International Journal of Systematic and Evolutionary Microbiology (2003), **53**, 787–793 DOI 10.1099/ijs.0.02417-0

Description of *Sulfurospirillum halorespirans* sp. nov., an anaerobic, tetrachloroethene-respiring bacterium, and transfer of *Dehalospirillum multivorans* to the genus *Sulfurospirillum* as *Sulfurospirillum multivorans* comb. nov.

Maurice L. G. C. Luijten,[1]† Jasperien de Weert,[1] Hauke Smidt,[1] Henricus T. S. Boschker,[2] Willem M. de Vos,[1] Gosse Schraa[1] and Alfons J. M. Stams[1]

Correspondence
Maurice L. G. C. Luijten
m.l.g.c.luijten@mep.tno.nl

[1]Laboratory of Microbiology, Wageningen University, H. v. Suchtelenweg 4, 6703 CT Wageningen, The Netherlands

[2]Netherlands Institute of Ecology (NIOO-CEMO), Postbus 140, 4400 AC Yerseke, The Netherlands

An anaerobic, halorespiring bacterium (strain PCE-M2^T = DSM 13726^T = ATCC BAA-583^T) able to reduce tetrachloroethene to *cis*-dichloroethene was isolated from an anaerobic soil polluted with chlorinated aliphatic compounds. The isolate is assigned to the genus *Sulfurospirillum* as a novel species, *Sulfurospirillum halorespirans* sp. nov. Furthermore, on the basis of all available data, a related organism, *Dehalospirillum multivorans* DSM 12446^T, is reclassified to the genus *Sulfurospirillum* as *Sulfurospirillum multivorans* comb. nov.

INTRODUCTION

Chlorinated ethenes are widespread soil and groundwater pollutants. Because of industrial activities, large amounts of chlorinated ethenes were discharged into the environment over the last few decades. Tetrachloroethene (perchloroethylene; PCE) is used mainly in dry-cleaning processes and as an organic solvent (DiStefano *et al.*, 1991). It is a suspected carcinogen and is thought to be persistent under aerobic conditions (Bouwer & McCarty, 1983; Fathepure *et al.*, 1987). However, Ryoo *et al.* (2000) recently reported the aerobic conversion of PCE by toluene-*o*-xylene monooxygenase of *Pseudomonas stutzeri* OX1. Under anaerobic conditions, PCE can be reductively dechlorinated via trichloroethene, dichloroethene and vinyl chloride to the nontoxic end-products ethene (DiStefano *et al.*, 1991; Freedman & Gossett, 1989) and ethane (de Bruin *et al.*, 1992).

Over the last decade, several bacteria that are able to couple the anaerobic reductive dechlorination of PCE to growth have been isolated. This respiratory process is also known as halorespiration. PCE is reduced to either trichloroethene or dichloroethene by, for example, *Dehalospirillum multivorans*, two *Dehalobacter* species and several *Desulfitobacterium* species (Scholz-Muramatsu *et al.*, 1995; Holliger *et al.*, 1993; Wild *et al.*, 1996; Gerritse *et al.*, 1996, 1999; Miller *et al.*, 1997). One organism, '*Dehalococcoides ethenogenes*', is able to reduce PCE to vinyl chloride, and couples these steps to energy conservation. Vinyl chloride is dechlorinated further to ethene by this organism, but this final reduction step is not coupled to growth (Maymo-Gatell *et al.*, 1997, 1999). The ecological, physiological and technological aspects of halorespiring organisms have been reviewed in detail (El Fantroussi *et al.*, 1998; Holliger *et al.*, 1998; Middeldorp *et al.*, 1999).

Here, we describe the isolation of a novel organism from soil from a polluted site in The Netherlands that is able to reduce PCE to *cis*-dichloroethene. Initial analysis showed our isolate to have high similarity to members of the genus *Sulfurospirillum* and to *Dehalospirillum multivorans*. Therefore, we included data for the type strains of *Sulfurospirillum barnesii*, *Sulfurospirillum deleyianum*, *Sulfurospirillum arsenophilum*, *Sulfurospirillum arcachonense* and *Dehalospirillum multivorans*. Evaluation of all physiological and phylogenetic properties makes it clear that the new isolate, strain PCE-M2^T, is a member of the genus *Sulfurospirillum*. We propose strain PCE-M2^T as the type strain of a novel species within the genus *Sulfurospirillum*, *Sulfurospirillum halorespirans* sp. nov. Furthermore, on the basis of all available data, we propose to reclassify *Dehalospirillum multivorans* as *Sulfurospirillum multivorans* comb. nov.

Published online ahead of print on 11 October 2002 as DOI 10.1099/ijs.0.02417-0.

†Present address: TNO Environment, Energy and Process Innovation, PO Box 342, 7300 AH, Apeldoorn, The Netherlands.

Abbreviation: PCE, tetrachloroethene (perchloroethylene).

The GenBank accession number for the 16S rDNA sequence of strain PCE-M2^T is AF218076.

METHODS

Inoculum source. A soil sample from a polluted site in Maassluis near Rotterdam Harbour in The Netherlands was used as the inoculum for laboratory-scale flow-through soil columns as described by Middeldorp *et al.* (1998). A liquid sample from one of these columns was used to start the enrichment culture.

Anaerobic medium and experimental set-up. A phosphate-/bicarbonate-buffered medium with a low chloride concentration, as described by Holliger *et al.* (1993), was used for the experiments. Electron acceptors and donors were added from aqueous, concentrated, sterile stock solutions to respective final concentrations of 10 and 25 mM, unless otherwise stated. PCE was added from a concentrated (1 M) stock solution in hexadecane. Hexadecane was not converted during experiments by the different bacteria. Yeast extract was omitted from the medium unless otherwise stated.

Incubations were carried out in 117 ml serum bottles containing 20 ml anaerobic medium. The headspace consisted of N_2/CO_2 (80 : 20) or H_2/CO_2 (80 : 20); the latter was used when molecular hydrogen was used as electron donor. Acetate was added as a carbon source when molecular hydrogen or formate was used as electron donor. For isolation purposes, the roll-tube method was used. The medium was solidified with Noble agar (22 g l^{-1}; Difco).

Organisms. *Dehalospirillum multivorans* DSM 12446^T, *S. deleyianum* DSM 6946^T, *S. arcachonense* DSM 9755^T and *S. arsenophilum* DSM 10659^T were purchased from the DSMZ. *S. barnesii* ATCC 700032^T was obtained from the ATCC.

Escherichia coli XL-1 Blue (Stratagene) was used as the host for cloning vectors. The strain was grown in Luria–Bertani medium at 37 °C (Sambrook *et al.*, 1989) and ampicillin was added at 100 μg ml^{-1} when appropriate.

DNA analyses. Both G+C-content analysis and DNA–DNA hybridization were performed at the DSMZ. DNA was isolated by chromatography with hydroxyapatite (Cashion *et al.*, 1977). G+C contents were determined using HPLC, as described by Mesbah *et al.* (1989). DNA–DNA hybridizations were carried out as described by De Ley *et al.* (1970), with the modifications described by Huß *et al.* (1983) and Escara & Hutton (1980). Renaturation rates were computed according to Jahnke (1992).

Analytical techniques. Chloride anion concentrations were determined with a Micro-chlor-o-counter (Marius). Prior to analysis, 0·6 ml samples were acidified with 10 μl pure sulfuric acid and purged for 5 min with nitrogen gas to eliminate sulfide anions, which interfered with the chloride measurement. Volatile fatty acids were determined by HPLC, as described by Scholten & Stams (1995). Inorganic anions were separated on a dionex column as described by the same authors.

All chlorinated ethenes and ethene were determined qualitatively in headspace samples using a 438A Chrompack Packard gas chromatograph. The gas chromatograph was equipped with a flame-ionization detector connected to a capillary column [25 m × 0·32 mm inner diameter; df 10 μm; 100 kPa N_2 (Poraplot Q; Chrompack)] and a splitter injector (ratio 1 : 10). The injector and detector temperatures were respectively 100 and 250 °C. The column temperature was initially 50 °C for 1 min and was then increased by 39 °C min^{-1} to 210 °C; finally, the temperature was kept at 210 °C for 7 min.

Fatty acid composition. Bacterial cultures were harvested by centrifugation (20 000 ***g***, 20 min, 4 °C) and pellets were extracted directly with a modified Bligh–Dyer extraction. The total lipid extract was fractionated on silicic acid and mild alkaline transmethylation was used to yield fatty acid methyl esters from the phospholipid fraction. Concentrations of individual polar-lipid fatty acids as fatty acid methyl esters were determined by using a capillary GC/flame-ionization detector. Identification of polar-lipid fatty acids was based on comparison of retention-time data with known standards (for further details, see Boschker *et al.*, 1999).

Transmission electron microscopy (TEM). For TEM, cells were fixed for 2 h in 2·5 % (v/v) glutaraldehyde in 0·1 M sodium cacodylate buffer (pH 7·2) at 0 °C. After the cells had been rinsed in the same buffer, they were subjected to post-fixation using 1 % (w/v) OsO_4 and 2·5 % (w/v) $K_2Cr_2O_7$ for 1 h at room temperature. Finally, the cells were post-stained in 1 % (w/v) uranyl acetate. After sectioning, micrographs were taken with a Philips EM400 transmission electron microscope.

Amplification of 16S rDNA, cloning and sequencing. Chromosomal DNA of strain PCE-M2^T was isolated as described previously (Van de Pas *et al.*, 1999). The 16S rDNA was amplified with a GeneAmp PCR System 2400 thermocycler (Perkin-Elmer-Cetus). After preheating to 94 °C for 2 min, 35 amplification cycles of denaturation at 94 °C for 20 s, primer annealing at 50 °C for 30 s and elongation at 72 °C for 90 s were performed. A final extension of 7 min at 68 °C was performed. The PCRs (50 μl) contained 10 pmol primers 8f [5′-CACGGATCCAGAGTTTGAT(C/T)(A/C)-TGGCTCAG-3′] and 1510r [5′-GTGAAGCTTACGG(C/T)TAC-CTTGTTACGACTT-3′] (Lane, 1991), 2 mM $MgCl_2$, 200 μM each of dATP, dCTP, dGTP and TTP and 1 U Expand Long Template enzyme mixture (Roche Diagnostics). PCR products were purified by the QIAquick PCR purification kit (Qiagen) and cloned into *E. coli* XL-1 Blue by using the pGEM-T plasmid vector (Promega). Plasmid DNA was isolated from *E. coli* by using the alkaline lysis method, and standard DNA manipulations were performed according to established procedures (Sambrook *et al.*, 1989) and manufacturers' instructions. Restriction enzymes were purchased from Life Technologies.

DNA sequencing was performed using a LiCor DNA sequencer 4000L. Plasmid DNA used for sequencing reactions was purified with the QIAprep Spin Miniprep kit (Qiagen). Reactions were performed using the Thermo Sequenase fluorescent labelled primer cycle sequencing kit (Amersham Pharmacia Biotech). Infrared dye (IRD800)-labelled oligonucleotides were purchased from MWG Biotech. The sequence was determined using labelled primers 515r (5′-ACCGCGGC-TGCTGGCAC-3′) (Lane, 1991), 338f (5′-ACTCCTACGGGA-GGCAGCAGGTA-3′) and 968f (5′-AACGCGAAGAACCTTA-3′) (Nübel *et al.*, 1996).

Sequences were analysed using the DNAstar software package and ARB software (Strunk & Ludwig, 1995). Initial sequence alignments were performed using the LALIGN utility at the GENESTREAM network server (http://www2.igh.cnrs.fr/bin/lalign-guess.cgi). The phylogenetic tree was constructed using the neighbour-joining method (*E. coli* positions 72–1419) (Saitou & Nei, 1987).

RESULTS

Isolation of strain PCE-M2^T

A PCE-degrading culture was enriched from soil polluted with chlorinated ethenes by using, alternately, hydrogen and lactate as electron donor and PCE as electron acceptor. The enrichment degraded PCE via trichloroethene to (mainly) *cis*-dichloroethene. Minor amounts of vinyl chloride and ethene were also produced. A microscopically pure culture was obtained via serial dilution in liquid medium. This culture reduced PCE, via trichloroethene, to

cis-dichloroethene. No other reduced products could be found in this enrichment. This culture was used to prepare a dilution series in roll tubes. In these roll tubes, only lens-shaped colonies were observed. Single colonies from these roll tubes were transferred back into liquid medium. Immediately, a new dilution series in liquid medium was prepared and incubated in parallel on solidified medium in roll tubes. After growth, single colonies were again transferred back to, and maintained in, liquid medium. One of these cultures was checked for purity by incubation in a rich Wilkins–Chalgren medium (Oxoid). This resulted in growth of, presumably, the PCE-reducing organism only. This culture, strain PCE-M2^T, was used for further characterization.

Morphology

Cells of strain PCE-M2^T were slightly curved rods, 2·5–4 μm long by 0·6 μm wide (Fig. 1). The bacteria stained Gram-negative and formation of endospores was never observed. Cells in actively growing cultures were motile.

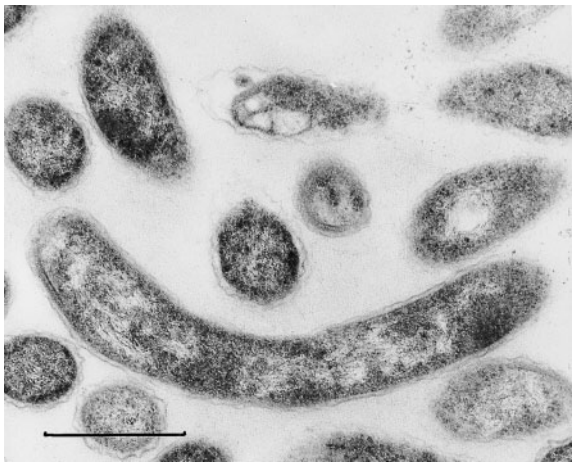

Fig. 1. Transmission electron micrograph of cells of *Sulfurospirillum halorespirans* sp. nov. PCE-M2^T. Bar, 0·5 μm.

Growth conditions

Strain PCE-M2^T was routinely cultivated with PCE as electron acceptor and lactate as electron donor. It was able to couple the oxidation of lactate, molecular hydrogen, formate and pyruvate to growth in the presence of PCE as terminal electron acceptor. Organic electron donors, except for formate, were oxidized incompletely to acetate. Formate and molecular hydrogen sustained growth only when acetate was present as carbon source. Strain PCE-M2^T was able to couple the reduction of a number of electron acceptors to growth (Table 1). Oxygenated sulfur compounds (sulfate, sulfite and thiosulfate) could not replace PCE as electron acceptor, nor could 3-chloro-4-hydroxyphenylacetate or 1,2-dichloroethane.

Strain PCE-M2^T grew fermentatively on both fumarate and pyruvate, whereas lactate could not be fermented. All known *Sulfurospirillum* species are also able to ferment fumarate (Stolz *et al.*, 1999; Finster *et al.*, 1997). Scholz-Muramatsu *et al.* (1995) reported that fumarate could not be fermented by *Dehalospirillum multivorans* DSM 12446^T. However, we were able to grow *Dehalospirillum multivorans* fermentatively on fumarate. Pyruvate fermentation is also reported for *S. deleyianum* and *Dehalospirillum multivorans*, whereas *S. arcachonense* is not able to ferment pyruvate (Schumacher *et al.*, 1992; Scholz-Muramatsu *et al.*, 1995; Finster *et al.*, 1997). No data on pyruvate fermentation have been reported for the other two *Sulfurospirillum* species.

Table 1. Terminal electron acceptors used by strain PCE-M2^T and related strains

Strains are indicated as: 1, PCE-M2^T; 2, *Dehalospirillum multivorans* DSM 12446^T; 3, *S. arsenophilum* DSM 10659^T; 4, *S. deleyianum* DSM 6946^T; 5, *S. barnesii* ATCC 700032^T; 6, *S. arcachonense* DSM 9755^T.

Acceptor	1	2	3	4	5	6
S^0	+	ND	+[g*]	+[f]	+[e]	+[c]
AsO_4	+	+[b]	+[g]	−[g]	+[e]	ND
SeO_4	+	+[b]	−[g]	−[g]	+[d]	ND
PCE	+	+[a]	ND	ND	−	ND
NO_3	+ ($\rightarrow NH_4$)	+[a] ($\rightarrow NO_2$)	+[g] ($\rightarrow NH_4$)	+[f] ($\rightarrow NH_4$)	+[d] ($\rightarrow NH_4$)	−[c]
NO_2	+	−[a]	+[g]	+[f]	+[e]	−[c]
Sulfite	−	ND	ND	+[f]	−[g]	−[c]
Thiosulfate	−	ND	+[g]	+[f]	+[e]	−[c]
Microaerophilic	+	ND	+[g]	+[f]	+[e]	+[c]
Fumarate	+	+[a]	ND	+[f]	+[e]	ND

ND, Not determined.

*Data for reference strains were taken from: *a*, Scholz-Muramatsu *et al.* (1995); *b*, Holliger *et al.* (1999); *c*, Finster *et al.* (1997); *d*, Oremland *et al.* (1994); *e*, Laverman *et al.* (1995); *f*, Schumacher *et al.* (1992); *g*, Stolz *et al.* (1999).

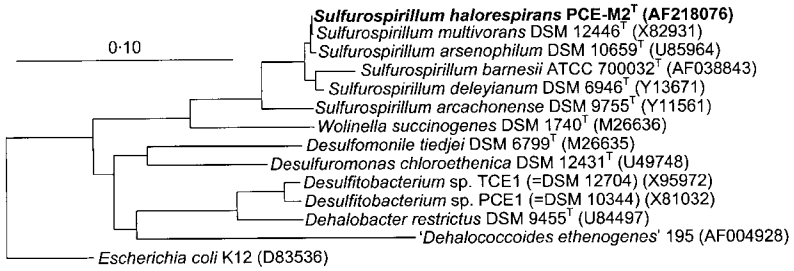

Fig. 2. Phylogenetic tree constructed by the neighbour-joining method (Saitou & Nei, 1987), using 1347 nucleotides from 16S rDNA sequences, showing the position of strain PCE-M2^T in relation to members of the genus *Sulfurospirillum* and other (dechlorinating) organisms. Bar, 10 % divergence.

Strain PCE-M2^T was able to grow at moderate temperatures. Optimal growth occurred between 25 and 30 °C.

Molecular analysis

The nucleotide sequence of a 16S rRNA gene of strain PCE-M2^T was determined and analysis revealed that strain PCE-M2^T is clustered in the ε-subclass of the *Proteobacteria*. A phylogenetic tree was constructed and showed that strain PCE-M2^T groups within the genus *Sulfurospirillum* and is closely related to *Dehalospirillum multivorans* (Fig. 2). DNA–DNA hybridization values and levels of 16S rDNA sequence similarity between strain PCE-M2^T, the different *Sulfurospirillum* species and *Dehalospirillum multivorans* are given in Table 2. These data show that both strain PCE-M2^T and *Dehalospirillum multivorans* should be included within the genus *Sulfurospirillum* and that they are related most closely to *S. arsenophilum*.

The G+C content of strain PCE-M2^T is 41·8±0·2 mol%. With the exception of *S. arcachonense* (32·0 mol%; Finster *et al.*, 1997), this agrees well with the G+C contents of other *Sulfurospirillum* species and *Dehalospirillum multivorans* (Table 2).

Fatty acid composition

Strain PCE-M2^T and the other four strains analysed had similar polar-lipid fatty acid profiles, mainly comprising 16 : 1ω7*c*, 16 : 0 and 18 : 1ω7*c* (Table 3). The dominant fatty acids were similar in *S. arcachonense*, as reported by Finster *et al.* (1997): there were smaller amounts of an 18 : 1 fatty acid and larger amounts of 18 : 0 fatty acid. As discussed by Finster *et al.* (1997), the fatty acid composition detected is typical of bacteria belonging to the ε-subclass of the *Proteobacteria*.

DISCUSSION

An anaerobic bacterium able to use PCE, selenate, arsenate and some other compounds (Table 1) as terminal electron acceptors for growth was isolated. The organism was isolated from a soil polluted with chlorinated aliphatic compounds; this soil produced a rapid dechlorination of PCE in laboratory-scale flow-through columns (Middeldorp *et al.*, 1998). Comparison of the physiological and phylogenetic features of strain PCE-M2^T revealed a close relationship to members of the genus *Sulfurospirillum* and to *Dehalospirillum multivorans* (Finster *et al.*, 1997; Holliger *et al.*, 1998; Laverman *et al.*, 1995; Oremland *et al.*, 1994; Scholz-Muramatsu *et al.*, 1995; Schumacher *et al.*, 1992; Stolz *et al.*, 1999). We used 16S rDNA sequences to construct a phylogenetic tree showing the position of strain PCE-M2^T in relation to closely related organisms and other (dechlorinating) organisms (Fig. 2). DNA–DNA hybridizations between all species tested are below the critical value of 70 % (the approximate threshold for delineation of separate species; Stackebrandt & Goebel, 1994).

Table 2. DNA G+C content, DNA–DNA relatedness and 16S rDNA sequence similarity between strain PCE-M2^T and related species

Values above the diagonal are 16S rDNA sequence similarity (%); values below the diagonal are DNA–DNA relatedness (%).

Strain	G+C content (mol%)	16S rDNA sequence similarity (%) to/DNA–DNA hybridization (%) with:					
		1	2	3	4	5	6
1. Strain PCE-M2^T	41·8±0·2	–	98·8	99·0	97·0	95·0	93·0
2. *D. multivorans* DSM 12446^T	41·5[b]*	65·7	–	99·0	98·0	98·0	92·0
3. *S. arsenophilum* DSM 10659^T	40·9[d]	33·2	35·4	–	97·0	97·0	93·0
4. *S. deleyianum* DSM 6946^T	40·6[a]	28·1	30·8	30·7[d]	–	98·0	92·0
5. *S. barnesii* ATCC 700032^T	40·8[d]	29·2	31·5	49·7[d]	55·0[d]	–	91·7
6. *S. arcachonense* DSM 9755^T	32·0[c]	ND	ND	ND	ND	ND	–

*Data obtained from: *a*, Schumacher *et al.* (1992); *b*, Scholz-Muramatsu *et al.* (1995); *c*, Finster *et al.* (1997); *d*, Stolz *et al.* (1999).
ND, Not determined.

Table 3. Polar-lipid fatty acid composition of strain PCE-M2^T and related strains

Values are mol% of total polar lipid fatty acids. Strains are identified as: 1, PCE-M2^T; 2, *Dehalospirillum multivorans* DSM 12446^T; 3, *S. arsenophilum* DSM 10659^T; 4, *S. deleyianum* DSM 6946^T; 5, *S. barnesii* ATCC 700032^T.

Fatty acid	1	2	3	4	5
14:0	3·3	5·1	4·5	–	1·8
i15:0	–	–	2·1	–	–
15:0	2·1	1·5	1·3	1·1	0·6
i16:1	1·4	0·6	1·1	0·6	0·6
16:1ω7*c*	47·4	47·4	47·5	52·0	43·2
16:1ω5	0·3	0·3	0·6	–	0·5
16:0	38·3	40·7	24·5	29·2	30·2
i17:1	–	1·1	2·1	–	1·1
17:0	–	–	1·9	–	0·2
18:1ω7*c*	6·5	2·8	12·2	17·2	21·6
18:0	0·5	–	0·4	–	–
Minor components	0·2	0·6	1·7	–	0·1

Schumacher *et al.* (1992) established the genus *Sulfurospirillum* to describe '*Spirillum*' 5175 as *S. deleyianum*, a Gram-negative, elemental sulfur-reducing spirillum. Since then, several bacteria have been identified as additional members of the genus *Sulfurospirillum* (Finster *et al.*, 1997; Stolz *et al.*, 1999). The data presented here justify the addition of our isolate, strain PCE-M2^T, to the *Sulfurospirillum* clade. Since our strain differs from the other described species, e.g. in using PCE as a terminal electron acceptor for growth, we propose that strain PCE-M2^T represents a novel species, *Sulfurospirillum halorespirans* sp. nov.

Strain PCE-M2^T is very similar to *Dehalospirillum multivorans*, especially with respect to the reduction of chlorinated ethenes. At the time of publication, the data that Scholz-Muramatsu *et al.* (1995) presented on *Dehalospirillum multivorans* justified the establishment of a new genus. However, over the last decade, more physiological data on *Dehalospirillum multivorans* have become available, such as the ability of this micro-organism to reduce selenate and arsenate (Holliger *et al.*, 1998). Combining these new data with the phylogenetic data presented here, it is necessary to reclassify *Dehalospirillum multivorans* DSM 12446^T in the genus *Sulfurospirillum* as *Sulfurospirillum multivorans* comb. nov.

Dehalobacter restrictus was the first organism isolated that is able to reduce PCE metabolically (Holliger *et al.*, 1993). This organism is limited to the use of PCE and trichloroethene as electron acceptors and molecular hydrogen as electron donor. Several *Desulfitobacterium* strains that are more diverse in their substrate spectrum have also been isolated from distinct environments. This diversity could indicate that strains of the genus *Desulfitobacterium* play an important role in the attenuation of chlorinated compounds in nature. Members of the genus *Sulfurospirillum* also have a more diverse substrate spectrum. They are known specifically for the reduction of sulfur and oxidized metals such as arsenate and selenate. The isolation of strain PCE-M2^T, a novel halorespiring species, and the addition of *Dehalospirillum multivorans* indicate the importance of the genus *Sulfurospirillum* in biotransformations in soils polluted with chlorinated compounds and metal ions.

Emended description of *Sulfurospirillum* Schumacher *et al.* 1993

The original description of this genus was provided by Schumacher *et al.* (1992). Additionally, some species are able to use arsenate, selenate, PCE or trichloroethene as terminal electron acceptors. The type species is *Sulfurospirillum deleyianum*.

Description of *Sulfurospirillum halorespirans* sp. nov.

Sulfurospirillum halorespirans (ha.lo.res′pi.rans. N.L. part. adj. *halorespirans* halorespiring, respiring halogenated compounds).

Gram-negative. Slightly curved, rod-shaped cells, 2·5–4 μm long by 0·6 μm wide. Motile. Optimum growth between 25 and 30 °C. PCE, selenate, arsenate, nitrate, nitrite, sulfur and fumarate serve as terminal electron acceptors. Capable of microaerophilic growth. Nitrate and nitrite are reduced to ammonium. PCE is reduced to *cis*-dichloroethene. Selenate is reduced, via selenite, to elemental selenium. Hydrogen, formate, pyruvate and lactate serve as electron donors. Hydrogen and formate serve as electron donors only when acetate is present as carbon source. Can grow fermentatively on fumarate and pyruvate. The G+C content of the type strain is 41·8±0·2 mol%.

The type and only strain, strain PCE-M2^T (=DSM 13726^T =ATCC BAA-583^T), was isolated from a soil that was polluted with chlorinated aliphatic compounds in Maassluis, near Rotterdam Harbour, The Netherlands.

Description of *Sulfurospirillum multivorans* comb. nov.

Basonym: *Dehalospirillum multivorans* Scholz-Muramatsu *et al.* 2002.

The description was provided by Scholz-Muramatsu *et al.* (1995). Additionally, this species is able to use arsenate and selenate as electron acceptors. The type strain is DSM 12446^T.

ACKNOWLEDGEMENTS

This work was financially supported by TNO Environment, Energy and Process Innovation, Apeldoorn, The Netherlands. The authors would like to thank A. Keizer-Gunnik (Laboratory for Electron Microscopy,

University of Groningen, The Netherlands) for the transmission electron micrographs. The authors would also like to thank Wim Roelofsen and Caroline Plugge for their assistance during this work.

REFERENCES

Boschker, H. T. S., de Brouwer, J. F. C. & Cappenberg, T. E. (1999). The contribution of macrophyte-derived organic matter to microbial biomass in salt-marsh sediments: stable carbon isotope analysis of microbial biomarkers. *Limnol Oceanogr* **44**, 309–319.

Bouwer, E. J. & McCarty, P. L. (1983). Transformations of 1- and 2-carbon halogenated aliphatic organic compounds under methanogenic conditions. *Appl Environ Microbiol* **45**, 1286–1294.

Cashion, P., Holder-Franklin, M. A., McCully, J. & Franklin, M. (1977). A rapid method for the base ratio determination of bacterial DNA. *Anal Biochem* **81**, 461–466.

de Bruin, W. P., Kotterman, M. J. J., Posthumus, M. A., Schraa, G. & Zehnder, A. J. B. (1992). Complete biological reductive transformation of tetrachloroethene to ethane. *Appl Environ Microbiol* **58**, 1996–2000.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

DiStefano, T. D., Gossett, J. M. & Zinder, S. H. (1991). Reductive dechlorination of high concentrations of tetrachloroethene to ethene by an anaerobic enrichment culture in the absence of methanogenesis. *Appl Environ Microbiol* **57**, 2287–2292.

El Fantroussi, S., Naveau, H. & Agathos, S. N. (1998). Anaerobic dechlorinating bacteria. *Biotechnol Prog* **14**, 167–188.

Escara, J. F. & Hutton, J. R. (1980). Thermal stability and renaturation of DNA in dimethyl sulfoxide solutions: acceleration of the renaturation rate. *Biopolymers* **19**, 1315–1327.

Fathepure, B. Z., Nengu, J. P. & Boyd, S. A. (1987). Anaerobic bacteria that dechlorinate perchloroethene. *Appl Environ Microbiol* **53**, 2671–2674.

Finster, K., Liesack, W. & Tindall, B. J. (1997). *Sulfurospirillum arcachonense* sp. nov., a new microaerophilic sulfur-reducing bacterium. *Int J Syst Bacteriol* **47**, 1212–1217.

Freedman, D. L. & Gossett, J. M. (1989). Biological reductive dechlorination of tetrachloroethylene and trichloroethylene to ethylene under methanogenic conditions. *Appl Environ Microbiol* **55**, 2144–2151.

Gerritse, J., Renard, V., Pedro Gomes, T. M., Lawson, P. A., Collins, M. D. & Gottschal, J. C. (1996). *Desulfitobacterium* sp. strain PCE1, anaerobic bacterium that can grow by reductive dechlorination of tetrachloroethene or *ortho*-chlorinated phenols. *Arch Microbiol* **165**, 132–140.

Gerritse, J., Drzyzga, O., Kloetstra, G., Keijmel, M., Wiersum, L. P., Hutson, R., Collins, M. D. & Gottschal, J. C. (1999). Influence of different electron donors and acceptors on dehalorespiration of tetrachloroethene by *Desulfitobacterium frappieri* TCE1. *Appl Environ Microbiol* **65**, 5212–5221.

Holliger, C., Schraa, G., Stams, A. J. M. & Zehnder, A. J. B. (1993). A highly purified enrichment culture couples the reductive dechlorination of tetrachloroethene to growth. *Appl Environ Microbiol* **59**, 2991–2997.

Holliger, C., Wohlfahrt, G. & Diekert, G. (1998). Reductive dechlorination in the energy metabolism of anaerobic bacteria. *FEMS Microbiol Rev* **22**, 383–398.

Huß, V. A. R., Festl, H. & Schleifer, K. H. (1983). Studies on the spectrometric determination of DNA hybridization from renaturation rates. *Syst Appl Microbiol* **4**, 184–192.

Jahnke, K.-D. (1992). BASIC computer program for evaluation of spectroscopic DNA renaturation data from GILFORD System 2600 spectrometer on a PC/XT/AT type personal computer. *J Microbiol Methods* **15**, 61–73.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.

Laverman, A. M., Switzer Blum, J., Shaefer, J. K., Philips, E. J. P., Lovley, D. R. & Oremland, R. S. (1995). Growth of strain SES-3 with arsenate and other diverse electron acceptors. *Appl Environ Microbiol* **61**, 3556–3561.

Maymo-Gatell, X., Chien, Y., Gossett, J. M. & Zinder, S. H. (1997). Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene. *Science* **276**, 1568–1571.

Maymo-Gatell, X., Anguish, T. & Zinder, S. H. (1999). Reductive dechlorination of chlorinated ethenes and 1,2-dichloroethane by "*Dehalococcoides ethenogenes*" 195. *Appl Environ Microbiol* **65**, 3108–3113.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Middeldorp, P. J. M., Van Aalst, M. A., Rijnaarts, H. H. M., Stams, F. J. M., De Kreuk, H. F., Schraa, G. & Bosma, T. N. P. (1998). Stimulation of reductive dechlorination for *in situ* bioremediation of a soil contaminated with chlorinated ethenes. *Water Sci Technol* **37** (8), 105–110.

Middeldorp, P. J. M., Luijten, M. L. G. C., Van de Pas, B. A., Van Eekert, M. H. A., Kengen, S. W. M., Schraa, G. & Stams, A. J. M. (1999). Anaerobic microbial reductive dehalogenation of chlorinated ethenes. *Bioremediation J* **3**, 151–169.

Miller, E., Wohlfarth, G. & Diekert, G. (1997). Comparative studies on tetrachloroethene reductive dechlorination mediated by *Desulfitobacterium* sp. strain PCE-S. *Arch Microbiol* **168**, 513–519.

Nübel, U., Engelen, B., Felske, A., Snaidr, J., Wieshuber, A., Amann, R. I., Ludwig, W. & Backhaus, H. (1996). Sequence heterogeneities of genes encoding 16S rRNAs in *Paenibacillus polymyxa* detected by temperature gradient gel electrophoresis. *J Bacteriol* **178**, 5636–5643.

Oremland, R. S., Switzer Blum, J., Culbertson, C. W., Visscher, P. T., Miller, L. G., Dowdle, P. & Strohmaier, F. E. (1994). Isolation, growth, and metabolism of an obligately anaerobic, selenate-respiring bacterium, strain SES-3. *Appl Environ Microbiol* **60**, 3011–3019.

Ryoo, D., Shim, H., Canada, K., Barbieri, P. & Wood, T. K. (2000). Aerobic degradation of tetrachloroethylene by toluene-*o*-xylene monooxygenase of *Pseudomonas stutzeri* OX1. *Nat Biotechnol* **18**, 775–778.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Scholten, J. C. M. & Stams, A. J. M. (1995). The effect of sulfate and nitrate on methane formation in a freshwater sediment. *Antonie van Leeuwenhoek* **68**, 309–315.

Scholz-Muramatsu, H., Neumann, A., Meßmer, M., Moore, E. & Diekert, G. (1995). Isolation and characterization of *Dehalospirillum multivorans* gen. nov., sp. nov., a tetrachloroethene-utilizing, strictly anaerobic bacterium. *Arch Microbiol* **163**, 48–56.

Schumacher, W., Kroneck, P. M. H. & Pfennig, N. (1992). Comparative systematic study on "*Spirillum*" 5175, *Campylobacter* and *Wolinella* species. Description of "*Spirillum*" 5175 as *Sulfurospirillum deleyianum* gen. nov., spec. nov. *Arch Microbiol* **158**, 287–293.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Stolz, J. F., Ellis, D. J., Switzer Blum, J., Ahmann, D., Lovley, D. R. & Oremland, R. S. (1999). *Sulfurospirillum barnesii* sp. nov. and *Sulfurospirillum arsenophilum* sp. nov., new members of the *Sulfurospirillum* clade of the ε *Proteobacteria*. *Int J Syst Bacteriol* **49**, 1177–1180.

Strunk, O. & Ludwig, W. (1995). ARB: a software environment for sequence data. Technical University of Munich. http://www.arb-home.de/

Van de Pas, B. A., Smidt, H., Hagen, W. R., Van der Oost, J., Schraa, G., Stams, A. J. M. & De Vos, W. M. (1999). Purification and molecular characterization of ortho-chlorophenol reductive dehalogenase, a key enzyme of halorespiration in *Desulfitobacterium dehalogenans*. *J Biol Chem* **274**, 20287–20292.

Wild, A., Hermann, R. & Leisinger, T. (1996). Isolation of an anaerobic bacterium which reductively dechlorinates tetrachloroethene and trichloroethene. *Biodegradation* **7**, 507–511.