REVIEW

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Tetrachloroethene-Dehalogenating Bacteria

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ABSTRACT. Tetrachloroethene is a frequent groundwater contaminant often persisting in the subsurface environments. It is recalcitrant under aerobic conditions because it is in a highly oxidized state and is not readily susceptible to oxidation. Nevertheless, at least 15 organisms from different metabolic groups, *viz.* halorespirators (9), acetogens (2), methanogens (3) and facultative anaerobes (2), that are able to metabolize tetrachloroethene have been isolated as axenic cultures to-date. Some of these organisms couple dehalogenation to energy conservation and utilize tetrachloroethene as the only source of energy while others dehalogenate tetrachloroethene fortuitously. Halorespiring organisms (halorespirators) utilize halogenated organic compounds as electron acceptors in an anaerobic respiratory process. Different organisms exhibit differences in the final products of tetrachloroethene dehalogenation, some strains convert tetrachloroethene to trichloroethene only, while others also carry out consecutive dehalogenation to dichloroethenes and vinyl chloride. Thus far, only a single organism, '*Dehalococcoides ethenogenes*' strain 195, has been isolated which dechlorinates tetrachloroethene all the way down to ethylene. The majority of tetrachloroethene-dehalogenating organisms have been isolated only in the past few years and several of them, *i.e.*, *Dehalobacter restrictus*, *Desulfitobacterium dehalogenans*, '*Dehalococcoides ethenogenes*', '*Dehalospirillum multivorans*', *Desulfuromonas chloroethenica*, and *Desulfomonile tiedjei*, are representatives of new taxonomic groups. This contribution summarizes the available information regarding the axenic cultures of the tetrachloroethene-dehalogenating bacteria. The present knowledge about the isolation of these organisms, their physiological characteristics, morphology, taxonomy and their ability to dechlorinate tetrachloroethene is presented to facilitate a comprehensive comparison.

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ABBREVIATIONS

ATCC	*American Type Culture Collection*
DSM	*Deutsche Sammlung von Mikroorganismen*
EMBL	*European Molecular Biology Laboratory*
JMC	*Japanese Collection of Microorganisms*
OCM	*Oregon Collection of Methanogens*
rDNA	ribosomal deoxyribonucleic acid
DCE	dichloroethene
ETH	ethene
PCE	tetrachloroethene
TCE	trichloroethene
VC	vinyl chloride
rRNA	ribosomal ribonucleic acid

1 INTRODUCTION

Tetrachloroethene [perchloroethylene (PCE), tetrachloroethylene, ethylene tetrachloride] is one of the pollutants frequently found in a subsurface. PCE is being used for example as a dry cleaning agent in the textile industry, in the scouring of machines and in fat extraction. The presence of four halogen atoms bound to the *sp2* carbon atoms in its chemical structure makes this compound resistant to microbial degradation. PCE is highly toxic, is a suspect carcinogen and is hazardous to human health. PCE is not easily combustible. Bioremediation represents an attractive technology for the removal of PCE from soil and water.

PCE is persistent under aerobic conditions, because it is in a highly oxidized state and is not easily susceptible to oxidation. Dehalogenation of PCE under bulk aerobic conditions was observed by Enzien *et al.* (1994) but the presence of anaerobic microsites where dehalogenation took place has been proposed by the authors. Also denitrifying conditions appear to be unfavorable for the dehalogenation of PCE (Holliger 1995). Under anaerobic conditions, PCE undergoes reductive dehalogenation to less chlorinated ethenes. The biotic dehalogenation can proceed *via* trichloroethene (TCE), *cis*-1,2-dichloroethene (*cis*-1,2-DCE) and/or *trans*-1,2-dichloroethene (*trans*-1,2-DCE) and vinyl chloride (VC) as intermediates (Fig. 1). The detailed study of the mechanisms of reductive dehalogenation of PCE was recently initiated by the isolation of tetrachloroethene reductive dehalogenases from '*Dehalospirillum multivorans*' (Neumann *et al.* 1995; Neumann *et al.* 1996), *Dehalobacter restrictus* PER-K23 (Schumacher and Holliger 1996), *Desulfitobacterium* sp. PCE-S (Miller *et al.* 1998) and '*Dehalococcoides ethenogenes*' 195 (Magnuson *et al.* 1998). The state of art in the enzymology of reductive dehalogenation is provided in an excellent review of Wohlfarth and Diekert (1997), and will not be discussed in this review.

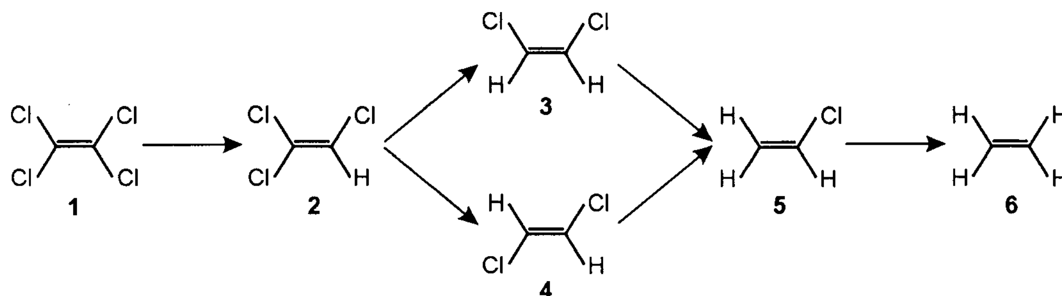

Fig. 1. The pathway of microbial reductive dehalogenation of PCE (Vogel *et al.* 1987); **1** – tetrachloroethene, **2** – trichloroethene, **3** – *cis*-1,2-dichloroethene, **4** – *trans*-1,2-dichloroethene, **5** – vinyl chloride, **6** – ethene.

Although the mixed cultures that perform reductive dechlorination of PCE have already been described some fifteen years ago (Bouwer and McCarty 1983; Vogel and McCarty 1985; Freedman and Gossett 1989; DiStefano *et al.* 1991; deBruin *et al.* 1992), the pure isolates have been obtained much more recently. The involvement of particular metabolic groups of microorganisms in PCE dehalogenation, such as strictly anaerobic methanogens, acetogens and facultative anaerobes, was originally implicated from observations made in mixed-culture studies (Bouwer and McCarty 1983; Kastner 1991; Enzien *et al.* 1994). Nowadays, there are at least fifteen species from five different metabolic groups that are known to perform partial or complete dechlorination of PCE. Some of the isolated PCE-dechlorinating bacteria did not belong to any known genus at the time of their isolation and they represent new taxons.

It is presently not known how widely PCE-dechlorinating organisms are geographically distributed so it is likely that their abundance at a given site may imply the extent and/or intensity of intrinsic biodegradation processes. Consequently, the PCE-dechlorinators, namely organisms that use the energy derived from dechlorination for growth, can potentially be used in bioremediation technologies (Holliger 1995; Sharma and McCarty 1996). Furthermore, knowledge of the physiological requirements of PCE-dehalogenating bacteria in terms of nutrients, electron donors and acceptors, growth factors, pH, *etc.*, is very important for the efforts to stimulate and optimize the activities of indigenous dechlorinators *in situ* (Hinchee *et al.* 1995). This communication reviews the axenic bacterial cultures that are reported to dehalogenate PCE. The important characteristics are summarized for each organism, and include growth conditions, morphology and physiology, electron donors and acceptors, carbon sources, dechlorination rates and products. Some of these characteristics have been summarized for anaerobic dechlorinating organisms by El Fantroussi and coworkers (1998). The present review also covers facultatively anaerobic PCE-dechlorinating bacteria and provides exhaustively all available information to uncover gaps in current knowledge. Also, the review will assist in the design of future experiments with PCE-dechlorinating organisms. Accession codes to international collection of microorganisms are listed for the strains mentioned in the review to provide the reader with immediate information about the availability of the organisms for experimental work. Tables I–IV can serve for quick overview of available information.

Table I. Pure cultures of PCE-dechlorinating bacteria

Strain/isolate[a]	References
Gram-positive halorespirators	
Desulfitobacterium dehalogenans JW/IU-DC1[b]	Utkin *et al.* 1994, 1995
Desulfitobacterium sp. PCE1	Gerritse *et al.* 1996
Desulfitobacterium sp. PCE-S	Miller *et al.* 1997, 1998
'*Dehalococcoides ethenogenes*' 195[c]	Maymo-Gatell *et al.* 1997
Gram-negative halorespirators	
Desulfomonile tiedjei DCB-1[b]	Shelton and Tiedje 1984; Fathepure *et al.* 1987; DeWeerd *et al.* 1990; Mohn and Tiedje 1990, 1992; Dolfing and Tiedje 1991; Cole *et al.* 1995; Ni *et al.* 1995; Louie *et al.* 1997; Townsend and Suflita 1997
Dehalobacter restrictus PER-K23	Holliger *et al.* 1993, 1998; Holliger and Schumacher 1994; Schumacher and Holliger 1996
D. restrictus TEA	Wild *et al.* 1996
'*Dehalospirillum multivorans*'	Neumann *et al.* 1994–1996, 1998; Scholz-Muramatsu *et al.* 1995
Desulfuromonas chloroethenica TT4B	Krumholz *et al.* 1996; Krumholz 1997
Acetogens	
Sporomusa ovata H1	Möller *et al.* 1984; Terzenbach and Blaut 1994
Acetobacterium woodii WB1	Balch *et al.* 1977; Tanner *et al.* 1978; Braun and Gottschalk 1981; Egli *et al.* 1988, 1990
Methanogens	
Methanosarcina sp.	Boyd and Shelton 1984; Fathepure *et. al.* 1987; Fathepure and Boyd 1988*a,b*
M. mazei S-6	Mah 1980; Fathepure *et al.* 1987; Fathepure and Boyd 1988*a,b*
Methanobacterium thermoautotrophicum MARBURG	Fuchs *et al.* 1978; Schonheit *et al.* 1979, 1980; Belay and Daniels 1987; Egli *et al.* 1987, 1990; Holliger *et al.* 1990
Facultative anaerobes	
isolate MS-1	Sharma and McCarty 1996
Pantoea agglomerans ATCC 27993	Sharma and McCarty 1996
Aerobes	
Pseudomonas sp.[d]	Deckard *et al.* 1994

[a]Names of bacteria in quotation marks have not been validly published in the *Internat. J. Syst. Bacteriol.* at the time of preparing this review.
[b]Halogenated aromatic compounds (not PCE) serve as electron acceptors for this isolate.
[c]Gram stain uncertain for this isolate.
[d]Dechlorination of PCE by this isolate is uncertain.

2 HALORESPIRING PCE-DECHLORINATING BACTERIA (GRAM-POSITIVE)

2.1 *Desulfitobacterium dehalogenans* JW/IU-DC1 (Utkin *et al.* 1994)

Isolation and cultivation. The enrichment culture was obtained from a fresh-water sediment collected from a pond located in a wooded area. The enrichment culture was grown under anaerobic conditions in a mineral medium supplemented with yeast extract (0.2–1 %) as carbon source. The medium used for isolation was prepared using the spent medium from a co-culture of *Clostridium* sp. JW/IU-YU1 and *Clostridium* sp. JW/IU-DC1, supplemented with yeast extract (0.1 %) and glucose (0.1 %, *W/V*). A pure culture was obtained by isolating single colonies in soft agar shake cultures amended with pyruvate (2 mmol/L), formate (2 mmol/L) and 3-chloro-4-hydroxyphenylacetate (1 mmol/L). The pH range for growth on 3-chloro-4-hydroxyphenylacetate is pH 6 to 9, as determined at 37 °C. The pH optimum is 7.5. Doubling time is about 3.5 h at pH 7.5 and 37 °C. The temperature range for growth and dechlorination of 3-chloro-4-hydroxyphenylacetate is 13–45 °C with the optimum around 38 °C. Growth and dechlorination are inhibited under aerobic conditions but dechlorination occurs under nitrogen atmosphere with 2 % air.

Table II. Isolation and cultivation of PCE-dechlorinating bacteria

Strain/isolate[a]	Source	Atmosphere	pH range	pH optimum	Temperature range, °C	Temperature optimum, °C	Final product[b]	Inhibition mmol/L[c]
Desulfitobacterium dehalogenans JW/IU-DC1	freshwater	-	6.0–9.0	7.5	13–45	38	TCE	–
Desulfitobacterium sp. PCE1	PCE-contaminated soil	N_2–CO_2	–	7.2	19–42	34–37	TCE	–
Desulfitobacterium sp. PCE-S	PCE-contaminated soil	N_2–CO_2	–	–	–	–	*cis*-1,2-DCE	–
'*D. ethenogenes*' 195	sewage sludge	–	–	–	–	–	ETH	–
D. tiedjei DCB1	sewage sludge	H_2–CO_2	6.5–7.8	6.8–7.0	30–38	37	TCE	> 0.2
D. restrictus PER-K23	anaerobic river sediment and sludge	N_2–CO_2	6.5–8.0	6.8–7.6	< 37	25–30	*cis*-1,2-DCE	–
D. restrictus TEA	contaminated aquifer	H_2–CO_2	–	–	–	30	*cis*-1,2-DCE	–
'*D. multivorans*'	sewage sludge	N_2–CO_2	6.0–8.0	7.0–7.5	15–33	30	*cis*-1,2-DCE	> 0.3
D. chloroethenica TT4B	contaminated anaerobic sediment	N_2–CO_2	6.5–7.4	7.4	21–31	–	*cis*-1,2-DCE	> 0.1
Sporomusa ovata H1	sugar beet leaf silage	N_2–CO_2	5.0–8.1	5.3–7.2	15–45	34–39	TCE	–
Acetobacterium woodii WB1	marine sediment	H_2–CO_2	–	–	–	30	TCE	–
Methanosarcina sp.	chlorophenol-degrading enrichment	–	–	–	–	–	TCE	–
M. mazei S-6	sewage sludge	N_2	6.1–8.0	7.0	30–40	37	TCE	–
Methanobacterium thermoautotrophicum MARBURG	anaerobic sewage digester	H_2–CO_2	5.2–7.8	7.2–7.6	45–70	65	TCE	–
Isolate MS-1	PCE-contaminated aquifer	N_2	–	7.0	–	37	*cis*-1,2-DCE	> 10
Pantoea agglomerans ATCC 27993	blood	N_2	–	–	–	37	*cis*-1,2-DCE	–

[a]*Pseudomonas* sp. is not listed due to uncertain dehalogenation of PCE. [b]Product of PCE transformation. [c]Concentration of PCE with inhibitory effect.

Table III. Morphology of PCE-dechlorinating bacteria

Strain/isolate[a]	Gram	Morphology	Diameter, μm	Length, μm	Sporulation	Motility	Flagellum	G + C, molar %	16s rRNA[b]	DSM[b]	ATCC[b]
Desulfitobacterium dehalogenans JW/IU-DC1	G^+	curved rod	0.7	2.5–4	no	yes	1–4	45.0	L28946	9161	51507
Desulfitobacterium sp. PCE1	G^+	curved rod	0.6–0.8	2–3	no	yes	4, lateral	–	X81032	10344	–
Desulfitobacterium sp. PCE-S	G^+	–	–	–	–	–	–	–	–	–	–
'*D. ethenogenes*' 195	–	irregular coccus	0.5	0.5	–	no	non	–	AF004928	–	–
D. tiedjei DCB1	G^-	rod	0.8–1	5–10	no	no	non	49.0	M26635	6790	49306
D. restrictus PER-K23	G^-	rod	0.3–0.5	2–3	no	yes	1, lateral	45.3	U84497	9455	–
D. restrictus TEA	G^-	rod	0.2–0.3	2–5	no	yes	1–5, lateral	–	Y10164	–	–
'*D. multivorans*'	G^-	spirillum	0.4–0.5	2–5	no	yes	–	41.5	X82931	–	–

D. chloroethenica TT4B	G–	rod	0.6	1–1.7	no	yes	subpolar	–	U49748	–	700295
Sporomusa ovata H1	–	rod	0.7–1	1–5	yes	no	non	41.3–42.2	X06403	2662	35899
Acetobacterium woodii WB1	G^+	rod	1	2	no	yes	1–2, subterminal	39.0	–	1030	29683
Methanosarcina sp.	–	–	–	–	–	no	non	–	–	–	–
M. mazei S-6	G^-	coccus	1–3	1–3	–	no	non	–	–	2053	43572
M. thermoautotrophicum MARBURG	G^+	rod	0.4–0.6	3–7	no	no	non	49.7–52.0	–	2133	–
Isolate MS-1	G^-	rod	0.8–1	2.8–3.5	no	yes	peritrichal	–	L43508	–	–
Pantoea agglomerans ATCC 27993	G^-	–	–	–	no	yes	peritrichal	52.0–59.0	–	–	27993

[a]*Pseudomonas* sp. is not listed due to uncertain dehalogenation of PCE. [b]Accession numbers.

Table IV. Electron donors, electron acceptors and carbon sources of PCE-dechlorinating bacteria[a]

Strain/isolate[b]	Electron donor(s)	Electron acceptor(s)	Carbon source(s)
Desulfitobacterium dehalogenans JW/IU-DC1	H_2, formate, lactate, pyruvate	chlorinated phenols, fumarate, nitrate, sulfite, sulfur, thiosulfate	pyruvate
Desulfitobacterium sp. PCE1	butyrate, ethanol, formate, lactate, pyruvate, succinate	*ortho*-chlorinated phenols, fumarate, PCE, sulfite, thiosulfate	pyruvate, lactate
Desulfitobacterium sp. PCE-S	formate, pyruvate	fumarate, PCE, sulfite, TCE	acetate
'*D. ethenogenes*' 195	H_2	PCE, TCE	acetate
D. tiedjei DCB1	anisates, benzoate, 3-chlorobenzoate, CO_2, formate, H_2, isovanillate, 3-methoxysalicylate, pyruvate, vanillate	*meta*-halobenzoates, sulfate, sulfite, thiosulfate	pyruvate
D. restrictus PER-K23	H_2	PCE, TCE	CO_2, acetate
D. restrictus TEA	H_2	PCE, TCE	CO_2, acetate
'*D. multivorans*'	ethanol, formate, glycerol, H_2, lactate, pyruvate	fumarate, PCE, TCE	acetate
D. chloroethenica TT4B	acetate, pyruvate	Fe(III), fumarate, nitriloacetate, PCE, polysulfide, TCE	acetate
Sporomusa ovata H1	*c*	*c*	*c*
Acetobacterium woodii WB1	*d*	*d*	*d*, CO_2
Methanosarcina sp.	H_2	CO_2	acetate, CO_2, methanol
M. mazei S-6	H_2	CO_2	acetate, CO_2, methanol
Methanobacterium thermoautotrophicum MARBURG	H_2	CO_2	CO_2
Isolate MS-1	acetate, amino acids, formate, glucose, lactate, pyruvate, yeast extract	nitrate, O_2, (PCE, TCE)	benzoate, yeast extract
Pantoea agglomerans ATCC 27993	acetate, yeast extract	O_2	acetate, yeast extract

[a]See text for complete list of the tested substrates.
[b]*Pseudomonas* sp. is not listed due to uncertain dehalogenation of PCE.
[c]Fermentable substrates: betaine, 2,3-butanediol, ethanol, formate, fructose, lactate, methanol, butanol, N,N-dimethylglycine, propanol, pyruvate, sarcosine.
[d]Fermentable substrates: formate, fructose, glucose, glycerate, H_2, lactate.

Electron donors and acceptors, carbon sources and growth factors. The electron donors of strain JW/IU-DC1 are hydrogen, formate, lactate and pyruvate, as tested in the presence of yeast extract (0.1 %) with 3-chloro-4-hydroxyphenylacetate (10 mmol/L) as electron acceptor. The following compounds can serve as electron acceptors (in alphabetical order): 3-chloro-4-hydroxyphenylacetate, 2,3-dichlorophenol, 2,4-dichlorophenol, 2,6-dichlorophenol, fumarate, nitrate, pentachlorophenol, sulfite, sulfur, thiosulfate, 2,3,4-trichlorophenol, 2,3,6-trichlorophenol, 2,4,6-trichlorophenol, 2,3,4,6-tetrachlorophenol, 2,3,4,5-tetrachlorophenol and 2,3,5,6-tetrachlorophenol, while the following compounds cannot serve as electron acceptors: 2-chlorophenol, 3-chlorophenol, 4-chlorophenol, 2,5-dichlorophenol, 3,4-dichlorophenol, 3,5-dichlorophenol and 3,4,5-trichlorophenol. The only defined carbon source utilized in the presence of yeast extract (0.1 %) under anaerobic conditions is pyruvate, while substrates that do not support growth under these conditions are acetate, arabinose, cellobiose, galactose, glucose, formate, fructose, lactose, maltose, mannose, mannitol, raffinose, rhamnose, ribose, sucrose, xylose, lactate, ethanol, methanol, butyrate, isobutyrate, propionate and isovalerate. The organism requires unidentified growth factors for growth.

Morphology and classification. The cells grow as lightly curved rods with cell size of 0.7 µm (diameter) and 2.5–4 µm (length). The organism has from one to four flagella and does not form spores. 16S-rRNA analysis positioned the strain JW/IU-DC1 in the *Desulfotomaculum–Clostridium* subphylum. The most closely related species is *Desulfosporosinus (Desulphotomaculum) orientis* (Stackebrandt *et al.* 1997). The G + C content is 45 molar %. The organism is available from the *Deutsche Sammlung von Mikroorganismen* (DSM) as strain DSM 9161 and from the *American Type Culture Collection* (ATCC) as strain ATCC 51507. Three additional species of the genus *Desulfitobacterium* are available from DSM, *i.e. D. chlororespirans* DSM 11544 (Sanford *et al.* 1996), *D. hafniense* DSM 10664 (Christiansen and Ahring 1996), and *Desulfitobacterium* sp. PCE1 (DSM 10344; Gerritse *et al.* 1996). All species of genus *Desulfitobacterium* reductively dechlorinate aromatic hydrocarbons but only strains JW/IU-DC1 and PCE1 were shown to dehalogenate PCE. The *American Type Culture Collection* contains one additional *Desulfitobacterium* strain (ATCC 700041).

PCE dechlorination. This organism was primarily isolated and described as a chlorophenol-dehalogenating organism. More recently, the dechlorination of PCE to TCE by *Desulfitobacterium dehalogenans* was reported by Gerritse *et al.* (1996). Dehalogenation occurred in a medium amended with yeast extract (1 g/L) and pyruvate (40 mmol/L). Dehalogenation of PCE was also observed by Odom and Wiegel as reported by Gerritse *et al.* (1996). No further details on PCE dechlorination by this organism had been published to-date.

2.2 *Desulfitobacterium* sp. PCE1 (Gerritse *et al.* 1996)

Isolation and cultivation. This organism was isolated from a PCE-dechlorinating anaerobic enrichment culture enriched from a PCE-contaminated soil sample (Gerritse *et al.* 1995). The enrichment culture was diluted and streaked on agar plates maintained under anaerobic conditions. The cultivations were performed in a basal medium supplied with a vitamin solution under N_2–CO_2 (4:1, *V/V*) atmosphere. PCE dissolved in hexadecane (500 mmol/L) was supplied as electron acceptor, which corresponded to a PCE concentration in the medium of about 100 mmol/L. Using pyruvate as growth substrate, strain PCE1 grows over the temperature range of 19–42 °C, with an optimum between 34 and 37 °C. The pH optimum at 30 °C is about 7.2. Optimum growth on L-lactate with PCE as electron acceptor (2.5 Hz, 37 °C, pH 7.2) proceeded with a μ_{max} of 0.012/h.

Electron donors and acceptors, carbon sources and growth factors. Strain PCE1 grows fermentatively with pyruvate as the sole carbon and energy source whereas growth with other substrates requires an external electron acceptor. The following compounds can serve as electron donors: butyrate, ethanol, formate, L-lactate, pyruvate and succinate. Besides PCE, several other compounds can serve as electron acceptors: fumarate, *ortho*-chlorinated phenolic compounds (2-chlorophenol; 2,4,6-trichlorophenol; 3-chloro-4-hydroxyphenylacetate), sulfite and thiosulfate. Vitamins are required for the growth of this organism.

Morphology and classification. The cells are Gram-positive, helical, 0.6–0.8 µm in diameter and 2–3 µm in length, although sometimes 7 µm long curved cells can be formed. The cell wall is surrounded by a protein surface layer (S-layer). The organism is motile with four lateral flagella and does not form spores. 16S-rRNA analysis reveals that the PCE1 strain belongs to the *Clostridium* subphylum of Gram-positive bacteria and is closely related to *Desulfitobacterium* species. Strain PCE1 further resembles the members of the genera *Desulfotomaculum* and *Heliobacterium*. The 16S-rRNA sequence of PCE1 is by 99.3 % identical with the sequence of *Desulfitobacterium dehalogenans*. The sequence of the 16S-rRNA gene is deposited in the *European Molecular Biology Laboratory* (EMBL) under accession number X81032. The organism is available from DSM as strain DSM 10344.

PCE dechlorination. The strain PCE1 transforms PCE to TCE and chloride with trace amounts (<5 %) of *trans*-1,2-DCE and *cis*-1,2-DCE. Reductive dechlorination of PCE permits growth of this organism on formate. Dechlorination of PCE is apparently coupled to ATP synthesis. The organism contains cytochrome *c*.

2.3 *Desulfitobacterium* sp. PCE-S (Miller *et al.* 1997)

Isolation and cultivation. The isolate PCE-S was isolated from PCE-contaminated soil. The enrichment was performed under anoxic conditions with hydrogen, acetate, yeast extract and tetrachloroethene. An anaerobic medium containing pyruvate (40 mmol/L), yeast extract (0.2 %) and fumarate (40 mmol/L) or PCE (500 mmol/L in hexadecane) as the terminal electron acceptor was used for routine cultivations.

Electron donors and acceptors, carbon sources and growth factors. Formate and pyruvate were reported as electron donors for the isolate PCE-S. The following compounds can serve as electron acceptors: fumarate, PCE, sulfite and TCE. A number of chlorinated aliphatic and aromatic compounds cannot serve as electron acceptors: 1,1-DCE, *cis*-1,2-DCE, *trans*-1,2-DCE, 3-chlorobenzoate, 3-chloro-4-hydroxyphenylacetate, 2,4,6-trichlorophenol and pentachlorophenol. In the crude extract assay, only the artificial electron donors with a standard redox potential of less than –400 mV were effective for PCE reduction.

PCE dechlorination. PCE is dechlorinated to *cis*-1,2-DCE and chloride by the isolate PCE-S. Dehalogenation of PCE and TCE appears to be mediated by the same enzyme. The tetrachloroethene dehalogenase activity assayed in crude extracts supplied with methyl viologen as electron donor is 2.6 nkat/mg. The pH optimum of the PCE dehalogenation as determined with crude extracts lies in the range of 7.5–7.8. The activity was stable for 10 min up to 50 °C. PCE dehalogenation is not affected by changing the gas phase from N_2 to CO_2 but exposure of the crude extracts to air results in a 50 % loss of activity within 1.5 h. Sulfite inhibits the dehalogenation activity of crude extracts. This inhibition is not observed with sulfate. The involvement of cobalamin in the dehalogenation reaction was implicated from crude extract activities obtained with different artificial electron donors and the inhibition of the activity by prolyl iodide.

2.4 '*Dehalococcoides ethenogenes*' 195 (Maymo-Gatell *et al.* 1997)

Isolation and cultivation. The enrichment culture was developed from a sample of sewage sludge. The initial enrichment culture was fed every 2 d with PCE (3.5 μmol/L) and methanol (0.32 mmol/L). The doses of PCE and methanol were gradually increased until they reached a concentration of 0.55 mmol/L PCE and 1.6 mmol/L methanol, leading to a significant increase in the dehalogenating activity and a decline of methanogenesis. Subsequently the methanol was replaced with hydrogen and the cultivation medium was amended by the spent medium from the methanol-PCE culture. The co-culture of irregular cocci and rods was obtained in the 10^{-6} dilution tubes. This partially purified culture was cultivated in an H_2-PCE medium supplemented with acetate (2 mmol/L), vitamin B_{12} (50 μg/L) and anaerobic digester sludge supernatant (25 %, *V/V*). The same medium supplied with filter-sterilized extract from H_2-PCE cultures was used for the isolation of the pure culture of the isolate 195. The axenic culture (irregular cocci) was obtained in a 10^{-7} dilution with the H_2-PCE medium containing ampicilin (0.3 g/L). The doubling time was about 19.2 h.

Electron donors and acceptors, carbon sources and growth factors. Hydrogen is required for PCE reduction. For growth both PCE and hydrogen are required. The following compounds cannot serve as electron donors: ethanol, formate, glucose, lactate, methanol, pyruvate and yeast extract. The following compounds cannot serve as electron acceptors: fumarate, nitrate, nitrite, oxygen, sulfate, sulfite and thiosulfate. The organism requires vitamin B_{12} and other unidentified growth factors for growth.

Morphology and classification. The organism grows as small irregular coccoid cells. The cell wall of strain 195 has an ultrastructure resembling the S-layer protein subunit type of cell walls found in *Archaea*. 16S-rDNA analysis positioned strain 195 within the eubacteria, but the organism could not be affiliated to any presently known taxa. The 16S-rDNA sequence is deposited in the GenBank under accession number AF004928.

PCE dechlorination. Strain 195 is the first organism which was reported to dehalogenate PCE completely to ethene. The specific dechlorination activity is 4,14 mmol/h per g cell protein. PCE is metabolized to vinyl chloride at a rate of 40 μmol/h per liter of culture medium. The conversion of vinyl chloride to ethene occurs after PCE depletion and follows first-order kinetics with a half-life of about 80 h for the first 300 h and of about 150 h thereafter. The culture reductively dehalogenates 1,2-dichloroethane and 1,2-dibromoethane. Growth and dechlorination are uncoupled.

3 HALORESPIRING PCE-DECHLORINATING BACTERIA (GRAM-NEGATIVE)

3.1 *Desulfomonile tiedjei* DCB-1 (DeWeerd *et al.* 1990)

Isolation and cultivation. The organism was isolated from a sewage sludge dehalogenating consortium (Shelton and Tiedje 1984) enriched for its ability to mineralize 3-chlorobenzoate. The vitamins thiamine (50 µg/L), nicotinamide (500 µg/L) and 1,4-naphthoquinone (200 µg/L) are essential for cultivation of the strain DCB-1 in an anaerobic mineral medium. Addition of yeast extract, trypticase, casamino acids, bactopeptone or neopeptone (all 0.1 %, *W/V*) to the vitamin-amended mineral medium enhances the growth. The pH range for growth is 6.5–7.8 with the optimum at 6.8–7.0. The temperature range is 30–38 °C with the optimum at 37 °C.

Electron donors and acceptors, carbon sources and growth factors. Electron donors (in alphabetical order): 3-anisate, 4-anisate, benzoate, 3-chlorobenzoate, carbon dioxide, formate, hydrogen, isovanillate, 3-methoxysalicylate, pyruvate and vanillate. The compounds that cannot serve as electron donors are acetate, alanine, arabinose, arginine, betaine, butanol, casamino acids, 3,4-dimethoxybenzoate, ethanol, ethylene, ferullate, fructose, fumarate, glucose, glycerol, isoleucine, lactate, lactose, methanol, 4-methoxysalicylate, proline, propanol, succinate and valine. The following compounds serve as electron acceptors: *meta*-halobenzoates, sulfate, sulfite and thiosulfate. The fastest growth rate and highest cell yield is obtained with pyruvate as carbon source. Strain DCB-1 can grow on this substrate fermentatively. Thiamine, nicotinamide and 1,4-naphthoquinone are required as growth factors.

Morphology and classification. The cells are rod-shaped 0.8–1 µm in diameter and 5–10 µm in length. The organism has an unusual invagination of the cell wall that resembles a collar. The organism is Gram-negative and non-sporulating. The 16S rRNA sequence analysis indicates that *Desulfomonile* is a new genus of sulfate-reducing bacteria in the δ subdivision of *Proteobacteria.* The organism is most closely related to *Desulfuromonas acetoxidans* and *Desulfobacter postgatei.* The G + C content of the DNA is 49.0 molar %. The strain is deposited in DSM as DSM 6799 and in ATCC as ATCC 49306.

PCE dechlorination. The strain DCB-1 is primarily known for its transformation of halobenzoates. The strain was the first bacterium known to couple reductive dechlorination of 3-chlorobenzoate to growth (Shelton and Tiedje 1984). Dehalogenation of PCE to TCE by the strain DCB-1 was first reported by Fathepure *et al.* (1987). TCE was formed stochiometrically at a rate of 97.5 nmol/h per g cell protein. The dehalogenation of PCE to TCE, *cis*-1,2-DCE and traces of *trans*-1,2-DCE was reported for DCB-1 cells induced by 3-fluorobenzoate (Cole *et al.* 1995). The observed rate of PCE dechlorination in induced cells is 22 µmol/h per g cell protein, while the transformation rate of TCE to DCE is 5.5 µmol/h per g cell protein. It is not known whether aromatic and aliphatic dechlorination is catalyzed by the same enzyme(s). However, both dehalogenation activities are co-induced which indicates at least some common components of the dehalogenation pathways. A further indication of the involvement of a common enzyme in the dehalogenation of aromatic and aliphatic substrates is provided by the study of Townsend and Suflita (1997) showing that both aryl and alkyl dehalogenation activities were inhibited by sulfur oxyanions. The organism conserves the energy for growth from reductive dechlorination. The 3-chlorobenzoate-reductive dehalogenase of DCB 1 has been isolated from the membrane fraction (Ni *et al.* 1995). The bacterium also contains cytochrome c_3 and desulfoviridin. The inducible *c*-type cytochrome has recently been isolated from the membrane fraction of DCB-1 and purified (Louie *et al.* 1997). Its sequence is unlike any presently known *c*-type cytochrome.

3.2 *Dehalobacter restrictus* PER-K23 (Holliger *et al.* 1993, 1998)

Isolation and cultivation. Strain PER-K23 was enriched from a packed-bed column filled with anaerobic river sediment and granular sludge from a sugar refinery, transforming PCE to ethane (deBruin *et al.* 1992). In the enrichment, the material from the packed-bed column was inoculated into the two-liquid-phase system with the N_2–CO_2 gas phase. An aliquot of 5 mL of PCE dissolved in hexadecane (200 mmol/L) was supplied as electron acceptor. The lactate was provided as electron donor in cultivation with the N_2–CO_2 gas phase. The maximum specific growth rate estimated from chloride production was 0.024/h with a doubling time of 29 h. The strain grows optimally between 25 and 35 °C, and no growth is observed at 37 °C. The pH optimum lies between 6.8 and 7.6, no growth occurs at pH <6.5 and >8.0. Best growth is obtained with stationary incubation for 2–3 d followed by semi-continuous cultivation at a frequency of 2 Hz.

Electron donors and acceptors, carbon sources and growth factors. The substrate range of this organism is very narrow. Only hydrogen serves as electron donor. Other compounds tested were (in alphabetical order): acetate, acetoin, alanine, aspartate, butyrate, carbon monoxide, ethanol, formate, fructose, fumarate, glucose, glutamate, glycerol, glycine, lactate, methanol, propanol propionate, pyruvate, succinate and xylose. Only PCE and TCE were shown to serve as effective electron acceptors. PER-K23 is the first

isolated organism that is completely dependent on a chlorinated hydrocarbon as electron acceptor. The following compounds could not serve as electron acceptors: acetoin, carbon dioxide, dimethyl sulfoxide, fumarate, glycine, hydroxyacetone, nitrate, nitrite, oxygen, 2-oxoglutarate, pyruvate, sulfate, sulfite, sulfur, thiosulfate and trimethylamine N-oxide. Other chlorinated compounds that did not serve as electron acceptors include chloroethane, *trans*-1,2-DCE, *cis*-1,2-DCE, hexachloro-1,3-butadiene and vinyl chloride. Acetate serves as carbon source. The strain PER-K23 requires fermented yeast extract or growth factors (iron, thiamine, cyanocobalamin, arginine, histidine and threonine) for growth.

Morphology and classification. The cells are rod-shaped with tapered ends and with cell size of 0.3–0.5 μm (diameter) and 2–3 μm (length). The organism is motile with one lateral flagellum. The cells appear singly or in pairs and do not form spores. The Gram stain is negative. However, a Gram-negative cell-envelope was not confirmed in ultrathin sections. The cell wall is surrounded by a protein surface layer (S-layer). The G + C content of the DNA is 45.3 molar %. The strain could not be affiliated to already known bacterial taxa on the basis of biochemical characterization. Analysis of 16S rRNA revealed that the strain PER-K23 is related to genera in the fourth subdivision of Gram-positive bacteria. The strain PER-K23, together with *D. restrictus* TEA, *Syntrophobotulus glycolicus*, *Desulfosporosinus* (*Desulfotomaculum*) *orientis* and desulfitobacteria form a phylogenetic cluster with at least 88.5 % of sequence similarity. The organism was deposited in DSM as the type culture DSM 9455^T.

PCE dechlorination. The dechlorination products of PCE degradation by the strain PER-K23 are TCE and *cis*-1,2-DCE. Electron balances showed that all electrons derived from the oxidation of the electron donor (hydrogen or formate) are completely recovered in the dechlorination products and the biomass with the growth yield of 2.1 g protein per mol released chloride. Concentrations of PCE above 200 μmol/L in the water phase are toxic for this organism. Membrane-bound PCE reductase was implicated in the dechlorination and the enzyme has recently been purified (Wohlfarth and Diekert 1997). The organism contains *b*-type cytochromes.

3.3 *Dehalobacter restrictus* TEA (Wild *et al.* 1996)

Isolation and cultivation. The isolate TEA was enriched from a laboratory fixed-bed reactor (Wild *et al.* 1995) packed with material from a full size anaerobic charcoal reactor. The charcoal reactor was originally inoculated with contaminated groundwater. This habitat contained 30 mmol/L of PCE. The culture was cultivated anaerobically under H_2–CO_2 (4 : 1, *V*/*V*) on a rotary shaker at 30 °C in the dark. Spent medium from the fixed-bed reactor was used as source of growth factors. The spent reactor medium could be successfully replaced with 1 mmol/L acetate.

Electron donors and acceptors, carbon sources and growth factors. Hydrogen serves as electron donor. TEA cannot use lactate, pyruvate, acetate, formate and glucose as electron donors. PCE and TCE serve as electron acceptors, while acetate and carbon dioxide can be used as carbon sources. The organism requires unidentified growth factor(s) for growth.

Morphology and classification. The cells are rod-shaped with the size of 0.2–0.3 μm (diameter) and 2–5 μm (length). The cells are motile, equipped with 1 to 5 lateral flagella and a crystalline surface layer. 16S-rDNA analysis indicates that the isolate TEA belongs to the subdivision of Gram-positive bacteria with low GC content. The 16S-rRNA sequence of TEA is 99.7 % identical with the sequence of *Dehalobacter restrictus* strain PER-K23. The isolate TEA is also closely affiliated with *Desulfosporosinus* (*Desulfotomaculum*) *orientis* (94 % sequence identity) and *Desulfitobacterium dehalogenans* (92.5 % sequence identity). The 16S-rDNA sequence is deposited in EMBL under accession number Y10164.

PCE dechlorination. The isolate TEA reductively dehalogenates PCE or TCE to *cis*-1,2-DCE. Dehalogenation is coupled to growth but the ability to grow on polychlorinated ethylenes is lost in the presence of glucose, pyruvate, lactate, acetate and formate. Electron balances indicate that the oxidation of hydrogen coupled with the reductive dehalogenation of TCE to *cis*-1,2-DCE is the only relevant energy-generating system with a growth yield of 0.1–0.25 g of biomass per mol chloride released.

3.4 '*Dehalospirillum multivorans*' (Neumann *et al.* 1994; Scholz-Muramatsu *et al.* 1995)

Isolation and cultivation. This organism was enriched from an activated sludge that had not been exposed to chlorinated ethenes using the anaerobic mineral medium containing pyruvate (20 mmol/L) and tetrachloroethene (0.2 mmol/L). The doubling time on acetate is 2.5 h at 37 °C. The optimum pH for growth is 7.0–7.5. At pH 6.0 or 8.5, almost no dechlorination is observed. The bacteria grew in liquid media at temperatures between 15 and 33 °C. The optimum temperature is 30 °C while no growth is observed at 37 °C.

Electron donors and acceptors, carbon sources and growth factors. The following compounds serve as electron donors (in alphabetical order): ethanol, formate, glycerol, hydrogen, lactate and pyruvate. Other tested substrates that cannot serve as electron donors are acetate, fructose, glucose, glucitol and mannitol. In the PCE dehalogenase assay, only artificial electron donors with a standard redox potential of less than –360 mV are effective electron donors for PCE reduction (Miller *et al.* 1997). PCE and fumarate were shown to serve as electron acceptors. Acetate is required as carbon source for growth with hydrogen or formate + PCE.

Morphology, phenotype and classification. The cells are Gram-negative spirals with cell size of 0.45 μm (diameter) and 2–5 μm (length). '*D. multivorans*' does not form spores. The organism can be motile. The G+C content is 41.5 molar %. The 16S-rDNA sequence deposited in EMBL has accession number X82931. This organism is positioned among the ε subgroup of *Proteobacteria*.

PCE dechlorination. '*D. multivorans*' grows on a mineral medium with H_2 + PCE as the sole energy source. Dechlorination rates determined with growing cultures and with cell extracts were 0.84 and 8.4 μmol/h per g cell protein, respectively. A PCE concentration higher than 300 μmol/L inhibits dehalogenation. *cis*-1,2-DCE and chloride are the final products of PCE degradation. Tetrachloroethene-reducting dehalogenase from '*D. multivorans*' was purified from the soluble fraction (Neumann *et al.* 1995, 1996). Corrinoid (vitamin B_{12}) and iron-sulfur are present as co-factors in this dehalogenase. Menaquinone and cytochromes of the *b*- and *c*-type can be involved in the respiration cycle. PCE dehalogenation is coupled with energy conservation *via* electron transport phosphorylation.

3.5 *Desulfuromonas chloroethenica* TT4B (Krumholz *et al.* 1996; Krumholz 1997)

Isolation and cultivation. Strain TT4B has been isolated from anaerobic sediments known to be contaminated with industrial solvents including trichloroethene and toluene. The sediments were inoculated into anaerobic mineral medium amended with 0.25 % Bacto-Agar and 61 μmol/L PCE. Pure culture was obtained after repeated 10 % transfers with agar and PCE, followed by transfers with acetate (10 mmol/L) and PCE (200 mmol/L). Amendments of yeast extract and Casitone caused significant growth inhibition. No growth is observed when the culture is exposed to air. The generation time ranges from 2 to 4 d, depending on growth conditions (electron donor and acceptor concentrations). The pH range suitable for growth on acetate is from 6.5 to 7.4, with an optimum around 7.4. No growth was observed at initial pH of 6.2 or 7.8. The bacteria grew on acetate between 21 and 31 °C, but not at 16 or 35 °C.

Electron donors and acceptors, carbon sources and growth factors. The strain grows in a mineral medium with either acetate or pyruvate as electron donor and any one of Fe(III), fumarate, nitriloacetate, PCE, polysulfide and TCE as electron acceptor. The following compounds cannot serve as electron donors: benzene, benzoate, butyrate, casamino acids, citrate, crotonate, ethanol, formate, fructose, glucose, glycerol, hydrogen, lactate, mannitol, methanol, toluene, tryptone and phenol. Compounds not acting as electron acceptors include 1,1,1,2-tetrachloroethane, 1,1,2,2-tetrachloroethane, 1,4-dichlorobenzene, carbon tetrachloride, chloroform, nitrate, oxygen, sulfate and sulfur.

Morphology and classification. The cells are Gram-negative, rod shaped with rounded ends. The cell size is 0.6 μm (diameter) and 1.0–1.7 μm (length). The cells appear singly and do not form spores. The flagellum is subpolar. The 16S-rRNA sequence analysis suggests that *D. chloroethenica* is most closely affiliated with *D. acetexigens, D. palmitatis* and *D. acetoxidans* within the *Desulfuromonas–Pelobacter* phylogenetic cluster. *D. chloroethenica* is unique among the desulfuromonads in using chloroethenes as electron acceptors. The 16S-rRNA sequence is deposited in the GenBank under accession number U49748. The type strain has been deposited in ATCC under accession number ATCC 700295^{T}. A total of 9 species of the genus *Desulfuromonas* are available from DSM.

PCE dechlorination. The optimum concentration of PCE in hexadecane (in a two-phase hexane–water system) is 100 mmol/L which corresponds to approximately 47 μmol/L of dissolved PCE in the aqueous solution. The activity is lower both below and above this concentration. *cis*-1,2-DCE and chloride are the final products of PCE degradation by the strain TT4B. One or more cytochromes, possibly of the *c*-type, can be involved in the respiratory metabolism of this organism.

4 ACETOGENIC PCE-DECHLORINATING BACTERIA

4.1 *Sporomusa ovata* H1 (Terzenbach and Blaut 1994)

Isolation and cultivation. The organism was isolated from sugar-beet leaf silage (Möller *et al.* 1984). The enrichment was done in a basal medium amended with N,N-dimethylglycine. The cells can be

grown on a complex medium with methanol as the sole source of carbon and energy. Degradation of PCE (300–400 nmol) has been observed in an anaerobic N_2–CO_2 atmosphere (4:1, *V/V*) at 37 °C. The pH range for growth is 5.0–8.1 with an optimum of 5.3–7.2. The temperature range is 15–45 °C with an optimum at 34–39 °C.

Electron donors and acceptors, carbon sources and growth factors. The following compounds are fermented by strain H1 (in alphabetical order): betaine, 2,3-butanediol, ethanol, formate, fructose, lactate, methanol, butanol, N,N-dimethylglycine, propanol, pyruvate and sarcosine. Acetate is the major fermentation product. The organism can also use carbon monoxide, methanol and Ti(III) as electron donors. Hydrogen cannot serve as the electron donor.

Morphology and classification. The cells grow as curved rods with tapered ends. The cell size is 0.7–1 μm (diameter) and 1–5 μm (length). Heat-resistant spores have the size of 0.9–1 μm (diameter) and 1.3–1.4 μm (length). The G+C content of the DNA is 41.3–42.2 molar %. The strain *S. ovata* H1 was deposited in DSM as the type culture DSM 2662^T. Two additional strains of *S. ovata* are available in DSM, namely *S. ovata* DSM 2663 (Möller *et al.* 1984) and *S. ovata* DSM 3300 (Möller *et al.* 1984).

PCE dechlorination. The only products of PCE reduction are TCE and chloride. In resting cells of *S. ovata*, PCE-dechlorination parallels the formation of acetate. The rate of dechlorination is dependent on the concentration of the substrate. In the presence of 4.1 μmol/L PCE the rate of dechlorination is 2.4 μmol/h per g cell protein. Upon addition of 136 μmol/L PCE the rate increases to 9.8 μmol/h per g cell protein. Dechlorination of PCE does not occur when methanol or CO_2 are omitted. The heat stable co-factor (corrinoid) is involved in the PCE dechlorination. In addition, the involvement of the enzyme from the Wood–Ljungdahl pathway in the PCE-dechlorinating activity was proposed.

4.2 *Acetobacterium woodii* WB1 (Egli *et al.* 1988)

Isolation and cultivation. The culture was enriched by inoculation of 0.5 mL of a marine sediment into 8 mL of basal medium amended with vitamin and mineral solutions (Balch *et al.* 1977). Incubations were done under an atmosphere of H_2–CO_2 (67:33, *V/V*) at 30 °C. Acetogenic colonies can be distinguished on the agar plates by a clearing of $CaCO_3$ (20 g/L) in the vicinity of the colonies. The generation time of the organism at 30 °C is 6 h. For the dehalogenation experiments, the cells were grown autotrophically or with 8 mmol/L fructose as the carbon source. An optimal temperature for growth is 30 °C. The cells remain viable at pH 5 for many weeks.

Electron donors and acceptors, carbon sources and growth factors. The following compounds can be fermented by strain WB1: formate, fructose, glucose, glycerate, hydrogen, and lactate. The substrates, which do not support growth are: alginic acid, amino acids, arabinose, butanol, casamino acids, casein, cellobiose, cellulose, 2-deoxyglucose, ethanol, δ-galactonolactone, galactose, galacturonic acid, δ-gluconolactone, glucuronic acid, glycerol, *myo*-inositol, lactose, maltose, mannitol, mannose, melezitose, methanol, pectin, 1-propanol, raffinose, rhamnose, ribose, short-chain fatty acids, starch, sucrose, trehalose and xylose. Pantothenate is required as a growth factor.

Morphology and classification. The cells are oval-shaped rods with the cell size 1 μm (diameter) and 2 μm (length). Cells often occur in pairs. The organism is Gram-positive, highly motile by means of one or two subterminal flagella and does not form spores. The G + C content of the DNA is 39.0 molar %. *Acetobacterium* is most closely related to the genus *Eubacterium* and was placed in the family *Propionibacteriaceae*. The strain was deposited in DSM (DSM 1030), in ATCC (ATCC 29683) and in the *Japanese Collection of Microorganisms* (JMC) [JCM 2381]. One additional *A. woodii* strain (DSM 2396) is available in DSM (Schink and Stieb 1983).

PCE dechlorination. Dechlorination of PCE by strain WB1 was observed in the resting cell assay. The dechlorination occurs only when fructose (5 mmol/L) is present in the reaction medium. The TCE is the product of PCE dehalogenation. A number of other halogenated substrates are also reductively dehalogenated by strain WB1: 1,1-DCE, dichloromethane, chloromethane, tetrachloromethane, 1,1,1-trichloroethane and trichloromethane.

5 METHANOGENIC PCE-DECHLORINATING BACTERIA

5.1 *Methanosarcina* sp. (Fathepure and Boyd 1988*a*)

Isolation and cultivation. This organism was isolated from a chlorophenol-degrading enrichment (Boyd and Shelton 1984) and adapted to grow on the PREM medium amended with acetate (50 mmol/L) or

methanol (25 mmol/L) as the sole source of carbon and energy. The incubations were performed in a medium containing PCE (1 mg/L) plus acetate (25 mmol/L) or methanol (25 mmol/L).

Morphology and classification. The genus *Methanosarcina* forms large clusters of roughly spherical or asymmetric units 2–3 μm in diameter aggregated into masses of a few hundreds of units (Starr *et al.* 1981). Each unit is further compartmentalized into small units or segments approximately 0.5 μm in diameter. The cells are nonmotile. *Methanosarcina* belongs to *Archaea.* A total of 32 methanosarcina strains are deposited in DSM and 8 strains are available from ATCC.

PCE dechlorination. The reductive dechlorination of PCE occurs without a lag phase. TCE and chloride are the only products of PCE dechlorination. The extent of dechlorination depends on cell mass. The observed rate of the TCE formation is 94 nmol per week. PCE dechlorination by this organism, as well as by other methanogens, is most likely co-metabolic due to the activity of methyl-coenzyme M reductase (Holliger and Schraa 1994).

5.2 *Methanosarcina mazei* S-6 (Fathepure and Boyd 1988*a*)

Isolation and cultivation. The organism was isolated from a sewage sludge plant (Mah 1980). The isolation was accomplished by inoculating 10-fold dilutions of the laboratory digester into roll-tubes containing complex medium. A substrate combination of methanol (5 mmol/L) and calcium acetate (50 mmol/L) were used during isolation. The organism was maintained under N_2 atmosphere. For the dehalogenation experiments the organism was adapted to grow in the PREM medium amended with acetate (50 mmol/L) or methanol (25 mmol/L) as the sole carbon and energy source. Dehalogenation of PCE was tested in medium containing PCE (1 mg/L) plus acetate (25 mmol/L) and methanol (25 mmol/L). The strain grows between 30 and 40 °C with growth optimum at 37 °C. The pH range for growth is pH 6.1–8.0, the optimum being 7.0. The generation times on methanol, methanol plus acetate, and acetate are 7.7, 7.7, and 16.6 h, respectively.

Morphology and classification. The cells are Gram-negative coccoid with cell size of 1–3 μm (diameter); the organism is nonmotile. *Methanosarcina mazei* is the basonym of *Methanococcus mazei, Methanococcus frisius* and *Methanosarcina frisia.* The strain is available from DSM as type culture DSM 2053^T, from ATCC (ATCC 43572) and from the *Oregon Collection of Methanogens* (OCM) [OCM 26]. In total, there are 18 different strains of *Methanosarcina mazei* deposited in DSM.

PCE dechlorination. Dechlorination occurs without a lag phase. TCE and chloride are the reaction products. The extent of dechlorination depends on cell mass.

5.3 *Methanobacterium thermoautotrophicum* MARBURG (Egli *et al.* 1987)

Isolation and cultivation. The strain was enriched from an anaerobic sewage digester. The organism was grown in a mineral medium at 63 °C during the dehalogenation experiment. A chlorinated compound (200 mmol/L in glacial acetic acid) was added after the A_{546} of the culture reached 0.1. The stationary state is reached in about 36 h under these conditions. A generation time of 5 h is among the shortest observed for pure isolates of methanogens. The optimum temperature lies between 65 and 70 °C, while growth does not occur under 40 °C, the optimum pH range being 7.2–7.6. Growth of this organism is dependent on nickel, cobalt and molybdenum.

Morphology and classification. The cells are long rod-shaped or filamentous, with cell size of 0.4–0.6 μm (diameter) and 3–7 μm (length). They are Gram-positive and nonmotile (Starr *et al.* 1981). *M. thermoautotrophicum* is the only extreme thermophilic methanotroph isolated to-date. The G + C content is 49.7–52.0 molar %. *Methanobacterium thermoautotrophicum* is basonym of *M. thermoalcaliphilum* and *M. thermoformicicum. M. thermoautotrophicum* MARBURG is available from DSM (DSM 2133). Further 16 strains of *M. thermoautotrophicum* have been deposited in DSM and 4 strains are available in ATCC.

PCE dechlorination. M. thermoautotrophicum MARBURG dehalogenates PCE to TCE during autotrophic growth. The organism is also able to dehalogenate 1,2-dichloroethane, 1,1,1-trichloroethane, trichloromethane and tetrachloromethane during its autotrophic growth, when only 1,2-dichloroethane undergoes complete dechlorination. The involvement of an enzyme in the dehalogenation was implicated from the specificity observed toward various halogenated substrates. A reductive mechanism was proposed based on halohydrocarbon recoveries.

6 FACULTATIVELY ANAEROBIC PCE-DECHLORINATING BACTERIA

6.1 Isolate MS-1 (Sharma and McCarty 1996)

Isolation and cultivation. MS-1 was isolated from aquifer material taken from a PCE-contaminated site. During the enrichment the aquifer material was added to the mineral medium containing sodium benzoate, PCE and sodium sulfate. After three months of cultivation, an aquifer-material-free enrichment was subcultured in a basal medium, containing benzoate (60 mg/L) and yeast extract (50 mg/L). Further isolation was done with a thioglycolate agar. All manipulations were done under strictly anaerobic conditions (100 % N_2). The doubling time of MS-1 cultivated in a medium containing yeast extract (1 g) and under optimal growth conditions (pH 7, 37 °C) is about 42 min.

Electron donors and acceptors, carbon sources and growth factors. The following substrates were shown to serve as electron donors: acetate, amino acids, formate, glucose, lactate, pyruvate and yeast extract. Nitrate, oxygen, PCE, and TCE serve as electron acceptors. The isolate MS-1 can also grow fermentatively on a number of saccharides, short-chain fatty acids, amino acids, purines and pyrimidines.

Morphology and classification. Isolate MS-1 is Gram-negative, rod-shaped, with cell size of 0.8–1.0 μm (diameter) and 2.8–3.5 μm (length). The MS-1 is motile, capsule-forming, and nonspore-forming with peritrichous fimbriae. The cells appear singly, in pairs, or occasionally as long chains. The MS-1 belongs to the family *Enterobacteriaceae*, but presently cannot be placed in a particular genus. Metabolically, the MS-1 closely resembles *Pantoea* (*Enterobacter*) *agglomerans* biogroup 5. Analysis of 16S rRNA showed that the organism is related to *Citrobacter freundii* and *Serratia marcescens*. The 16S-rRNA sequence deposited in GenBank has an accession number L43508.

PCE dechlorination. The isolate MS-1 is the first published facultative bacterium that reductively dehalogenates PCE. The final products of PCE dehalogenation are *cis*-1,2-DCE and chloride. Vinyl chloride or other products of DCE are not produced by this organism. The observed dechlorination rates of PCE are 498 μmol/h per g dry mass of cells. MS-1 tolerates PCE concentrations up to 10 mmol/L. PCE dehalogenation requires the absence of oxygen, nitrate, and high concentrations of fermentable compounds.

6.2 *Pantoea agglomerans* ATCC 27993 (Sharma and McCarty 1996)

Isolation and cultivation. The strain used in PCE-dehalogenation experiments has been obtained from ATCC (ATCC 27993). This strain was originally isolated from blood. Dehalogenation of PCE was conducted under anaerobic conditions (N_2 atmosphere) in a mineral medium containing acetate (260 mg/L), yeast extract (10 mg/L), and PCE (100 μmol/L).

Morphology and classification. Pantoea agglomerans is the basonym of *Enterobacter agglomerans*, *Erwinia herbicola* and *Erwinia milletiae*. The organism is representative of biogroup 5. Overall there are 10 strains of *Pantoea* (*Enterobacter*) *agglomerans* available from DSM and 175 strains from ATCC.

PCE dechlorination. Dehalogenation of PCE by *P. agglomerans* is presumably fortuitous. The final products of PCE dehalogenation are *cis*-1,2-DCE and chloride. The organism dehalogenated 100 μmol PCE to *cis*-1,2-DCE within 12 d.

7 AEROBIC PCE-DECHLORINATING BACTERIUM

7.1 *Pseudomonas* sp. (Deckard *et al.* 1994)

Isolation and cultivation. The organism has been isolated from contaminated soil. The isolates subcultured on nutrient agar were screened for dehalogenation of halogenated substrates using PCE and TCE as the only carbon sources. Aerobic conditions were used for organism isolation. The organism was cultivated in a chloride-free mineral salt medium, amended with PCE (2 mmol/L) and saturated with oxygen. Incubations were performed at 28 °C under stationary conditions.

Morphology and classification. The bacterium is a Gram-negative rod. The bacterium is oxidase-positive and catalase-negative, positive for both nitrate and nitrite reduction without gas formation. It has been classified as *Pseudomonas* based on fatty-acid analysis.

PCE dechlorination. This is the first and only pure culture claimed to dehalogenate PCE under aerobic conditions. It was reported that this organism was able to grow on PCE as the only source of carbon and energy. The growth was measured as an increase of total protein concentration per sample time. An approximate 3.5-fold increase in total protein was obtained after 3 d of incubation. Dechlorination of PCE was implied from the production of inorganic chloride and depletion of the substrate from the media (65 %

of PCE in 2 d). However, the other degradation metabolites were not identified. The results obtained certainly need further validation since aerobic (oxidative) dechlorination of PCE is not mechanistically supported.

8 CONCLUSIONS

Tetrachloroethene is one of the most frequently occurring groundwater contaminants. The involvement of different metabolic groups of bacteria in the reductive dehalogenation of PCE has been implicated from mixed-culture experiments and *in situ* observations, but until recently, pure cultures of PCE-dechlorinating bacteria had not been obtained. Presently there are at least 15 axenic cultures known to be able to transform PCE. These bacteria belong to four different metabolic groups, *viz.* halorespirators (9), acetogens (2), methanogens (3) and facultative anaerobes (2). A single organism (*Pseudomonas* sp.) was reported to have the ability to dehalogenate PCE under fully aerobic conditions. However, the reaction products of this dehalogenation reaction were not identified and further validation is necessary to confirm this unexpected result. No denitrifying organisms capable of dehalogenation of PCE have been isolated thus far, which is consistent with the observation that PCE-dechlorination is inhibited under denitrifying conditions. PCE-dehalogenating bacteria have been isolated from different sources, such as sewage sludge, contaminated soil, water or sediment, and bioreactor columns, all representing anaerobic environments. The most frequently used atmosphere for isolation and cultivation of these organisms is N_2–CO_2 (4 : 1, *V*/*V*), H_2–CO_2 (4 : 1, *V*/*V*) or N_2. The pH range observed for the growth of PCE-dechlorinating bacteria is pH 6–8, with the optimum around pH 7. The temperature range is between 13 °C (*D. dehalogenans* JW/IU-DC1) and 65 °C (*M. thermoautrophicum* MARBURG), with the optimum for most of the species 30–37 °C. Depending on the metabolic group, the organisms show different extents of dehalogenation. The sulfate reducers, acetogens and methanogens conduct only a single conversion of PCE to TCE, while facultative anaerobes and most of the halorespirators (all but three species of the *Desulfitobacterium* spp.) continues further conversion down to *cis*-1,2-DCE. To date, a single organism ('*Dehalococcoides ethenogenes*' 195) has been isolated which is able to conduct the conversion of PCE to the nonhalogenated compound ethene. The extent of dechlorination relates to the type of metabolism: co-metabolic dehalogenation is expected for acetogens and methanogens, while halorespirators are able to gain the energy from the dehalogenation reaction. Reported inhibition concentrations of PCE for three dechlorinators lies in the 0.1–0.3 mmol/L range, while only one organism (isolate MS1) is described that is able to grow in a medium containing 10 mmol/L of PCE. Several of the PCE-dechlorinating organisms require the addition of some growth factor to the medium in order to survive in an axenic culture. Vitamin solutions, fermented yeast extract or spent medium from bioreactors can be used as a source of these factors. PCE-dechlorinating organisms differ significantly in their affinity for the various sources of electron donors and acceptors. This variability is most apparent within the group of halorespirators. In this metabolic group some species (*Dehalobacter restrictus*) express a very narrow substrate range with H_2–PCE as the sole electron donor–acceptor pair, while other species utilize a broad range of electron donors and/or acceptors (*e.g.*, *Desulfitobacterium* spp., '*Dehalospirillum*' spp.). It is believed that a missing electron donor could be one of the factors limiting dehalogenation of chlorinated ethenes in the environment and providing the subsurface with the appropriate electron donor thus represents a possible target for bioremediation technology *in situ.* A number of PCE-dechlorinating organisms are available from DSM (8 species) and/or ATCC (7 species). This availability of strains can positively contribute to future research on the biodegradation of PCE by axenic cultures.

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