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Oxidation of arsenite by *Thiomonas* strains and characterization of *Thiomonas arsenivorans* sp. nov.

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Abstract

A novel bacterium, strain b6^T (T = type strain), was isolated from a disused mine site by growth using arsenite [As(III)] as energy source in a simple mineral medium. Cells of strain b6^T were rod-shaped, Gram-negative, non-sporulating and motile. Optimum growth occurred at temperatures between 20 and 30 °C, and at pH between 4.0 and 7.5. Strain b6^T grew chemoautotrophically on As(III), sulphur and thiosulphate, and also heterotrophically on yeast extract and a variety of defined organic compounds. Several other *Thiomonas* strains, including the type species *Thiomonas* (*Tm.*) *intermedia*, were able to oxidize As(III), though only strain b6^T and strain NO115 could grow using As(III) as sole energy source in the absence of any organic compound. The G+C content of the DNA of strain b6^T was 65.1 mol %. Comparative small subunit (SSU) ribosomal RNA (rRNA) analysis indicated that strain b6^T belongs to the genus *Thiomonas* in the β-subdivision of the *Proteobacteria*. It was closely related to an unnamed *Thiomonas* strain (NO115) isolated from a Norwegian mining site, though sequence identities between strain b6^T and characterized *Thiomonas* species were less than 95%. DNA–DNA hybridization between strain b6^T and the type species of the genus *Tm. intermedia* showed less than 50% homology. On the basis of phylogenetic and phenotypic characteristics, strain b6^T (DSM 16361^T, LMG 22795^T) is proposed as the type strain of the new species *Thiomonas arsenivorans*, sp. nov.

Abbreviations: MCSM – Modified Cheni As-oxidizing population Selective Medium

Introduction

Bacteria belonging to the genus *Thiomonas* are facultative chemolithoautotrophs growing optimally in mixotrophic media containing both a reduced sulphur compound (or elemental sulphur) and organic substrates such as yeast extract, peptone, sugars and amino-acids (Moreira and Amils 1997). In the absence of an organic compound, these bacteria can grow autotrophically using elemental sulphur or reduced inorganic sulphur compounds, such as thiosulphate or tetrathionate (London 1963; Shooner et al. 1996). The first *Thiomonas* species

were isolated from soils (London 1963; London and Rittenberg 1967), and more recently strains have been found in mining sites, where soluble metals and metalloïds are often present in relatively high concentrations (Dennison et al. 2001; Coupland et al. 2004; Johnson and Hallberg 2005). A number of studies have shown that *Thiomonas* spp. are involved in metal-mobilization and the oxidation of minerals that are commonly found in sulphide-containing geological formations. The species *Tm. cuprina*, formerly known as '*Thiobacillus cuprinus*' and isolated from an uranium mine (Huber and Stetter 1990), can grow using a variety of sulphide ores such as chalcopyrite, sphalerite, arsenopyrite, and galena as energy sources. The ability of some *Thiomonas* strains to oxidise Fe(II) was demonstrated with isolates obtained from different mine sites including copper, coal, tin and gold mines (Coupland et al. 2004; Johnson and Hallberg 2005). As(III) oxidation was recently demonstrated in several *Thiomonas* isolates (Battaglia-Brunet et al. 2003; Bruneel et al. 2004; Coupland et al. 2004; Duquesne 2004). However, most of these strains were not able to grow lithoautotrophically with As(III), but required small amounts of yeast extract. The discovery of several chemolithotrophic As(III)-oxidizing bacteria, isolated from gold mines in Australia, demonstrated that energy for growth can be conserved during oxidation of As(III) (Santini et al. 2000, 2002). The Australian isolates are distinct from *Thiomonas* spp., and are more closely related to the genera *Rhizobium*, *Agrobacterium*, and *Sinorhizobium*. Even in bacteria that depend on organic carbon sources, As(III) may be used as supplemental energy source (Anderson et al. 2003). A novel As(III)-oxidizing bacterium was isolated from a mixed population obtained from a disused gold mining site, and selected by its ability to grow by oxidizing As(III) in a mineral salts medium (Battaglia-Brunet et al. 2002). The present paper describes this isolate (strain b6^T) as a new species of the genus *Thiomonas*, which is characterized by its ability to use As(III) as sole energy source for chemolithoautotrophic growth. The name *Thiomonas arsenivorans* sp. nov. is proposed for this microorganism. In addition, we show that the ability to oxidize As(III) is common to many *Thiomonas* strains, including the type species of the genus, *Tm. intermedia*.

Materials and methods

Bacteria

Strain b6^T was isolated from an As(III)-oxidizing mixed population (coded CAsO1) obtained from Cheni, a disused gold mine site in France (Battaglia-Brunet et al. 2002). Two other strains (NO115 and WJ68) were isolated from mining sites in Norway and Wales (U.K.), respectively (Coupland et al. 2004; Johnson and Hallberg 2005). *Tm. intermedia* ATCC 15466^T was obtained from the American Type Culture Collection. The type strain of *Tm. perometabolis* (ATCC 23370^T) was obtained from the Pasteur Institute Collection (CIP 104403^T). Strain b6^T has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen as DSM 16361 and in the Belgian Co-ordinated Collections of Microorganisms, Bacteria Collection (BCCM/LMG), as LMG 22795.

Media and cultivation

Modified CSM medium (MCSM), containing 100 mg l^{-1} As(III) (Battaglia-Brunet et al. 2002) and with vitamins omitted, was used throughout the work. Isolates were also grown on an equivalent solid medium (gelled with 1.5% bacteriological agar: MERCK) amended with 1 g l^{-1} sodium lactate. *Tm. perometabolis* was cultured in medium 126 (Pasteur Institute) at pH 6.0. Media were inoculated with 10% (v/v) of active bacterial culture throughout. To determine the pH range for growth of b6^T, sulphuric acid or dilute sodium hydroxide were used to adjust the pH of the MCSM in shake flasks that were incubated at 25 °C under reciprocal agitation (120 rpm). For the determination of temperature range for growth, glass reactors filled with 1 l MCSM, equipped with water jackets and agitated with a magnetic impeller were used. To compare mixotrophic and chemolithotrophic growth, 10 ml of the following media were distributed in 25 ml glass tubes previously ignited (2 h at 500 °C) in order to eliminate traces of organic carbon, and incubated at 25 °C in static conditions: (i) MCSM prepared with HPLC-grade water with (100 mg l^{-1}) and (ii) without As(III), (iii) MCSM prepared with demineralized water with 100 mg l^{-1} As(III), (iv) MCSM prepared with demineralized water with

100 mg l^{-1} As(III) and further amended with 0.02 and (v) 0.2 g l^{-1} yeast extract. Four successive cultures in each media were performed.

The ability of *Thiomonas* strains to oxidize As(III) was tested in 20 ml glass flasks containing (i) MCSM, (ii) medium 126 supplemented with 100 mg l^{-1} As(III), and (iii) MCSM supplemented with 1 mM sodium thiosulphate and 0.2 g l^{-1} yeast extract; incubation was performed at 25 °C in static conditions. For kinetics of As(III) oxidation, shake flasks were filled with 100 ml of MCSM and incubated at 25 °C under reciprocal agitation (115 rpm).

All growth experiments were done on at least two separate occasions.

Anaerobic growth with NO_3^- as electron acceptor was tested in sealed flasks containing 100 ml of MCSM supplemented with 0.1 g l^{-1} NO_3^- as $NaNO_3$. The head space gas was N_2. Nitrification was tested in 100 ml aerobic cultures with MCSM supplemented with 0.1 g l^{-1} NH_4 as $(NH_4)_2SO_4$. Growth experiments with organic compounds were performed at 20 °C by using MCSM medium lacking As(III), and which was supplemented with the organic compounds given in Table 1. Growth was monitored as described below. Additional substrates were tested using API strips (Biotype 100) inoculated with the bacterial culture and incubated during 2 and 4 days at 25 °C; when turbidity developed or colour changed after 2 or 4 days, growth was considered as positive.

Oxidation of ferrous iron by strain b6^T was studied by following changes in ferrous iron concentrations in triplicate cultures growing in ferrous iron/thiosulphate medium (Hallberg and Johnson 2003) in shake flasks. These were inoculated with a culture grown previously on the same medium, and were incubated at 30 °C with shaking (at 150 rpm). The pH of the medium was 4.5 at the start, and the iron concentration was 5 mM and thiosulfate 10 mM. An uninoculated flask served as control to measure abiotic oxidation of iron.

Analytical methods

Growth was determined by enumerating cells using a Thoma bacterial counting chamber, with

Table 1. Characteristics of strain b6^T and its closest characterized relatives among the *Thiomonas* genus.

	Strain b6[Ta]	*Tm. perometabolis*[b, c, d]	*Tm. intermedia*[a, d, e]	*Tm. thermosulfata*[d, f]	*Tm. cuprina*[d, g]
Heterotrophic growth with:					
Acetate	−(20 mM)	ND	ND	−	−
Aspartate	+(20 mM)	+	−	−	ND
Formate	−(40 mM)	−	ND	−	−
Glutamate	+(10 mM)	+	−	+	−
Pyruvate	+(20 mM)	ND	ND	−	+
Succinate	+(10 mM)	+	ND	+	−
Glucose	+(10 mM)	−	−	−	−
Raffinose	+10 mM)	ND	ND	−	−
Sucrose	+(10 mM)	ND	−	−	−
Sorbitol	+(10 mM)	ND	ND	−	ND
Yeast extract	+(0.05%)	+	+	+	+
Autotrophic growth with:					
Sulphur	+(0.5%)	+	+	+	+
Thiosulphate	+(20 mM)	+	+	+	−
Tetrathionate	+(10 mM)	+	+	+	−
As(III) oxidation	+	−	+	ND	ND
Fe(II) oxidation	+	+	+	ND	−
Motility	+	+	+	+	+
Temperature (°C) (optimum)	20–30	30–37	30–37	50	30–36
pH range (optimum)	4.0–7.5	5.0–7.0	5.0–7.0	5.0–6.0	3.0–4.0
G+C content of DNA (mol %)	65%	65–66%	65–67%	61%	66–69%

ND: not determined; growth for a given compound is indicated by + while lack of growth is indicated by −. [a]This study. [b]Katamaya-Fujimura and Kuraishi (1983). [c]London and Rittenberg (1967). [d]Moreira and Amils (1997). [e]London (1963). [f]Shooner et al. (1996). [g]Huber and Stetter (1990).

an optical microscope (Nikon Optiphot), at 400× magnification. Arseniate [As(V)] and As(III) were separated by extracting As(III) in methyl isobutyl ketone with pyrrolidine dithiocarbamate (Battaglia-Brunet et al. 2002). As(V) remaining in the aqueous phase was analysed by Atomic Absorption Spectrophotometry (AAS, Varian SpectrAA 300), using As(V) standards prepared following the same extraction method. Total arsenic was analysed by AAS with standards prepared in 1% HCl (v/v). Ferrous iron was measured colorimetrically using the ferrozine reagent as described previously (Lovley and Phillips 1987). Nitrate and nitrite were analysed using a high performance ion chromatography system (Dionex DX600®, Sunnyvale, CA, USA) equipped with an AS50® autosampler, an EG40® eluent generator, a CD25® conductivity detector, and used with an IonPack® AS17 column.

SSU rRNA gene sequence determination and phylogenetic analysis

Two ml of strain b6^T culture were centrifuged (14,000 rpm, 30 min) and genomic DNA was extracted from the cell pellet by bead beating using the Bio101 FastPrep Instrument and the FastPrep MH Kit (Bio 101, Vista, Ca). The SSU rRNA gene was amplified by PCR using the eubacterial-specific primers 27F (5′-GAGTTTGATCCTGGCT CAG-3′) and 1492R (5′-TACGGTTACCTTGTT ACGAC-3′) and the following reaction conditions: an initial denaturation of 1 min at 95 °C, 30 cycles of 30 s at 95 °C, 30 s at 55 °C, 2 min at 72 °C, and a final extension of 5 min at 72 °C. PCR products were purified with the Geneclean Turbo kit (Qbiogen, Illkirch, France) and ligated into pGEMT-easy plasmids (Promega, Charbonnière, France). The clone library was screened by direct PCR amplification from a colony using the vector specific primers T7 (5′-TAATACGAC TCACTA-TAGGG-3′) and SP6 (5′-ATTTAGGT GACACT ATAGAA-3′). A plasmid containing an insert of the correct length was purified with the Wizard Plus SV Minipreps DNA Purification System kit (Promega). Sequencing was performed by Genome Express (Grenoble, France) with primers T7 and SP6.

The SSU rRNA gene sequence of strain b6^T was aligned with reference sequences obtained from the Ribosomal Database Project II (Maidak et al. 2001) and from GenBank database (Benson et al. 1999), using the sequence alignment editor BioEdit (Hall 1999). Positions of sequence and alignment uncertainties were omitted from the analyses. Pairwise evolutionary distances of 1279 unambiguous nucleotides of the SSU rRNA gene were computed by the Jukes and Cantor (1969) method. A phylogenetic tree was constructed by the neighbor joining method (Saito and Nei 1987). Confidence in the tree topology was determined by bootstrap analysis using 100 resamplings of the sequences (Felsenstein 1985). All phylogenetic programs were implemented in the software package Treecon 1.3b (Van de Peer and De Wachter 1994).

DNA–DNA hybridization and G + C content

A fermentor with a working volume of 15 l (SGI, set 20 CM) was used to produce strain b6^T biomass in MCSM supplemented with 0.2 g l^{-1} yeast extract. Approximately 3 g dry weight of biomass was sent to the DSMZ (Deutsche Sammlumg von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) for DNA–DNA hybridization and G + C content determination. Genomic DNA was isolated by chromatography on hydroxyapatite as described by Cashion et al. (1977). DNA–DNA hybridization was carried out as described by De Ley et al. (1970), with the modifications described by Huss et al. (1983) and Escara and Hutton (1980), using a spectrophotometer. Renaturation rates were computed with the TRANSFER.BAS program of Jahnke (1992). G + C content was determined by enzymatic digestion of genomic DNA and HPLC as described by Mesbah et al. (1989). Calibration was performed with non-methylated Lambda-DNA (Sigma), GC-content 49.9 mol %.

Results

Isolation and morphology of strain b6^T

Strain b6^T was isolated from a mixed bacterial population (Battaglia-Brunet et al. 2002), originating from the disused Cheni gold mine site (Limousin, France), where 600,000 tonnes of a

fine arsenic-containing silt were dumped. The population was able to grow autotrophically using As(III) as the only energy source. Colonies of strain b6^T on lactate-containing solid medium were disc shaped, 0.5 mm in diameter, smooth, beige and translucent. Cells were straight small rods, 1–2 μm long (Figure 1), motile or non-motile depending on the growth phase and growth conditions. Gram-staining was negative. Spores were not observed.

Phylogenetic analysis

Phylogenetic analyses of the almost complete sequence (1279 base pairs) of the SSU rRNA gene of strain b6^T affiliated the new isolate to the genus *Thiomonas* in the β-subclass of the *Proteobacteria* (Figure 2). Strain b6^T was most closely related (98.3% identity) to the unnamed isolate NO115 from a Norwegian mining site. The SSU rRNA genes of the closest related described species, *Tm. intermedia*, *Tm. perometabolis*, and *Tm. thermosulfata* showed less than 95% sequence identity with that of b6^T. The phylogenetic tree shows the position of the main other known As(III)-oxidizing *Proteobacteria.*

Genomic DNA characteristics

The genomic DNA G + C content of strain b6^T was 65.1 mol % as determined by HPLC. DNA–DNA hybridization (in 2 × SSC + 5% [v/v] formamide at 70 °C) of strain b6^T against *Tm. intermedia* ATCC 15466^T gave 48.8% homology.

Growth properties and substrate utilization

The temperature range for optimum growth of strain b6^T was 20–30 °C. Growth was weak at 4 °C and not observed at 45 °C. The pH range for growth was 2.5–8.0, with optimal growth occurring between pH 4.0–7.5 (Figure 3). Growth was not observed at an initial pH of 1.9 or 9.0.

Strain b6^T was able to use elemental sulphur, thiosulphate, tetrathionate and a wide range of organic compounds as electron donors (Table 1). In addition, API tests were recorded as positive for the following carbon sources: phosphoramidothioic acid, hydroxykinoline-β-glucuronide, 4-aminobutyrate, 2-ketogluconate, tyrosine, histamine, gentisate and esculin. Strain b6^T did not grow anaerobically in presence of nitrate. In presence of oxygen, strain b6^T oxidized about 20% of ammonium to nitrite but no nitrate was produced.

Oxidation of As(III)

Strain b6^T was able to grow in MCSM, containing 100 mg l^{-1} As(III), by oxidizing As(III) (Figure 4). The maximal growth rate, obtained in this medium with an initial pH of 5.5 (Figure 3), was 0.147 (±0.031) h^{-1}. The corresponding growth yield and arsenite oxidation rates were 3.4 × 10^{11}

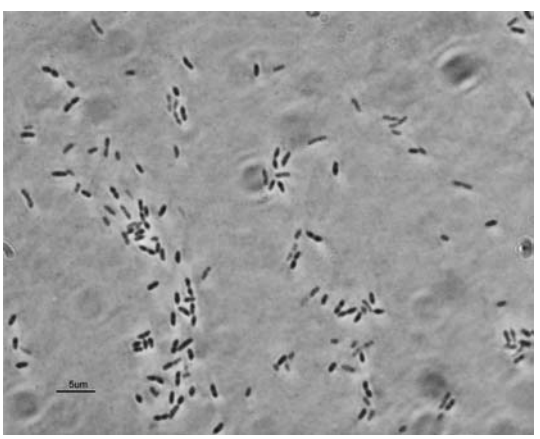

Figure 1. Phase-contrast photomicrograph of strain b6^T grown in MCSM with 0.2 g l^{-1} yeast extract. Bar, 5 μm.

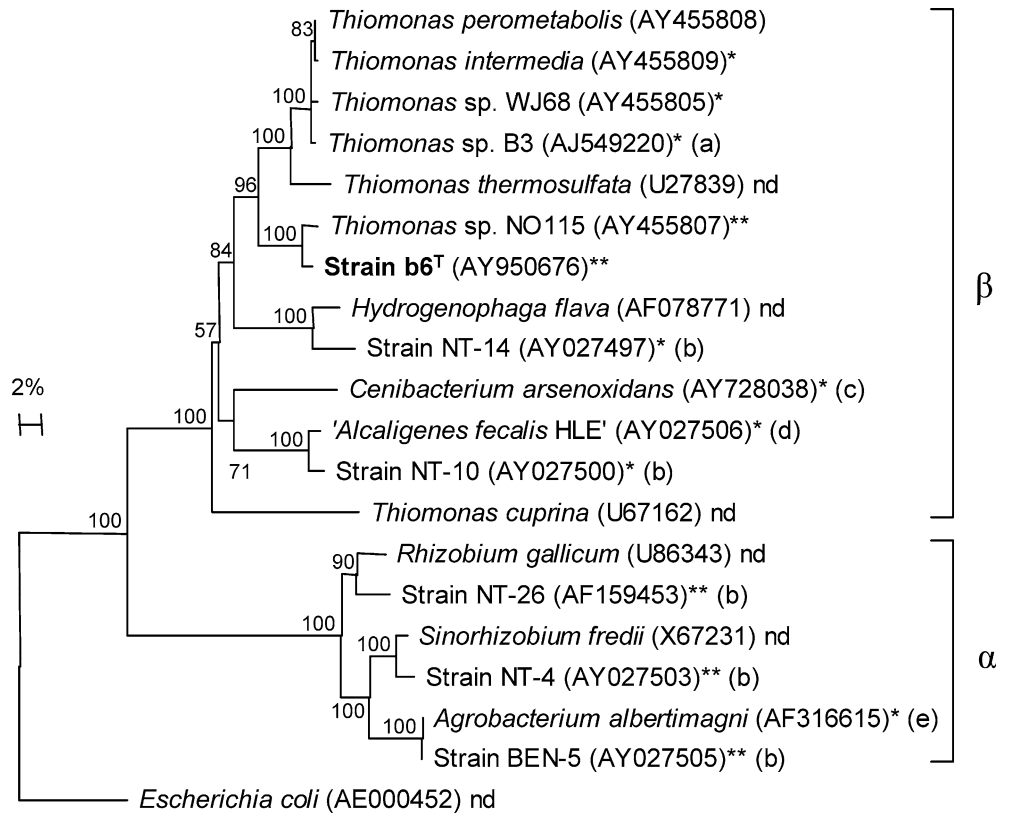

Figure 2. SSU rRNA gene distance tree showing the phylogenetic position of strain b6^T in the *Thiomonas* genus and the other main As(III)-oxidizing micro-organisms within the β and α subclass of the *Proteobacteria*. Symbols: (*): As(III)-oxidizing bacteria; (**): bacteria able to grow chemoautotrophically during As(III) oxidation. (nd): As(III) oxidation not determined. Bar: 2 nucleotide substitution per 100 nucleotides. [a] Bruneel et al. (2004); [b] Santini et al. (2002); [c] Weeger et al. (1999); [d] Anderson et al. (2003); [e]Salmassi et al. (2002).

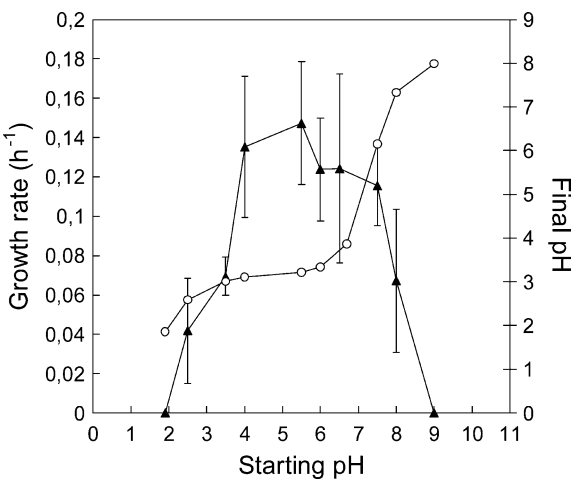

Figure 3. Influence of pH on b6^T growth rate. (▲), growth rate; (○) final pH. Error bars represent the standard error of the mean (triplicates).

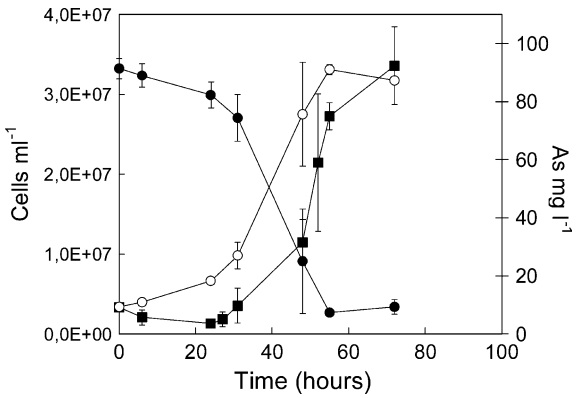

Figure 4. Growth of strain b6^T and oxidation of As(III) in MCSM and shake flasks. (■), cells ml^{-1}; (○), As(V); (●) As(III). Error bars represent the standard error of the mean (triplicates).

($\pm 3 \times 10^{10}$) cells per g of As(III) and 4 (± 0.44) mg [As(III)] l^{-1} h^{-1}, respectively. In order to verify that the strain did not use traces of organic carbon present in the demineralized water or on the vessel, four subcultures were performed in MSCM prepared with HPLC-grade water and using oven-treated tubes. Growth was identical in basal media prepared with HPLC-grade or demineralized water (Figure 5). No growth was obtained in the absence of As(III). Final cell concentrations were higher in the presence of yeast extract, however growth in chemolithoautotrophic conditions was sustained in successive sub-cultures. Final analyses showed that As(III) had been entirely oxidized to As(V) in all As-containing media.

The ability of other *Thiomonas* strains to oxidize As(III) was tested. Table 2 shows that several strains were able to grow in the presence of As(III), including the type species *Tm. intermedia* ATCC 15466^T, and were able to oxidize As(III). The growth of *Tm. perometabolis* was inhibited by As(III). Only strains b6^T and NO115 were capable of sustained growth in MCSM, with As(III) as the sole energy source. *Tm. intermedia* and WJ68 required yeast extract for growth while oxidizing As(III).

Oxidation of Fe(II)

Strain b6^T was able to oxidize ferrous iron when grown in shake flasks, as indicated by the disappearance of ferrous iron over 6 days and the accumulation of ferric iron. During this same time frame, there was a concomitant increase in b6^T cell numbers (data not shown). There was no oxidation of iron in the abiotic controls over the 6 days period.

Discussion

Strain b6^T was isolated from an arsenic-contaminated mining site (Battaglia-Brunet et al. 2002), selected for its ability to oxidize As(III). Phylogenetic analysis of SSU rRNA genes clearly showed its affiliation to the genus *Thiomonas* within the β-subclass of the *Proteobacteria*. Strain b6^T and the unnamed As(III)-oxidizing isolate NO115 are a group of related bacteria that share SSU rRNA gene identities of more than 98%. Sequence identities between strain b6^T and described *Thiomonas* species, including *Tm. intermedia* ATCC 15466^T (type species of the genus), *Tm. perometabolis* and *Tm. thermosulfata* were less than 95%; unfortunately *Tm. thermosulfata* is no longer available from

Table 2. Capacity of strain b6^T and other *Thiomonas* strains to oxidize As(III) at a starting concentration of 100 mg l^{-1} .

Strains	Medium	Growth	As(III)-oxidation
Strain b6^T	MCSM	+	+
NO 115	MCSM	+	+
WJ 68	MCSM	–	–
WJ 68	MCSM + 0.2 g l^{-1} YE + 1 mM thiosulphate	+	+
Tm. intermedia ATCC 15466^T	MCSM	–	–
Tm. intermedia ATCC 15466^T	MCSM + 0.2 g l^{-1} YE + 1 mM thiosulphate	+	+
Tm. perometabolis CIP 104403	MCSM	–	–
Tm. perometabolis CIP 104403	MCSM + 0.2 g l^{-1} YE + 1 mM thiosulphate	–	–
Tm. perometabolis CIP 104403	Medium 126 + 100 mg l^{-1} As(III)	–	–

YE = yeast extract.

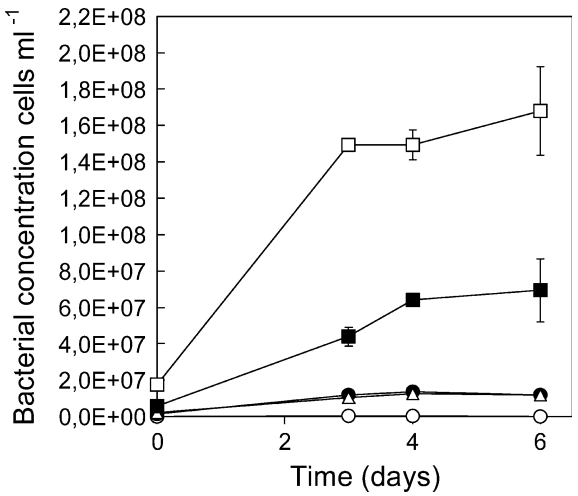

Figure 5. Growth of the fourth sub-culture of strain b6^T in static 10 ml tubes. (○), MCSM without As(III) made with HPLC-grade water; (●), MCSM made with HPLC-grade water; (▲), MCSM; (■), MCSM with 0.02 g l^{-1} yeast extract; (□), MCSM with 0.2 g l^{-1} yeast extract. Error bars represent the standard error of the mean (triplicates).

any culture collection so we were unable to assess whether this microbe could also oxidize As(III) or ferrous iron. The DNA–DNA homology of strain b6^T against *Tm. intermedia* was less than 50%, in accordance with the phylogenetic distance between strain b6^T and the group of described species including *Tm. intermedia*. Based upon this level of DNA–DNA homology, strain b6^T belongs to a distinct species as defined by Wayne et al. (1987).

Strain b6^T shares with other *Thiomonas* isolates important phenotypic features, such as lithoautotrophic growth on reduced sulphur compounds, mixotrophic growth, optimum pH range, and DNA G+C content (Moreira and Amils 1997). As an original feature, strain b6^T can grow heterotrophically with numerous simple organic substrates, while most *Thiomonas* species need complex substrates (Moreira and Amils 1997), or use a limited range of simple compounds (Katayama-Fujimura and Kuraishi 1983; Huber and Stetter 1990; Shooner et al. 1996). Its closest described relative, *Tm. thermosulfata*, was able to grow on sulphur, thiosulphate, yeast extract, glutamate and succinate, but not on glucose, aspartate, pyruvate, raffinose, sucrose and sorbitol (Shooner et al. 1996). According to their original descriptions, *Tm. intermedia* and *Tm. perometabolis* were not able to use simple organic substrates for growth (London 1963; London and Rittenberg 1967). However, Katayama-Fujirama and Kuraishi (1983) found that *Tm. perometabolis* could grow slowly on alaline, malate, citrate, aspartate, glutamate and succinate after a long lag phase.

We showed here that oxidation of As(III) is a common phenotypic feature of several *Thiomonas* strains isolated from diverse environments, such as As(III)-contaminated mining sites (strains b6^T, NO115 and WJ68) and soil (*Tm. intermedia*). Furthermore, oxidation of As(III) by *Thiomonas* strains isolated from a heavily As-polluted mine drainage was reported by Bruneel et al. (2004) and Duquesne (2004). One original feature that distinguish strain b6^T and its closest phylogenetic relative strain NO115 from other As(III)-oxidizing *Thiomonas* isolates is that these two organisms can grow chemolithoautotrophically using As(III) as the energy source. To date, chemolithoautotrophy in the genus *Thiomonas* was exclusively reported with sulphur compounds. Several strains also isolated from mining sites, namely NT-26, BEN-5 and NT-4, have been reported to grow autotrophically with As(III) as the sole energy source (Santini et al. 2002). However, they all belong to the α-subclass of *Proteobacteria* and are thus phylogenetically distant from the genus *Thiomonas*. Except from the *Thiomonas*-related isolates b6^T and NO115, only heterotrophic As(III) oxidation has been reported to date amongst members of the *β-Proteobacteria*.

In mining environments, sulphur, iron and arsenic are commonly associated with minerals. Recent theories suggest an ancient bioenergetic origin for arsenite oxidase (Lebrun et al. 2003). Microorganisms are able to grow with As(III) when the concentrations of reduced sulphur and iron compounds become limiting (Jackson et al. 2001). The dilution of acidic mine waters in neutral streams often creates transition zones slightly acidic to neutral, where the energetic use of As(III) may be favoured as this chemical species can remain soluble whereas most of the iron is removed from the water phase. The large pH range for growth of strain b6^T and other *Thiomonas* isolates are characteristics of these transition zones.

Conclusions

Oxidation of As(III) is a common phenotypic feature of *Thiomonas* strains, including the type

species *Tm. intermedia* ATCC 15466^T. To date, strain b6^T is the first characterized β-proteobacterium able to grow autotrophically during oxidation of As(III). On the basis of phenotypic and phylogenetic distinctiveness, we propose strain b6^T as a novel species of the genus *Thiomonas, Thiomonas arsenivorans* sp. nov.

Description of *Thiomonas arsenivorans* sp. nov.

Thiomonas arsenivorans (N. L. adj. ar.se.ni'vo.rans. element arsenic from Gr.n. *arsén*, male; L. n. *vorans*, masc. substantive from L. part. adj. *vorans*, devouring, organism that uses arsenic as a growth substrate).

Cells are rod shaped, approximately 0.3–0.5 μm wide and 1 μm long, gram-negative, non-spore-forming and motile. The optimum temperature for growth is between 20 and 30 °C. The optimum pH for growth is between 4.0 and 7.5. Facultative chemolithoautotroph, capable of aerobic growth with As(III), sulphur, thiosulphate, tetrathionate. Organotrophic growth occurs aerobically on yeast extract, glucose, sucrose, succinate, aspartate, glutamate, pyruvate, raffinose, sorbitol, tyrosine, 2-ketogluconate, phosphoramidothioic acid, hydroxykinoline-β-glucuronide, 4-aminobutyrate, histamine, gentisate and esculin. Capable of ferrous iron oxidation. Denitrification was not observed. G + C content of the DNA is 65.1%. The type strain is strain b6^T (DSM 16361^T, LMG 22795^T). The 16S rRNA gene sequence of b6^T has been deposited in GenBank under the accession number AY950676.

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