***Desulforegula conservatrix* gen. nov., sp. nov., a long-chain fatty acid-oxidizing, sulfate-reducing bacterium isolated from sediments of a freshwater lake**

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A novel sulfate-reducing bacterium, strain Mb1Pa^T, was isolated from the sediments of a freshwater floodplain lake. Cells of strain Mb1Pa^T were rod-shaped, 1–1.3 µm wide and 2.6–3 µm long, motile and Gram-negative. The bacterium grew on straight-chain carboxylic acids with 4–17 carbon atoms. Electron donors with an even number of carbon atoms were oxidized to acetate and electron donors with an odd number of carbon atoms were oxidized to acetate and propionate. No other compounds were found to be used as electron donors. No growth occurred in the absence of sulfate. The optimum temperature for growth was between 25 and 30 °C and the maximum temperature for growth was 32 °C. Strain Mb1Pa^T grew very slowly in medium with 5 g NaCl l⁻¹ with optimum growth occurring with up to 1.0 g NaCl l⁻¹. Analysis of the 16S rRNA gene showed that strain Mb1Pa^T belonged to the δ-subclass of the *Proteobacteria*, was a member of the family *Desulfobacteraceae*, but lacked similarity with any currently described representatives. The combined phylogenetic analysis and physiological data indicate that strain Mb1Pa^T represents a new genus and the name *Desulforegula conservatrix* is proposed. The type strain is Mb1Pa^T (= DSM 13527^T = ATCC BAA-134^T).

Keywords: *Desulforegula conservatrix*, sulfate-reducing bacteria, long-chain fatty acids, sediment

INTRODUCTION

Widdel (1980) carried out a major study on sulfate-reducing bacteria (SRB) and described a diverse range of SRB that could use a variety of fatty acids as carbon and energy sources. In particular, enrichment cultures containing hexadecanoate yielded several previously undescribed strains of SRB which were described as *Desulfovibrio sapovorans*. More recently, phylogenetic studies transferred the latter organisms to the genus '*Desulfobotulus*' (Widdel & Bak, 1992). Further studies have since been carried out that have attempted to extend our knowledge of compounds that can be oxidized by SRB. Such studies have demonstrated that

compounds as diverse as alkanes (Aeckersberg *et al.*, 1991; Reuter *et al.*, 1994), amino acids (Rees *et al.*, 1998), and variously substituted aromatic compounds (Drzyzga *et al.*, 1993; Rabus *et al.*, 1993; Schnell & Schink, 1992) can be oxidized by SRB. Despite the early interest into SRB that oxidize long-chain fatty acids (Widdel, 1980), the more recent studies involving SRB able to use long-chain fatty acids have been limited to carbon and electron donor tests performed on isolates obtained by enrichment with alternative substrates. As yet unidentified strains known to be able to degrade long-chain fatty acids have been isolated from marine or estuarine sediments (Widdel & Bak, 1992), but freshwater environments have been less extensively examined. In this study, we examined sediments from a freshwater wetland lake for SRB able to oxidize hexadecanoate. Rather than isolating '*Desulfobotulus sapovorans*', enrichment led to the isolation of a previously undescribed organism. In this

Abbreviation: SRB, sulfate-reducing bacteria.

The GenBank accession number for the 16S rRNA sequence of strain Mb1Pa^T is AF243334.

paper, we describe the isolation and characterization of strain Mb1Pa^T.

METHODS

Isolation and culture conditions. Strain Mb1Pa^T was isolated by enrichment from the sediment of a wetland lake that is on the floodplain of the River Murray, Albury, Australia. Primary enrichment cultures contained approximately 1 g sediment in 10 ml medium that had been supplemented with 1 mM sodium palmitate. Palmitate continued to be supplied as the electron donor throughout the enrichment stages but was replaced with butyrate for final purification by agar shake dilution tubes. Dilute freshwater medium (Janssen *et al.*, 1997) preparation and anaerobic culture techniques were used throughout this study, with all media prepared as described previously (Rees *et al.*, 1995). Substrates and electron acceptors were added as required from sterile stock solutions. All incubations were carried out at 25 °C. When necessary, *Desulfovibrio desulfuricans* DSM 642 was used as a control for growth experiments.

Cellular and metabolic characterization. Desulfovibrin was determined by the fluorescence test (Postgate, 1959) but with the fluorescence detection carried out with a fluorescence spectrophotometer. The pellet from 30 ml culture was washed and resuspended in 2 ml phosphate buffer (50 mM, pH 7). Following addition of 2 drops 2 M NaOH, the suspension underwent UV excitation in a Hitachi F4500 spectrofluorimeter and an emission spectrum was obtained from 500 to 800 nm. The presence of desulfovibrin was demonstrated by a single large peak with an emission maximum at 712 nm. The lipid nature of cell inclusions was demonstrated by staining with Nile blue (Ostle & Holt, 1982). Organic acids were determined by GC as described previously (Rees *et al.*, 1997). Dry mass measurements were carried out as described previously (Rees *et al.*, 1994).

DNA base composition and phylogenetic analysis. For phylogenetic studies, genomic DNA was purified from strain Mb1Pa^T by the methods described by Ausubel *et al.* (1997). The universal primers Fd1 and Rd1 were used to obtain a PCR product of approximately 1.5 kb corresponding to base positions 1–1542 based on *Escherichia coli* numbering of the 16S rRNA gene (Winker & Woese, 1991) using procedures described previously (Redburn & Patel, 1993; Andrews & Patel, 1996). PCR products were sequenced using the ABI PRISM Dye Terminator Cycle Sequencing Kit containing AmpliTaq FS DNA polymerase and using an ABI 373A sequencer. A 10 µl reaction contained 35 ng PCR product, 4 µl cycle sequencing reaction mix, 3.2 pmol primer (Andrews & Patel, 1996) and 2.5 µg BSA. Thermal cycling was carried out using a Rapid Cyclor (Idaho Technology) at a temperature transition slope of 2, an initial denaturation of 94 °C for 15 s, then 25 cycles of denaturation at 94 °C for 0 s, annealing at 50 °C for 10 s, and extension at 60 °C for 3 min.

The new sequence data that were generated were aligned, and an almost full-length consensus 16S rRNA gene sequence was assembled and manually checked for accuracy using the alignment editor ae2 (Maidak *et al.*, 2000). These sequences were compared with others in the GenBank database (Benson *et al.*, 2000) using BLAST (Altschul *et al.*, 1997), to determine if any newly released closely related sequences existed in the database. For analysis, the sequences of *Desulfofrigus oceanense*, *Desulfofrigus fragile*, *Desulfobacterium phenolicum*, strain R-

Capr A1, *Desulfobacterium indolicum*, *Desulfonema limicola*, *Desulfonema magnum*, *Desulfonema ishimotonii*, *Desulfobacterium cetonicum*, *Desulfobacterium anilini*, '*Desulfobotulus sapovorans*', *Desulfococcus multivorans* and *Desulfosarcina variabilis* were extracted from GenBank and manually aligned with the prealigned sequences obtained from the Ribosomal Database Project (Maidak *et al.*, 2000). Pairwise evolutionary distances based on 733 unambiguous nucleotides were computed using DNADIST (Jukes & Cantor, 1969) and neighbour-joining programs that form part of the PHYLIP suite of programs (Felsenstein, 1993).

For DNA base compositions studies, a total of four different methods to isolate DNA were carried out independently in three different laboratories. The methods used were those of Marmur (1961) and Pitcher *et al.* (1989), and also small-scale preparation involving hexadecyl trimethylammonium bromide precipitation (Woo *et al.*, 1997) and extraction with guanidine.HCl (Janssen & O'Farrell, 1999). In addition to following procedures outlined in the original methods, additional modifications were made to try and obtain suitable DNA preparations. Significant modifications included introducing chloroform during early stages of the protocols to eliminate lipid storage globules interfering with DNA isolation, and using protease treatments early in the procedure to destroy nuclease activity.

RESULTS AND DISCUSSION

Morphological and physiological characteristics

Primary enrichment cultures with palmitate and lake sediment appeared to be totally dominated by rod-shaped cells. A pure culture was subsequently obtained and designated strain Mb1Pa^T. Cells of strain Mb1Pa^T were rod-shaped, 1–1.3 µm wide and 2.6–3 µm long (Fig. 1). Motile cells were observed in early growth phases but at less frequency in older cultures. Cells

Fig. 1. Phase-contrast photomicrograph of *Desulfobacterium conservatrix* strain Mb1Pa^T. Bar, 10 µm.

Fig. 2. Phylogenetic tree showing the position of *Desulforegula conservatrix* strain Mb1Pa^T compared to some of the closest members of the δ -subclass of the *Proteobacteria*. Scale bar equals substitutions per 100 nucleotides.

stained Gram-negative and spores were not observed in any cultures. Cellular inclusions could be stained with Nile blue, indicating that the inclusion bodies were poly-3-hydroxybutyrate.

When growing under optimal conditions with 10 mM butyrate, subcultures (1%, v/v, inoculum) from an early stationary phase culture usually reached a maximum cell yield within 3–4 d. However, when cultures were stored for more than 4 weeks, subsequent subcultures had lag periods that were often greater than 1 week. The lag period could be reduced by including dithionite in the medium to give 100 μ M final concentration.

Although strain Mb1Pa^T was isolated with a dilute salts medium, it grew equally well in standard freshwater medium containing 1 g NaCl l⁻¹ (Widdel & Bak, 1992). Growth occurred in medium with 5 g NaCl l⁻¹ but cultures required incubation for 2 weeks before measurable growth occurred. Strain Mb1Pa^T grew with an incubation temperature up to 32 °C, with optimum growth occurring at between 25 and 30 °C. A vitamin supplement was required for growth. Yeast extract could not replace the vitamin supplement.

The compounds that were used as electron donors by strain Mb1Pa^T were (mM): butyrate (5), valerate (5), hexanoate (5), octanoate (1), decanoate (0.25), dodecanoate (0.25), hexadecanoate (1) and heptadecanoate (1). Compounds not used by strain Mb1Pa^T were: lactate (10), pyruvate (10), malate (5), succinate (5), fumarate (5), ethanol (10), formate (5), formate (5) plus acetate (1), acetate (10), propionate (5), octadecanoate (1), glucose (5), benzoate (5) and citrate (5). Growth on decanoate occurred only when the concentration in the medium was no greater than 0.25 mM; growth was confirmed following additions of very small amounts of decanoate to cultures over

time. The presence of 1.0 mM decanoate was sufficient to completely inhibit growth on butyrate.

Stoichiometric measurements demonstrated that the oxidation of 7.99 mmol butyrate l⁻¹ yielded 14.17 mmol acetate l⁻¹ and 47 mg dry mass of cells l⁻¹. These data represent a carbon recovery of 94.6%. When strain Mb1Pa^T oxidized valerate, acetate and propionate were produced in equimolar amounts. The oxidation of heptadecanoic acid resulted in an acetate to propionate ratio of 7. These stoichiometry studies are consistent with the incomplete oxidation of fatty acids as described by Widdel (1980). Strain Mb1Pa^T was unable to ferment pyruvate, fumarate or malate. Sulfite, thiosulfate or nitrate were not used as terminal electron acceptors.

All attempts to isolate DNA for base composition studies failed. Cells were easily lysed; however, all subsequent procedures to isolate sufficient quantities of DNA, whether from original protocols or modification methods, failed. Cells contained desulfoviridin.

Phylogenetic analysis and taxonomic affiliation

Phylogenetic analysis showed that strain Mb1Pa^T was a member of the δ -subclass of the *Proteobacteria* and formed a new branch within the proposed family *Desulfobacteraceae*. The 16S rRNA gene sequence of Mb1Pa^T was not highly similar to any sequence in public databases. Instead, there were four strains with similarity between 89.4 and 89.9%. The most closely related organisms in the molecular database were strain R-Capr A1 (89.9% similarity), *Desulfo faba gelida* (89.9% similarity), *Desulfo bacterium indolicum* (89.6% similarity) and *Desulfo nema limicola* (89.4% similarity) (Fig. 2).

Table 1. Summary of morphological and physiological characteristics of strain Mb1Pa^T and related SRB

(+), Weak growth; NR, not reported.

	<i>Desulforegula conservatrix</i> strain Mb1Pa ^T	<i>Desulfofaba gelida</i> strain PSv29*	<i>Desulfofacterium indolicum</i> strain In04†	<i>Desulfonema limicola</i> strain 5ac10‡	' <i>Desulfofobotulus sapovorans</i> ' strain 1pa3§
Morphology	Rod shaped	Slightly curved rod	Rod-shaped	Filamentous	Vibrio
Cell size (µm):					
Width	1–1.3	3.1	0.7–1.5	2.5–3	1.5
Length	2.6–3	3.2–6.2	2–2.5	50–1000 µm (2.5–3 one cell)	3–3.5
Motility	+	+	+	+	+
Oxidation of substrate	Incomplete	Incomplete	Complete	Complete	Incomplete
Electron donors used:					
H ₂	–	–	–	+	–
Formate	–	+	(+)	+	–
Acetate	–	–	(+)	(+)	–
Fatty acids (C atoms)	4–17	3–4	(3)	3–14	4–16
Lactate	–	+	–	+	+
Fumarate	–	+	(+)	+	–
Ethanol	–	+	(+)	–	–
Ferment pyruvate	–	+	NR	–	+
Electron acceptors used:					
Sulfite	–	+	–	+	+
Thiosulfate	–	+	+	+	–
Sulfur	–	–	–	–	–
Desulfoviridin	+	–	–	+	–

* Knoblauch *et al.* (1999).

† Bak & Widdel (1986).

‡ Widdel *et al.* (1983).

§ Widdel & Bak (1992).

Strain Mb1Pa^T oxidized long-chain fatty acids, a trait which is in common with isolates obtained from a variety of sediment types. For example, '*Desulfofobotulus sapovorans*' was isolated from mud of a ditch in a dairy pasture (Widdel, 1980). In addition to '*Desulfofobotulus sapovorans*', Widdel (1980) also described two further strains, 2pa3 and 3pa8, that also were able to oxidize straight-chain fatty acids with 4–18 carbon atoms. Also, an uncharacterized oval-shaped organism that was able to oxidize long-chain fatty acids was referred to by Widdel (1988). Strain Mb1Pa^T and '*Desulfofobotulus sapovorans*' carry out incomplete oxidation of a relatively narrow range of long-chain fatty acids. However, significant phenotypic and phylogenetic differences exist between these organisms (Table 1). The organism with closest sequence similarity to strain Mb1Pa^T is *Desulfofaba gelida* (89.9% similarity); however, this organism has physiological characteristics that are significantly different from those of Mb1Pa^T (Knoblauch *et al.*, 1999) (Table 1). *Desulfofaba gelida* incompletely oxidizes its substrates; however, it is a psychrophilic sulfate reducer that can oxidize alcohols and dicarboxylic acids and it cannot use straight-chain

carboxylic acids with more than 4 carbon atoms. Strain R-Capr A1 has sequence similarity with strain Mb1Pa^T of 89.9% (Wind *et al.*, 1999). This organism has a limited substrate range and incompletely oxidizes hexanoate and alcohols, but as yet has not been tested for growth on long-chain fatty acids. *Desulfofacterium indolicum* (89.6% similarity) differs from Mb1Pa^T by its ability to completely oxidize acetate and propionate and also being unable to use long-chain fatty acids (Bak & Widdel, 1986). Strains of *Desulfofacterium indolicum* have been isolated from brackish and marine environments whereas strain Mb1Pa^T was obtained from freshwater sediment. *Desulfonema* spp. are conspicuous filamentous SRB that exhibit gliding motility (Widdel *et al.*, 1983). Sequence comparison showed a relatively distant relationship to *Desulfonema limicola*. Similarly, significant morphological and physiological differences were observed between these two organisms (Table 1). Given the distance from the nearest relatives, combined with the physiological differences with any related organisms, we propose that strain Mb1Pa^T represents a new species of a new genus. We propose the name *Desulforegula conservatrix* gen. nov., sp. nov.

Description of *Desulforegula* gen. nov.

Desulforegula (De.sul.fo.re'gu.la. L. pref. *de* from; L. n. *sulfur* sulfur; L. n. fem *regula* a straight piece of wood or ruler; N.L. fem. n. *Desulforegula* a sulfate-reducing bacterium shaped like a ruler).

Cells are Gram-negative, straight rods and can be motile. Strict anaerobes that use sulfate as a terminal electron acceptor. *Desulforegula* belongs to the δ -subclass of the *Proteobacteria*. The nearest relatives are *Desulfofaba*, *Desulfobacterium* and *Desulfonema*. The type species is *Desulforegula conservatrix*.

Description of *Desulforegula conservatrix* sp. nov.

Desulforegula conservatrix (con.ser.va'trix. L. fem. n. *conservatrix* she who preserves, describing the storage of lipid cell inclusions).

Cells are rod-shaped, 1–1.3 μ m wide and 2.6–3 μ m long. The maximum temperature for growth is 32 °C, with optimum growth occurring between 25 and 30 °C. Optimum growth occurs with up to 1.0 g NaCl l⁻¹. Slow growth occurs in medium with 5 g NaCl l⁻¹. Oxidizes straight-chain carboxylic acids with 4–17 carbon atoms. No other substrates are known to be utilized. Substrates are incompletely oxidized. No growth occurs in the absence of sulfate. Habitat: sediment of freshwater lake. The type strain is Mb1Pa^T (= DSM 13527^T = ATCC BAA-134^T).

ACKNOWLEDGEMENTS

This work was supported by the University of Canberra (grant 98/474) and the Cooperative Research Centre for Freshwater Ecology (Project B400). We thank Professor H. G. Trüper for assistance with the naming of the organism.

REFERENCES

- Aeckersberg, F., Bak, F. & Widdel, F. (1991). Anaerobic oxidation of saturated hydrocarbons to CO₂ by a new type of sulfate-reducing bacterium. *Arch Microbiol* **156**, 5–14.
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1997). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.
- Andrews, K. T. & Patel, B. K. C. (1996). *Fervidobacterium gondwanense* sp. nov., a new thermophilic anaerobic bacterium isolated from nonvolcanically heated geothermal waters of the Great Artesian Basin of Australia. *Int J Syst Bacteriol* **46**, 265–269.
- Ausubel, F. M., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A. & Struhl, K. (1997). *Short Protocols in Molecular Biology*, 3rd edn. New York: Wiley.
- Bak, F. & Widdel, F. (1986). Anaerobic degradation of indolic compounds by sulfate-reducing enrichment cultures, and description of *Desulfobacterium indolicum* gen. nov., sp. nov. *Arch Microbiol* **146**, 170–176.
- Benson, D. A., Karsch-Mizrachi, I., Lipman, D. J., Ostell, J., Rapp, B. A. & Wheeler, D. L. (2000). GenBank. *Nucleic Acids Res* **28**, 15–18.
- Drzyzga, O., Küver, J. & Blotvogel, K. H. (1993). Complete oxidation of benzoate and 4-hydroxybenzoate by a new sulfate-

- reducing bacterium resembling *Desulfoarculus*. *Arch Microbiol* **159**, 109–113.
- Felsenstein, J. (1993). PHYLIP (Phylogenetic Inference Package) version 3.51c. Distributed by the author. Department of Genetics, University of Washington, Seattle, USA.
- Janssen, P. H. & O'Farrell, K. A. (1999). *Succinispira mobilis* gen. nov., sp. nov., a succinate-decarboxylating anaerobic bacterium. *Int J Syst Bacteriol* **49**, 1009–1013.
- Janssen, P. H., Schuhmann, A., Mörschel, E. & Rainey, F. A. (1997). Novel anaerobic ultramicrobacteria belonging to the *Verrucomicrobiales* lineage of bacterial descent isolated by dilution culture from anoxic rice paddy soil. *Appl Environ Microbiol* **63**, 1382–1388.
- Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.
- Knoblauch, C., Sahm, K. & Jørgensen, B. B. (1999). Psychrophilic sulfate-reducing bacteria isolated from permanently cold Arctic marine sediments: description of *Desulfofrigus oceanense* gen. nov., sp. nov., *Desulfofrigus fragile* sp. nov., *Desulfofaba gelida* gen. nov., sp. nov., *Desulfotalea psychrophila* gen. nov., sp. nov., and *Desulfotalea arctica* sp. nov. *Int J Syst Bacteriol* **49**, 1631–1643.
- Maidak, B. L., Cole, J. R., Lilburn, T. G. & 9 other authors (2000). The RDP (Ribosomal Database Project) continues. *Nucleic Acids Res* **28**, 173–174.
- Marmur, M. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 201–218.
- Ostle, A. G. & Holt, J. G. (1982). Nile blue A as a fluorescent stain for poly- β -hydroxybutyrate. *Appl Environ Microbiol* **44**, 238–241.
- Pitcher, D. G., Saunders, N. A. & Owen, R. J. (1989). Rapid extraction of bacterial genomic DNA with guanidium isothiocyanate. *Lett Appl Microbiol* **8**, 151–156.
- Postgate, J. R. (1959). A diagnostic reaction of *Desulphovibrio desulphuricans*. *Nature* **183**, 481–482.
- Rabus, R., Nordhaus, R., Ludwig, W. & Widdel, F. (1993). Complete oxidation of toluene under strictly anoxic conditions by a new sulfate-reducing bacterium. *Appl Environ Microbiol* **59**, 1444–1451.
- Redburn, A. C. & Patel, B. K. C. (1993). Phylogenetic analysis of *Desulfotomaculum thermobenzoicum* using polymerase chain reaction-amplified 16S rRNA-specific DNA. *FEMS Microbiol Lett* **113**, 81–86.
- Rees, G. N., Rainey, F. A. & Harfoot, C. G. (1994). Characterization of a novel obligate anaerobe that ferments agar. *Arch Microbiol* **162**, 395–400.
- Rees, G. N., Grassia, G. S., Sheehy, A. J., Dwivedi, P. P. & Patel, B. K. C. (1995). *Desulfacinum infernum* gen. nov., sp. nov., a thermophilic sulfate-reducing bacterium from a petroleum reservoir. *Int J Syst Bacteriol* **45**, 85–89.
- Rees, G. N., Patel, B. K. C., Grassia, G. S. & Sheehy, A. J. (1997). *Anaerobaculum thermoterrenum* gen. nov., sp. nov., a novel, thermophilic bacterium which ferments citrate. *Int J Syst Bacteriol* **47**, 150–154.
- Rees, G. N., Harfoot, C. G. & Sheehy, A. J. (1998). Amino acid degradation by the mesophilic sulfate-reducing bacterium *Desulfobacterium vacuolatum*. *Arch Microbiol* **169**, 76–80.
- Reuter, P., Rabus, R., Wilkes, H., Aeckersberg, F., Rainey, F. A., Jannasch, H. W. & Widdel, F. (1994). Anaerobic oxidation of hydrocarbons in crude oil by new types of sulphate-reducing bacteria. *Nature* **372**, 455–458.

- Schnell, S. & Schink, B. (1992).** Anaerobic degradation of 3-aminobenzoate by a newly isolated sulfate reducer and a methanogenic enrichment culture. *Arch Microbiol* **158**, 328–334.
- Widdel, F. (1980).** *Anaerober Abbau von Fettsäuren und benzoessäure durch neu isolierte Arten Sulfat-reduzierender Bakterien*. PhD thesis, Göttingen University.
- Widdel, F. (1988).** Microbiology and ecology of sulfate- and sulfur-reducing bacteria. In *Biology of Anaerobic Microorganisms*, pp. 469–585. Edited by A. J. B. Zehnder. New York: Wiley.
- Widdel, F. & Bak, F. (1992).** Gram negative mesophilic sulfate-reducing bacteria. In *The Prokaryotes: a Handbook on the Biology of Bacteria – Ecophysiology, Isolation, Identification, Applications*, 2nd edn, pp. 3352–3378. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. Berlin, Heidelberg & New York: Springer.
- Widdel, F., Kohring, G. W. & Mayer, F. (1983).** Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. III. Characterization of the filamentous gliding *Desulfonema limicola* gen. nov. sp. nov., and *Desulfonema magnum* sp. nov. *Arch Microbiol* **134**, 286–294.
- Wind, T., Stubner, S. & Conrad, R. (1999).** Sulfate-reducing bacteria in rice field soil and on rice roots. *Syst Appl Microbiol* **22**, 269–279.
- Winker, S. & Woese, C. R. (1991).** A definition of the domains *Archaea*, *Bacteria* and *Eucarya* in terms of small subunit ribosomal RNA characteristics. *Syst Appl Microbiol* **13**, 161–165.
- Woo, T. H. S., Patel, B. K. C., Smythe, L. D., Symonds, M. L., Norris, M. A. & Dohnt, M. F. (1997).** Identification of pathogenic *Leptospira* by TaqMan Probe in a LightCycler™. *Anal Biochem* **256**, 132–134.