding author. Email: domenico.davolos@ispesl.it

ISSN 0275-7540 print/ISSN 1029-0370 online

DOI: 10.1080/02757540.2010.536157
http://www.informaworld.com

reduction to As(III) via a cytoplasmic As(V) reductase (ArsC) and the extrusion of As(III) by a membrane-associated arsenite efflux pump (ArsB permease or Acr3–type family) [5]. The As(V) or As(III) cell exposure (these ions enter the bacterial cell though phosphate transporters and aquaglyceroporins, respectively) induces the ArsB and ArsC synthesis (mediated by ArsR, the repressor involved in the transcriptional regulation of the *ars* operon).

Our main aim was to investigate, on the basis of 16*S* rRNA gene sequences, the aerobic arsenic-resistant bacterial strains isolated from freshwater sites with different As contamination levels. Furthermore, we examined the occurrence of horizontal arsenic resistance gene transfer events in environmental strains focusing the phylogenetic analysis on the arsenite carriers genes of the ArsB family.

2. Materials and methods

2.1. *Sources of the isolates*

We collected surface water from Lake Albano, a crater lake in the volcanic district of Alban Hills, southeast of Rome (Italy), from Tiber River, Rome (locality Ponte Sisto) and from a freshwater well (Bassano Romano, Viterbo, Italy). All the sites are from the Latium region, central Italy, in which volcanic rocks and aquifer systems containing As contribute to the water As concentration ([6] and references therein). The water samples were stored in sterile bottles in the dark at 5 °C until arrival in the laboratory. Aliquots (10 mL) of the surface waters were supplemented with either sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$; Sigma-Aldrich) at a final concentration of 0.05 or 20 mM (Bassano Romano) or with sodium arsenite (*m*–$NaAsO_2$; Riedel–de Haën) at a final concentration of 50 or 130 μM (Bassano Romano). After ∼10 days of incubation in the dark at 25 °C, aliquots were spread on Nutrient Agar (NA, containing 3 g of beef extract, 5 g of peptone and 15 g of Agar per litre of double-distilled water) plates and the formed colonies were purified in NA Petri dishes under aerobic conditions. Bacterial colonies were initially grouped on the basis of colony morphology and Gram staining. The cell morphology of the isolates was examined mainly via a phase-contrast microscope (Leica Microsystems, Heidelberg, GmbH).

2.2. *PCR amplification, DNA sequencing and phylogenetic analysis*

For each purified colony, the PCR-mediated 16*S* rDNA amplification was performed using bacterial primers 8f and 926r reported previously [7]; the conditions for PCR amplification are reported in Davolos and Pietrangeli [8]. The 16*S* rDNA PCR products were purified either from the 2% agarose gel by the SpinClean Gel extraction Kit (MBiotech) or directly by the GenElute PCR Clean-up Kit (Sigma); the DNA sequencing reaction was performed on both strands using the same PCR primers. Moreover, a region of the *arsB* gene was amplified by the new pair of oligonucleotides arsBF: 5′-GGTGTGGAACATCGTCTGGAA-3′ and arsBR: 5′-CAGGCCGTACACCACCAG-3′, based on the primers darsB1F and darsB1R available from Achour et al. [9]. The PCR programme was: one cycle at 95 °C for 2 min, 33 cycles at 95 °C for 50 s, 56–58 °C for 30 s, 72 °C for 30 s, and a final cycle at 72 °C for 7 min. The concentration of each primer was 0.4 μM. The PCR ingredients included *Taq* DNA polymerase and the relative PCR buffer (Promega). The next step was the purification from the 2% agarose gel of the specific *arsB* band (∼750 bp) by the SpinClean Gel extraction Kit (MBiotech) and its elution in the TE buffer (10 mM Tris/HCl, pH 8.0 plus 1 mM EDTA, pH 4.5). DNA sequencing was performed with the same PCR primers. All the DNA sequences were determined with an Applied Biosystems model 373A stretch fluorescent automated sequencer.

The obtained nucleotides and the inferred amino acid residues were compared with accessible data in GenBank databases at NCBI using the BLAST algorithm. Phylogenetic analyses for the 16*S* rRNA genes were conducted using the neighbour-joining (NJ) method as reported previously [8], calculating 1000 bootstrap replicates. Phylogenetic reconstruction for the As(III) efflux pump genes (*arsB* and Acr3-type) was inferred by the NJ method and a Bayesian inference was calculated using MrBayes version 3.1.2 [10] with the best fitting model of nucleotide substitution determined by AIC criteria of jModelTest [11].

3. Results and discussion

The newly determined 16*S* rRNA and *arsB* gene sequences for the cultivable arsenic-resistant bacteria here obtained were submitted to the GenBank database (Table 1). The accession numbers for 16*S* rRNA genes were: FJ238516–FJ238520, FJ389743 and GU339466 (Lake Albano); FJ765352–FJ765363 (Tiber River); GU255469–GU255476 (Bassano Romano); for the *arsB* gene: GU270285–GU270295 (Tiber River), GU255477–GU255480 (Lake Albano) and GU263788–GU263791 (Bassano Romano).

Phylogenetic reconstructions based on the 16S rRNA genes showed that *Brevundimonas* (α-Proteobacteria), *Acidovorax*, *Comamonas* and *Delftia* (β-Proteobacteria), *Acinetobacter*, *Aeromonas* and *Pseudomonas* (γ-Proteobacteria) and *Chryseobacterium* (Bacteroidetes) were the genera of bacteria arsenate-resistant or able to reduce As(V) under aerobic conditions (Figure 1 and Table 1). Among the As(III) oxidisers or arsenite-resistant bacteria (Figure 1 and Table 1): *Shinella* (α-Proteobacteria), *Acidovorax*, *Comamonas* and *Variovorax* (β-Proteobacteria), *Klebsiella*, *Enterobacter*, *Rheinheimera* and *Pseudomonas* (γ-Proteobacteria) and *Bacillus* (Firmicutes). The general morphological features of these taxa, as investigated by microscopic observation (data not shown), were in agreement with the description of closely related species available in literature.

Interestingly, despite 3–7 $\mu g \cdot L^{-1}$ levels of As in Lake Albano [12], 1–3 $\mu g \cdot L^{-1}$ in the Tiber River (data from www.arpalazio.net) and 5.6 $\mu g \cdot L^{-1}$ in the well (E. De Matthaeis, pers. commun.), several phylogenetically distant bacteria showed arsenic resistance (Figure 1). Thus, our results indicated that diverse groups of arsenic-resistant bacteria can be present in aquatic environments with As levels below 10 $\mu g \cdot L^{-1}$ [13]. Most of the genera from the current study (Figure 1) were previously reported as arsenic-resistant bacteria. For example, in different taxa belonging to *Acinetobacter*, *Comamonas* or *Pseudomonas*, the *arsB* and other As(III) transporter genes have been confirmed [14–18]. An extensive analysis of available completely sequenced bacterial genomes (data extracted from the NCBI databases) allowed to characterise most of the genera from the present study in terms of arsenic resistance, including the ubiquitous aquatic genus *Aeromonas* (Figure 1 and Table 1).

Furthermore, the DNA sequencing analysis indicated the presence of the *arsB* gene in many arsenic-resistant bacteria isolated here (Figure 2) suggesting the intracellular reduction of As(V) and the efflux of As(III) from the cells. Our phylogenetic analysis showed a prevalence of *arsB* in β-Proteobacteria and γ-Proteobacteria (Figure 2), while a previous investigation indicated an *arsB* presence mainly in γ-Proteobacteria and Firmicutes [19].

A recent molecular study showed some inconsistencies in arsenite-resistant bacteria when comparing the arsenite transporter genes phylogeny and the evolutionary tree based on the 16*S* rRNA genes, indicating putative horizontal gene transfer of *arsB* and Acr3-type genes [16]. The phylogeny of the taxa examined in this study (Figure 1) and the results obtained from comparative phylogenetic analysis of their *arsB* sequences conducted by the NJ and Maximum Composite Likelihood methods (Figure 2) and generally confirmed by the Bayesian inference (not shown) suggested that the *arsB* can be horizontally propagated even among taxonomically

Table 1. Bacterial strains in the current study (S, Lake Albano; T, Tiber River; BA or BI, Bassano Romano), the GenBank accession number (Acc. No.), the GC% of the *arsB* gene, the sequence identity (%) of the 16*S* rRNA and the *arsB* genes with other Proteobacteria, Firmicutes and Bacteroidetes deposited at NCBI database.

Taxon	Locality	Gene	GenBank Acc. No.	GC%	Best match in GenBank (Acc. No.)	Identity (%)
Aeromonas sp. S1	Lake Albano	16*S* rDNA	FJ238516	ND	*Aeromonas media* V69 (AM262150)	100
Pseudomonas sp. S8	Lake Albano	16*S* rDNA	FJ238517	ND	*Pseudomonas* sp. PS18-2010 (GU930777)	100
		arsB	GU255480	71.5	*P. aeruginosa* UCBPP-PA14 (CP000438)	100
Klebsiella sp. S3	Lake Albano	16*S* rDNA	FJ238518	ND	*Klebsiella variicola* At-22 (CP001891)	100
		arsB	GU255479	61.6	*Klebsiella pneumoniae* 342 (CP000964)	99.4
Enterobacter sp. S5	Lake Albano	16*S* rDNA	FJ238519	ND	*Enterobacter* sp. WP2ML (GU272397)	100
		arsB	GU255478	58.9	*Enterobacter cloacae* (AF521305)	90.7
Comamonas sp. S6	Lake Albano	16*S* rDNA	FJ238520	ND	*Comamonas testosteroni* CNB-2 (CP001220)	100
		arsB	GU255477	58.9	*Enterobacter cloacae* (AF521305)	90.7
Shinella sp. S12	Lake Albano	16*S* rDNA	FJ389743	ND	*Shinella kummerowiae* CCNWGS0228 (FJ154090)	99.3
Brevundimonas sp. S2	Lake Albano	16*S* rDNA	GU339466	ND	*Brevundimonas* sp. K01-14 (EU333887)	100
Acinetobacter sp. T2	Tiber River	16*S* rDNA	FJ765352	ND	*Acinetobacter johnsonii* (AF188300)	99.7
		arsB	GU270285	63.4	*Pseudomonas fluorescens* SBW25 (AM81176)	90.3
Chryseobacterium sp. T6	Tiber River	16*S* rDNA	FJ765353	ND	*Chryseobacterium* sp. YJ1 (DQ521273)	99.6
		arsB	GU270286	63.7	*Pseudomonas fluorescens* SBW25 (AM81176)	87.6
Pseudomonas sp. T10	Tiber River	16*S* rDNA	FJ765354	ND	*Pseudomonas alcaliphila* D11 (EU082832)	100
		arsB	GU270287	64.2	*Pseudomonas fluorescens* SBW25 (AM81176)	88.9
Pseudomonas sp. T12	Tiber River	16*S* rDNA	FJ765355	ND	*Pseudomonas mendocina* PC1 (DQ178219)	100
		arsB	GU270288	63.6	*Pseudomonas fluorescens* SBW25 (AM81176)	88.0
Bacillus sp. T1	Tiber River	16*S* rDNA	FJ765356	ND	*Bacillus megaterium* M530013 (HM032807)	100
		arsB	GU270289	63.7	*Pseudomonas fluorescens* SBW25 (AM81176)	88.6
Rheinheimera sp. T3	Tiber River	16*S* rDNA	FJ765357	ND	*Rheinheimera* sp. THWCSN2 (AM888197)	99.1
		arsB	GU270290	64.3	*Pseudomonas fluorescens* SBW25 (AM81176)	88.1
Pseudomonas sp. T4	Tiber River	16*S* rDNA	FJ765358	ND	*Pseudomonas anguilliseptica* ZH8 (HM103328)	100
		arsB	GU270291	63.0	*Pseudomonas fluorescens* SBW25 (AM81176)	88.3
Acinetobacter sp. T5	Tiber River	16*S* rDNA	FJ765359	ND	*Acinetobacter johnsonii* CONC8 (EU275352)	100
Pseudomonas sp. T7	Tiber River	16*S* rDNA	FJ765360	ND	*Pseudomonas alcaligenes* B19 (EU240201)	99.9
		arsB	GU270292	64.1	*Pseudomonas fluorescens* SBW25 (AM81176)	88.4
Pseudomonas sp. T8	Tiber River	16*S* rDNA	FJ765361	ND	*Pseudomonas alcaliphila* D11 (EU082832)	100
		arsB	GU270293	63.9	*Pseudomonas fluorescens* SBW25 (AM181176)	89.2
Pseudomonas sp. T9	Tiber River	16*S* rDNA	FJ765362	ND	*Pseudomonas alcaligenes* T2944D (DQ298036)	99.6
		arsB	GU270294	64.0	*Pseudomonas fluorescens* SBW25 (AM81176)	88.4

(*Continued*)

Table 1. Continued.

Taxon	Locality	Gene	GenBank Acc. No.	GC%	Best match in GenBank (Acc. No.)	Identity (%)
Pseudomonas sp. T11	Tiber River	16*S* rDNA	FJ765363	ND	*Pseudomonas putida* IHB B 1369 (GU186116)	99.5
		arsB	GU270295	64.7	*Pseudomonas fluorescens* SBW25 (AM181176)	89.1
Comamonas sp. BA4	Bassano Romano	16*S* rDNA	GU255469	ND	*Comamonas* sp. DJ-12 (AY600616)	96.5
		arsB	GU263788	61.3	*Delftia* sp. TS41 (EU311958)	99.8
Delftia sp. BA2	Bassano Romano	16*S* rDNA	GU255470	ND	*Delftia lacustris* strain 332 (EU888308)	100
		arsB	GU263789	60.3	*Delftia* sp. TS41 (EU311958)	100
Acidovorax sp. BA3	Bassano Romano	16*S* rDNA	GU255471	ND	*Acidovorax* sp. KNA-A (AB539974)	100
		arsB	GU263790	56.8	*Delftia* sp. TS41 (EU311958)	100
Acidovorax sp. BA1	Bassano Romano	16*S* rDNA	GU255472	ND	*Acidovorax* sp. RCP3 (DQ922753)	100
Comamonas sp. BI3	Bassano Romano	16*S* rDNA	GU255473	ND	*Comamonas* sp. D18 (AM179889)	99.8
		arsB	GU263791	66.3	*Comamonas* sp. TS38 (EU311957)	100
Comamonas sp. BI44	Bassano Romano	16*S* rDNA	GU255474	ND	*Comamonas* sp. DJ-12 (AY600616)	96.4
Variovorax sp. BI2	Bassano Romano	16*S* rDNA	GU255475	ND	*Variovorax* sp. GG6b (GQ337855)	99.8
Acidovorax sp. BI4	Bassano Romano	16*S* rDNA	GU255476	ND	*Acidovorax* sp. KNA-A (AB539974)	100

Note: The GC percentage was computed using the GC Calculator available at http://www.genomicsplace.com/gc_calc.html. ND, not determined.

distant bacteria. Indeed, inconsistencies between the 16*S* rDNA phylogeny and the *arsB* gene phylogeny were apparent in certain taxa isolated from the Tiber River. Among them, *Acinetobacter* sp. T2, *Rheinheimera* sp. T3, *Chryseobacterium* sp. T6 and *Bacillus* sp. T1 (Figures 1 and 2); the GC% values calculated for their *arsB* regions (Table 1) were close to the characteristic GC% range for *Pseudomonas* species (58.4–66.6%; genomic data extracted from the NCBI) suggesting that the putative lateral *arsB* transfer events occurred recently and (at least initially) the donor DNA originated from a taxon belonging to the genus *Pseudomonas*. *Comamonas* sp. S6 (β-Proteobacteria) from Lake Albano appeared to possess *arsB* gene that was similar to those of Enterobacteriaceae (γ-Proteobacteria; Figure 2 and Table 1). Putative horizontal genetic transfer events for the *arsB* gene emerged also in *Comamonas* sp. BA4 (located within the *Delftia* clade; Figure 2) and *Acidovorax* sp. BA3 (not included in the Figure 2) from the Bassano Romano well where the arsenic-resistant taxa belonged only to the β-Proteobacteria (Figure 1). Moreover, according to data extracted from the NCBI databases and included in the phylogenetic analysis (Figures 1 and 2), clear discrepancies between the 16*S* rDNA phylogeny and the *arsB* gene phylogeny emerged for *Acinetobacter baumannii* AB0057.

Obviously, the consequences of the human use of As in the environment may contribute to the dissemination of microbial arsenic resistance. Previously, studies showed that horizontal transfer of *arsB* and Acr3-type genes occurred in strains isolated from highly As-contaminated soil [5,16]. Notably, *arsB* and Acr3-type As(III) transporter genes can be found within the same taxon, as demonstrated here for *Delftia acidovorans* SPH-1 (data extracted from the NCBI databases; Figures 1 and 2). In addition, the evolution of highly arsenic-resistant bacteria can be a consequence of other genetic mechanisms such as gene duplication events. In fact, genome analysis revealed the presence of duplication for *ars* operon containing *arsB*, e.g. in *Pseudomonas putida* KT2440 and *Shewanella putrefaciens* CN–32 (Figures 1 and 2). Four different *ars* loci were located in the chromosome of the heterotrophic *Herminiimonas arsenicoxydans* [5] (Figures 1 and 2). Furthermore, in *Methylibium petroleiphilum* PM1 and *Acidovorax* sp. JS42 (Figure 1) the As extrusion could be mediated by two chromosomal copies of Acr3-type arsenical pump (Figure 2).

In Lake Albano and in the Tiber River, sequence analysis revealed the occurrence of arsenic resistance in opportunistic pathogens or genera containing pathogenic strains (e.g. *Acinetobacter*,

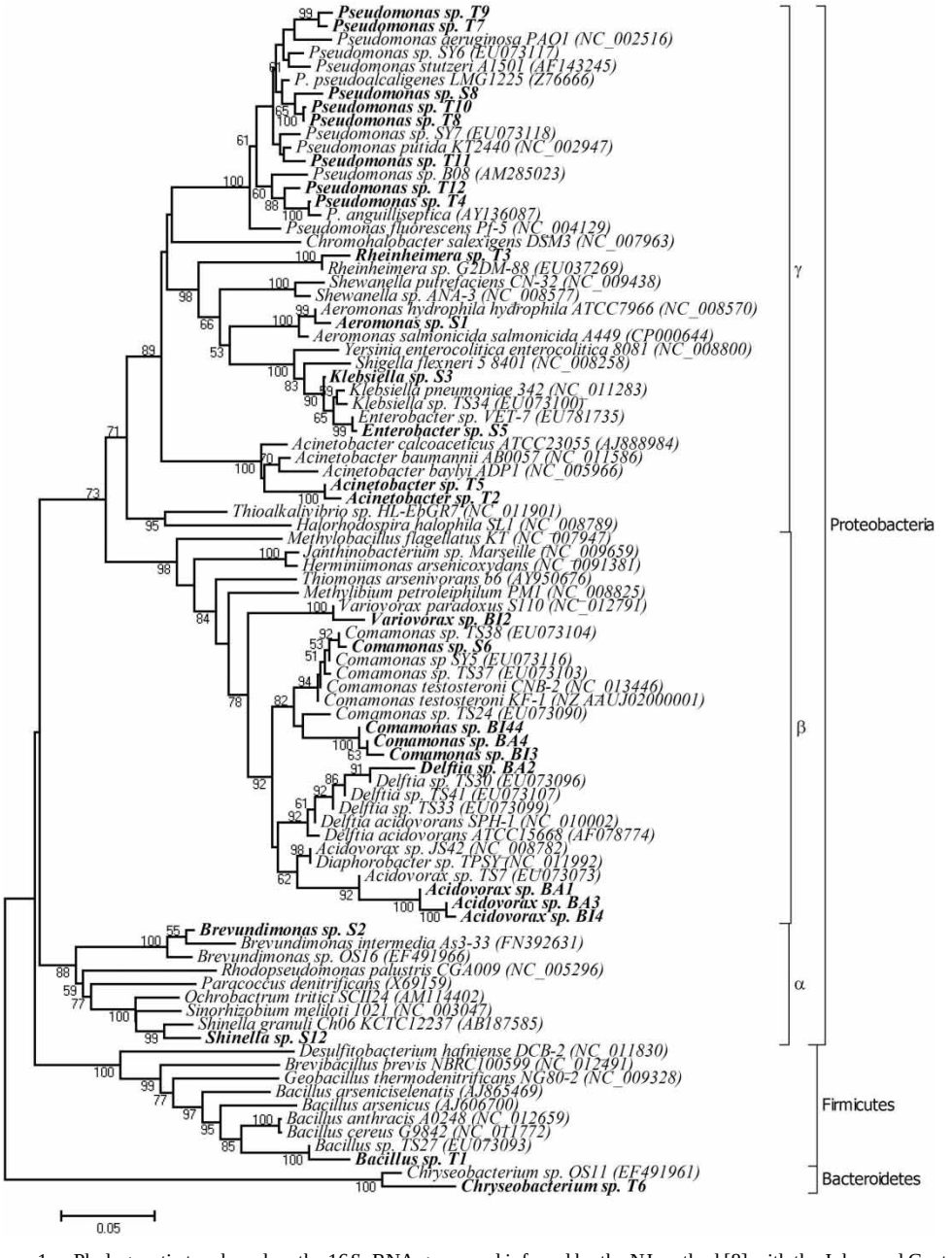

Figure 1. Phylogenetic tree based on the 16*S* rRNA genes and inferred by the NJ method [8] with the Jukes and Cantor corrections from the strains of the current study (contrasted in bold; S, Lake Albano; T, Tiber River; BA or BI, Bassano Romano) and other Proteobacteria, Firmicutes and Bacteroidetes deposited at NCBI database (the relative GenBank accession numbers are shown in parentheses). Numbers on the branching points are bootstrap (1000 replicates) support values; values under 50% were omitted.

Aeromonas, *Klebsiella*; Figures 1 and 2). Notably, on the basis of previous analysis conducted on different environments, lateral gene transfer events for genes involved in heavy metal/metalloid resistance, as well as for genes codifying virulence factors, appeared to be frequently occurring in water-living bacteria [20]. Consequently, we underline the potential usefulness of the

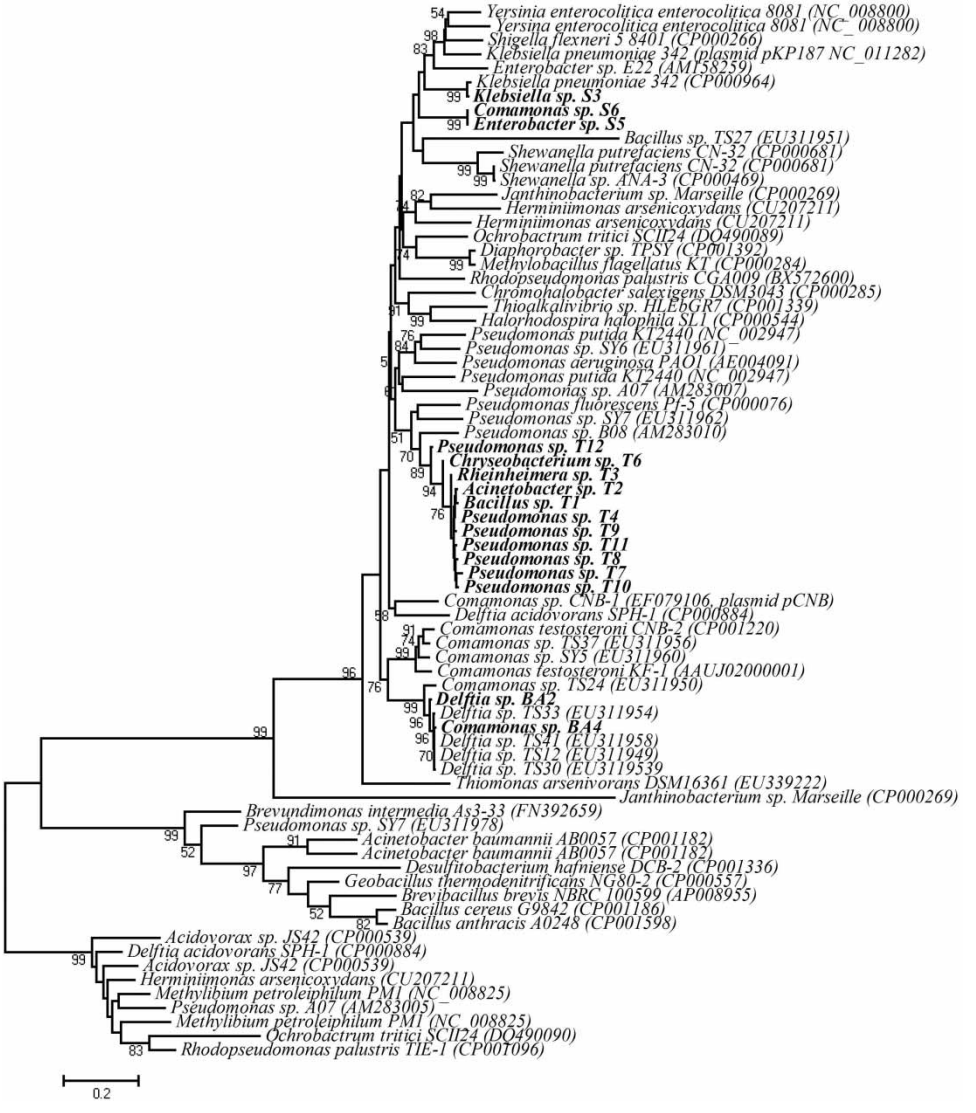

Figure 2. Phylogenetic tree inferred by the NJ and the Maximum Composite Likelihood methods and based on the As(III) efflux pump genes (*arsB* and Acr3-type) from the strains of the present study (contrasted in bold; S, Lake Albano; T, Tiber River; BA, Bassano Romano) and other bacteria deposited at NCBI database (the relative GenBank accession numbers are shown in parentheses). Numbers on the branching points are bootstrap (1000 replicates) support values (values under 50% were omitted). Acr3-type gene sequences were used to root the tree.

arsB gene and in general of the As metabolism genes as functional markers for monitoring the freshwater bacterial communities towards arsenicals and other factors concerning public health [21,22].

In conclusion, the molecular information on the arsenic resistance genes obtained from phylogenetically characterised arsenic-resistant bacteria can be fundamental for improving molecular tools, such as the DNA microarray [23], for the culture-independent analysis of As metabolism in natural systems. Considering the evolutionary history of the bacterial arsenic resistance genes, including differences in gene copy numbers, more DNA sequence analyses have to be carried out from environmental As metabolising isolates. Further analysis is required on the strains in which

the pathways for biochemical responses to As were hitherto unknown mechanisms and, regarding As decontamination application, for comparing the bacterial As removal capacities [24–27].

Acknowledgements

We express our gratitude to Prof. Elvira De Matthaeis (Sapienza University of Rome, Rome, Italy) for the water sample from Bassano Romano.

References

[1] W.P. Inskeep, R.E. Macur, N. Hamamura, T.P. Warelow, S.A. Ward, and J.M. Santini, *Detection, diversity and expression of aerobic bacterial arsenite oxidase genes*, Environ. Microbiol. 9 (2007), pp. 934–943.
[2] D.K. Nordstrom, *Public health. Worldwide occurrences of arsenic in ground water*, Science 296 (2002), pp. 2143–2145.
[3] R.S. Oremland and J.F. Stolz, *The ecology of arsenic*, Science 300 (2003), pp. 939–944.
[4] R. Branco, A.P. Chung, and P.V. Morais, *Sequencing and expression of two arsenic resistance operons with different functions in the highly arsenic-resistant strain* Ochrobactrum tritici *SCII24T*, BMC Microbiol. 8 (2008), p. 95.
[5] D. Muller, C. Médigue, S. Koechler, V. Barbe, M. Barakat, E. Talla, V. Bonnefoy, E. Krin, F. Arsène-Ploetze, C. Carapito, M. Chandler, B. Cournoyer, S. Cruveiller, C. Dossat, S. Duval, M. Heymann, E. Leize, A. Lieutaud, D. Lièvremont, Y. Makita, S. Mangenot, W. Nitschke, P. Ortet, N. Perdrial, B. Schoepp, P. Siguier, D.D. Simeonova, Z. Rouy, B. Segurens, E. Turlin, D. Vallenet, A. Van Dorsselaer, S. Weiss, J. Weissenbach, M.C. Lett, A. Danchin, and P.N. Bertin, *A tale of two oxidation states: bacterial colonization of arsenic-rich environments*, PLoS Genet. 3 (2007), p. e53.
[6] M. Angelone, C. Cremisini, V. Piscopo, M. Proposito, and F. Spaziani, *Influence of hydrostratigraphy and structural setting on the arsenic occurrence in groundwater of the Cimino-Vico volcanic area (central Italy)*, Hydrogeol. J. 17 (2009), pp. 901–914.
[7] W.T. Liu, T.L. Marsh, H. Cheng, and L.J. Forney, *Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA*, Appl. Environ. Microbiol. 63 (1997), pp. 4516–4522.
[8] D. Davolos and B. Pietrangeli, *Molecular and phylogenetic analysis on bacterial strains isolated from a PAHs wastewater treatment plant*, in *Current Research Topics in Applied Microbiology and Microbial Biotechnology*, A. Mendez-Vilas, ed., World Scientific, Singapore, 2009, pp. 313–316.
[9] A.R. Achour, P. Bauda, and P. Billard, *Diversity of arsenite transporter genes from arsenic-resistant soil bacteria*, Res Microbiol. 158 (2007), pp. 128–137.
[10] F. Ronquist and J.P. Huelsenbeck, *MRBAYES 3: Bayesian phylogenetic inference under mixedmodels*, Bioinformatics 19 (2003), pp. 1572–1574.
[11] D. Posada, *Selection of models of DNA evolution with jModelTest*, Methods Mol. Biol. 537 (2009), pp. 93–112.
[12] M. Dall'Aglio, *Acque potabili non salubri a causa di vistose anomalie geochimiche di arsenico e fluoro nel mondo e in Italia. I casi della Toscana e del Lazio*, in *La crisi dei sistemi idrici: approvvigionamento agro-industriale e civile*, Accademia Nazionale dei Lincei, Rome, 2007, pp. 179–194.
[13] C.R. Jackson, K.G. Harrison, and S.L. Dugas, *Enumeration and characterization of culturable arsenate resistant bacteria in a large estuary*, Syst. Appl. Microbiol. 28 (2005), pp. 727–734.
[14] M. Pepi, M. Volterrani, M. Renzi, M. Marvasi, S. Gasperini, E. Franchi, and S.E. Focardi, *Arsenic-resistant bacteria isolated from contaminated sediments of the Orbetello Lagoon, Italy, and their characterization*, J. Appl. Microbiol. 103 (2007), pp. 2299–2308.
[15] M. Pepi, H.J. Heipieper, J. Fischer, M. Ruta, M. Volterrani, and S.E. Focardi, *Membrane fatty acids adaptive profile in the simultaneous presence of arsenic and toluene in* Bacillus *sp. ORAs2 and* Pseudomonas *sp. ORAs5 strains*, Extremophiles 12 (2008), pp. 343–349.
[16] L. Cai, G. Liu, C. Rensing, and G. Wang, *Genes involved in arsenic transformation and resistance associated with different levels of arsenic-contaminated soils*, BMC Microbiol. 9 (2009), p. 4.
[17] P.C. Patel, F. Goulhen, C. Boothman, A.G. Gault, J.M. Charnock, K. Kalia, and J.R. Lloyd, *Arsenate detoxification in a Pseudomonad hypertolerant to arsenic*, Arch. Microbiol. 187 (2007), pp. 171–183.
[18] S.A. Connon, A.K. Koski, A.L. Neal, S.A. Wood, and T.S. Magnuson, *Ecophysiology and geochemistry of microbial arsenic oxidation within a high arsenic, circumneutral hot spring system of the Alvord Desert*, FEMS Microbiol. Ecol. 64 (2008), pp. 117–128.
[19] M. Quemeneur, A. Heinrich-Salmeron, D. Muller, D. Lievremont, M. Jauzein, P.N. Bertin, F. Garrido, and C. Joulian, *Diversity surveys and evolutionary relationships of* aoxB *genes in aerobic arsenite-oxidizing bacteria*, Appl. Environ. Microbiol. 74 (2008), pp. 4567–4573.
[20] S. Audic, C. Robert, B. Campagna, H. Parinello, J.M. Claverie, D. Raoult, and M. Drancourt, *Genome analysis of* Minibacterium massiliensis *highlights the convergent evolution of water-living bacteria*, PLoS Genet. 3 (2007), p. e138.

[21] N. Hamamura, R.E. Macur, S. Korf, G. Ackerman, W.P. Taylor, M. Kozubal, A.L. Reysenbach, and W.P. Inskeep, *Linking microbial oxidation of arsenic with detection and phylogenetic analysis of arsenite oxidase genes in diverse geothermal environments*, Environ. Microbiol. 11 (2009), pp. 421–431.
[22] N.S. Chauhan, R. Ranjan, H.J. Purohit, V.C. Kalia, and R. Sharma, *Identification of genes conferring arsenic resistance to* Escherichia coli *from an effluent treatment plant sludge metagenomic library*, FEMS Microbiol. Ecol. 67 (2009), pp. 130–139.
[23] T.Z. DeSantis, E.L. Brodie, J.P. Moberg, I.X. Zubieta, Y.M. Piceno, and G.L. Andersen, *High-density universal 16S rRNA microarray analysis reveals broader diversity than typical clone library when sampling the environment*, Microb. Ecol. 53 (2007), pp. 371–383.
[24] P. Mondal, C.B. Majumder, and B. Mohanty, *Growth of three bacteria in arsenic solution and their application for arsenic removal from wastewater*, J. Basic Microbiol. 48 (2008), pp. 521–525.
[25] T.C. Hazen, R. Chakraborty, J.M. Fleming, I.R. Gregory, J.P. Bowman, L. Jimenez, D. Zhang, S.M. Pfiffner, F.J. Brockman, and G.S. Sayler, *Use of gene probes to assess the impact and effectiveness of aerobic* in situ *bioremediation of TCE*, Arch. Microbiol. 191 (2009), pp. 221–232.
[26] W. Baeyens, A. De Brauwere, N. Brion, M. De Gieter, and M. Leermakers, *Arsenic speciation in the River Zenne, Belgium*, Sci. Total. Environ. 384 (2007), pp. 409–419.
[27] R.S. Oremland and J.F. Stolz, *Arsenic, microbes and contaminated aquifers*, Trends Microbiol. 13 (2005), pp. 45–49.