APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1993, p. 2991–2997
0099-2240/93/092991-07$02.00/0

Vol. 59, No. 9

A Highly Purified Enrichment Culture Couples the Reductive Dechlorination of Tetrachloroethene to Growth

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Received 17 March 1993/Accepted 29 June 1993

A microscopically pure enrichment culture of a gram-negative anaerobic bacterium, in the present article referred to as PER-K23, was isolated from an anaerobic packed-bed column in which tetrachloroethene (PCE) was reductively transformed to ethane via trichloroethene (TCE), *cis*-1,2-dichloroethene (*cis*-1,2-DCE), chloroethene, and ethene. PER-K23 catalyzes the dechlorination of PCE via TCE to *cis*-1,2-DCE and couples this reductive dechlorination to growth. H_2 and formate were the only electron donors that supported growth with PCE or TCE as an electron acceptor. The culture did not grow in the absence of PCE or TCE. Neither O_2, NO_3^-, NO_2^-, SO_4^{2-}, SO_3^{2-}, $S_2O_3^{2-}$, S, nor CO_2 could replace PCE or TCE as an electron acceptor with H_2 as an electron donor. Also, organic electron acceptors such as acetoin, acetol, dimethyl sulfoxide, fumarate, and trimethylamine *N*-oxide and chlorinated ethanes, DCEs, and chloroethene were not utilized. PER-K23 was not able to grow fermentatively on any of the organic compounds tested. Transferring the culture to a rich medium revealed that a contaminant was still present. Dechlorination was optimal between pH 6.8 and 7.6 and a temperature of 25 to 35°C. H_2 consumption was paralleled by chloride production, PCE degradation, *cis*-1,2-DCE formation, and growth of PER-K23. Electron balances showed that all electrons derived from H_2 or formate consumption were recovered in dechlorination products and biomass. Exponential growth could be achieved only in gently shaken cultures. A maximal specific growth rate of 0.024 h^{-1} was estimated, and a growth yield of 2.1 g of protein per mol of Cl^- was measured. Experiments with $^{14}CO_2$ revealed that CO_2 was incorporated only via heterotrophic CO_2 fixation under the growth conditions tested. The rest of the carbon was apparently assimilated from organic compounds present in the fermented yeast extract added. On the basis of physiological characteristics, the gram-negative rod could not be assigned to a known group of anaerobic bacteria.

Tetrachloroethene (PCE) is one of the most often encountered contaminants in different environments (27, 31, 35), and it is the only chloroethene isomer that persists throughout aerobic biodegradation. Under anoxic conditions, PCE is found to be reductively dechlorinated via stepwise hydrogenolysis reactions. The first indications of such biotransformations of PCE were obtained in batch experiments seeded with a methanogenic mixed culture from a laboratory-scale digester (3) or with muck from a pristine site in the Everglades (26). One study revealed that PCE could be partially mineralized to CO_2 (33). However, most biotransformations resulted in the accumulation of less-chlorinated ethenes. Especially chloroethene (VC), a product even more toxic than the parent compound, was often observed in large amounts (1, 25, 33). In a few studies, dechlorination to ethene took place (5, 8, 17). Only in the packed-bed column of de Bruin et al. (5) was a complete transformation to ethene and eventually to ethane performed with no residual chloroethene. Hence, anaerobic mixed cultures have the potential to detoxify PCE. The often-observed incomplete transformation emphasizes the need of a better understanding of the underlying microbiological aspects involved.

Methanogens are able to dechlorinate PCE to trichloroethene (TCE) (14–16). However, the dechlorination is incomplete, and the dechlorination rates are very low. Both corrinoids and factor F_{430}, two tetrapyrrole cofactors present in high amounts in methanogens (4, 7), catalyze this reaction (18). Hence, PCE transformation by methanogens is an unspecific activity of these cofactors or of enzymes with either corrinoids or factor F_{430} as the prosthetic group. In enrichments on methanol and PCE (8) or benzoate and PCE (30), bacteria other than methanogens dechlorinated PCE. The high amounts of electrons recovered in dechlorination products (e.g., 31%) suggest that PCE served as an electron acceptor (8, 30). Chlorinated compounds are stronger oxidants than nitrate (32). On the basis of such thermodynamic considerations, chlorinated hydrocarbons could act as terminal electron acceptors in a respiration process (13, 20). *Desulfomonile tiedjei* (6) dechlorinates 3-chlorobenzoate and couples this reductive dechlorination reaction to growth (12, 23). To date, this is the only bacterium in pure culture that uses reductive dechlorination as a novel type of anaerobic respiration.

We have obtained a microscopically pure enrichment culture of an anaerobic bacterium, PER-K23, which utilizes H_2 as an electron donor and PCE as an electron acceptor. No growth was observed in the absence of PCE.

MATERIALS AND METHODS

Materials. All chemicals were of analytical grade and used without further purification. PCE and TCE were from E. Merck, Darmstadt, Germany. 1,1-Dichloroethene (1,1-DCE), *cis*-1,2-DCE, and *trans*-1,2-DCE were obtained from Aldrich Chemie N.V., Brussels, Belgium. VC, ethene, and all other gases were from Hoekloos, Schiedam, The Netherlands. Where necessary, experiments were carried out in

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an anaerobic glovebox (Coy Laboratories Products, Toepffer GmbH, Göppingen, Germany). The oxygen concentration in the anaerobic glovebox was kept low with the palladium catalyst RO-20 provided by BASF (Arnhem, The Netherlands).

Source of inoculum. The inoculum was material from a packed-bed column that transformed PCE to ethane (5). The packed-bed column was wet packed with anaerobic sediment from the Rhine River near Wageningen, The Netherlands, and with ground anaerobic granular sludge (28) from a sugar refinery (Centrale Suiker Maatschappij, Breda, The Netherlands). The reactor was percolated continuously with an anaerobic mineral medium (5). Samples for inoculation were taken after 14 months of operation.

Anaerobic media. The medium used was phosphate-bicarbonate buffered and had a low chloride concentration. The composition of the medium was as follows (in grams per liter of demineralized water, unless otherwise stated): K_2HPO_4, 0.65; $NaH_2PO_4 \cdot 2H_2O$, 0.20; NH_4HCO_3, 0.44; $CaCl_2 \cdot 2H_2O$, 0.11; $MgCl_2 \cdot 6H_2O$, 0.10; $NaHCO_3$, 3.73; $Na_2S \cdot 9H_2O$, 0.24; resazurin, 0.0005; 1 ml of trace element solution (containing in milligrams per liter: $FeCl_2 \cdot 4H_2O$, 2,000; $MnCl_2 \cdot 4H_2O$, 100; $CoCl_2 \cdot 6H_2O$, 190; $ZnCl_2$, 70; $CuCl_2$, 2; $AlCl_3 \cdot 6H_2O$, 10; H_3BO_3, 6; Na_2MoO_4, 36; $NiCl_2 \cdot 6H_2O$, 24; and EDTA, 500; plus 1 ml of concentrated HCl); 1 ml of vitamin solution (containing in milligrams per liter of medium: biotin, 0.05; folic acid, 0.02; pyridoxine, 0.1; riboflavin, 0.05; thiamine, 0.1; cyanocobalamin, 0.1; nicotinamide, 0.55; *p*-aminobenzoic acid, 0.25; lipoic acid, 0.05; pantothenic acid, 0.05); and 10 ml of a fermented yeast extract solution (4% [wt/vol]. Fermented yeast extract was generated by inoculating 500 ml of medium containing 2.1 g of $NaHCO_3$ and 20 g of yeast extract with 5 ml of anaerobic granular sludge from a sugar refinery. After an incubation for 3 to 4 weeks at 37°C, bacteria were removed by centrifugation (10,000 × *g*, 30 min, 4°C), and the supernatant was sterilized by filtration (0.2-μm-pore-size filter). Media were prepared as follows: bottles with K_2HPO_4, NaH_2PO_4, and resazurin in demineralized H_2O were sealed with Viton stoppers (Maag Technic AG, Dübendorf, Switzerland). The atmosphere was changed with either N_2-CO_2 or H_2-CO_2 (4:1 [vol/vol]) and pressurized to 1.5×10^5 Pa. The bottles were sterilized at 121°C. The rest of the components were added aseptically by syringe from filter-sterilized stock solutions. The pH of the medium was 7.0 to 7.2.

Enrichment and cultivation. The culture was enriched and cultivated in a two-liquid-phase system as described previously (21). Initial enrichment in 500-ml bottles was started by inoculating 200 ml of medium with approximately 0.5 g of material from the PCE-dechlorinating packed-bed column. The gas phase was either N_2-CO_2 or H_2-CO_2. When N_2-CO_2 was the gas phase, lactate was added as an electron donor, supplied daily to a final concentration in the enrichment of 1 mM. An aliquot of 5 ml of PCE dissolved in hexadecane (0.2 M) was added by syringe. The log of K_{HW} (partition coefficient between hexadecane and water) of PCE is 3.7. Hexadecane was sterilized by heat (121°C, 60 min), and PCE was filter sterilized through 0.2-μm-pore-size membrane filters (type GVWP; Millipore, Etten-Leur, The Netherlands). Cultures were stationary and incubated in the dark at 30°C. Dechlorination was monitored by measuring the chloride ion concentration. When an increase of Cl^- was found, the gas phase was analyzed for the presence of dechlorination products. Initial enrichments were transferred into fresh medium after 30 days of incubation. At the same time, the initial enrichment was also diluted to 10^{-8}. The highest dilution in which dechlorination occurred was diluted again to 10^{-8}. The stable culture obtained by this procedure was routinely subcultured (1% [vol/vol] transfers) in 117-ml serum bottles containing 20 ml of medium. These subcultures served as inoculum for experiments. The best growth was obtained if unshaken cultures were incubated for 2 to 3 days and then incubated while being shaken horizontally at 120 rpm on a rotary shaker.

The purity of PER-K23 was checked microscopically or in a medium containing 13 g of Wilkins-Chalgren anaerobe broth (Oxoid, Basingstoke, Great Britain) per liter of demineralized water. The gas phase was 100% N_2.

Electron donor and acceptor utilization. Various compounds were added as electron donors from concentrated stock solutions to a final concentration of 20 mM. Formate and methanol were added to a final concentration of 50 mM, while CO was tested at a partial pressure of 0.5×10^5 Pa. Electron acceptors were tested at a final concentration of 10 mM with H_2 as an electron donor. Chlorinated compounds, other than PCE and TCE, were added dissolved in hexadecane (0.2 M; 1 ml per vial), except for chloroethane and VC which were added as gaseous compounds (5 ml per vial). Duplicate bottles (117 ml containing 20 ml of medium) were inoculated (10% [vol/vol]), and the increase in turbidity was taken as a measure for growth.

Effect of temperature and pH. Cultures in 117-ml serum bottles containing 20 ml of medium and inoculated with 1% (vol/vol) were stationary and incubated in the dark at different temperatures (15, 20, 25, 30, 35, and 37°C). They were assayed daily for chloride production.

The effect of pH was tested with cultures growing on formate and PCE. The pH of the medium was varied by using different partial pressures of CO_2 in the gas phase (N_2-CO_2). Culture vials contained 40 ml of medium and were inoculated with 1% (vol/vol). Samples of 1.5 ml were withdrawn daily by syringe and analyzed for pH and chloride concentration.

$^{14}CO_2$ fixation experiment. $^{14}CO_2$ fixation experiments were performed in 117-ml serum bottles containing 40 ml of medium. The gas phase was H_2-CO_2 (4:1 [vol/vol]) at 1.5×10^5 Pa. Approximately 5 μCi of $NaH^{14}CO_3$ was added to the cultures by syringe shortly after inoculation (1% [vol/vol]). Cultures were incubated in a shaking water bath at 30°C. The CO_2-HCO_3^- content of the cultures was determined from cultures to which only unlabelled bicarbonate was added. For the determination of total radioactivity per culture, the same amount of the $NaH^{14}CO_3$ solution was added to Viton-stopper-sealed, 35-ml serum bottles containing 20 ml of 1 M NaOH. Samples of 50 μl were transferred to scintillation vials containing 4 ml of Aqualuma scintillation cocktail. The biomass content of ^{14}C-containing cultures was analyzed by measuring protein. To be able to convert protein content into carbon content of the biomass, the total organic carbon and the protein content were determined in cultures cultivated with unlabelled compounds. This resulted in a conversion factor for protein content into carbon content of 0.81. To measure the radioactivity incorporated into biomass, samples of 5 ml of the cultures were filtered through a 0.2-μm-pore-size membrane filter (Millipore). The filters were transferred to scintillation vials containing 4 ml of Aqualuma scintillation cocktail after being washed with 20 ml of 100 mM $NaHCO_3$ and after exposure of air-dried filters to HCl vapors in a desiccator for 1 h. The radioactivity was counted with a LKB Wallac 1211 Rackbeta liquid scintillation counter. Quench corrections were made by the channel-ratio method.

Electron balances. Experiments to calculate electron balances were performed in 117-ml serum bottles containing 20 or 40 ml of medium. The gas phase consisted of N_2-H_2-CO_2 (16:4:5 [vol/vol/vol]) at 1.5×10^5 Pa in the case of H_2 as the electron donor and N_2-CO_2 (4:1 [vol/vol]) in the case of formate as the electron donor. Bottles were inoculated with 1% (vol/vol) and incubated at 30°C in the dark. At time zero and after 14 days of incubation, H_2, formate, PCE, TCE, *cis*-1,2-DCE, chloride, and protein were determined. For the calculation of the hydrogen and formate consumption by biomass production, the following assumptions were used: $>C_5H_7O_2N>$ is the formula for biomass with a molecular weight of 113, and biomass is formed heterotrophically from organic material (CH_2O) and CO_2 (see results of $^{14}CO_2$ fixation experiments) according to the formula $10/3H_2 + 10/3CH_2O + 5/3CO_2 + NH_3 \rightarrow <C_5H_7O_2N> + 14/3H_2O$ or $10/3HCOOH + 10/3CH_2O + NH_3 \rightarrow <C_5H_7O_2N> + 5/3CO_2 + 10/3H_2O$. The measured total amount of protein of a culture was converted into micromoles of biomass per culture by multiplying by a factor of 0.0135. This factor was calculated from the conversion factor for protein content of the biomass into carbon content of 0.81, determined in the $^{14}CO_2$ fixation experiment, and the conversion factor for carbon content of the biomass into micromoles of biomass of 0.0167 (113/60 × 1/113). Per mole of biomass, 10/3 mol of H_2 or formate was consumed as determined by the formulas described above.

Analyses. PCE, TCE, *cis*-1,2-DCE, H_2, and CO_2 were determined by gas chromatography. PCE, TCE, and *cis*-1,2-DCE were determined in a 200-μl headspace sample with a model 438A Chrompack Packard gas chromatograph equipped with a flame ionization detector connected to a capillary column (25 m by 0.32 mm [inner diameter], Sil 5CB, 1.22 μm, Chrompack, The Netherlands) and a splitter injector (ratio, 1:50). Standards were prepared in 117-ml Viton-stopper-sealed serum bottles containing 20 ml of water and 200 μl of hexadecane. PCE, TCE, and *cis*-1,2-DCE were added from an ethanol stock solution. The retention times and peak areas were determined with a Shimadzu C-3A computing integrator. The detection limits were 1 μmol per bottle for *cis*-1,2-DCE and TCE and 3 μmol per bottle for PCE. H_2 was measured with a model 417 Packard gas chromatograph equipped with a thermal conductivity detector at 100 mA connected to a molecular sieve column (180 cm by 1/4 in. [1 in. = 2.54 cm] 60/80 mesh). CO_2 was measured with a model 406 Packard gas chromatograph equipped with a thermal conductivity detector at 100 mA connected to a packed column (Porapak Q, 600 cm by 1/8 in., 80/100 mesh). Standards were prepared by adding different amounts of 100% H_2 or CO_2 to sealed 117-ml serum bottles.

Chloride was analyzed as described previously (21). Formate was analyzed by high-performance liquid chromatography (22). Protein of whole cells was measured by a modified Lowry method (24). Total organic carbon was determined with an Ionics 555 carbon analyzer (Thermal Instruments, Etten-Leur, The Netherlands).

Other methods. Gram staining was performed by standard procedures (10). In addition, the Gram type was checked by the KOH method (19).

RESULTS

Enrichment and isolation. Initial enrichments were performed with lactate or H_2-CO_2 as an electron and carbon source and PCE as an electron acceptor. Material from the PCE-dechlorinating packed-bed column was used as an inoculum (5). After 33 days of incubation, the chloride ion concentration had increased by 3.4 to 4.6 mM. The dechlorination products TCE, *cis*-1,2-DCE, VC, and ethene were formed. Ethane, the end product of the PCE degradation in the packed-bed column, was not found. Further enrichment was done with H_2-CO_2. In the first transfer, methanogenic activity was inhibited by 2-bromoethanesulfonate (5 mM), which had no effect on the dechlorination. After only several serial dilutions, methanogens were no longer present, and the routine addition of 2-bromoethanesulfonate was stopped. Two morphologically different bacteria were predominant in the PCE-dechlorinating culture, a small thin rod and a larger thick rod. In the absence of PCE, only the thick rod grew on H_2-CO_2. The formation of up to 20 mM acetate from H_2-CO_2 indicated that this bacterium was a homoacetogen. In initial enrichments and subsequent dilution series, the medium did not contain fermented yeast extract, but selenium and tungsten were present as trace elements. Amendment of the medium with fermented yeast extract increased dechlorination rates. Omission of selenium and tungsten resulted in the loss of the homoacetogen. With an additional four serial dilutions, a microscopically homogeneous culture consisting of the small thin rod was obtained (Fig. 1). Growth was observed only in the presence of PCE. Neither methane nor acetate was formed as a product in the presence of H_2-CO_2. In the following discussion, the PCE-dechlorinating enrichment culture dominated by a small thin rod is referred to as PER-K23.

Purity tests on Wilkins-Chalgren anaerobe broth medium indicated that PER-K23 was still contaminated. No dechlorination was observed when the rod-shaped bacterium growing on this complex medium was isolated by dilution series or from single colonies on agar-containing complex medium and transferred into medium with H_2-CO_2 and PCE. Attempts to grow the PCE-dechlorinating bacterium on agar-containing medium with H_2-CO_2 and PCE in hexadecane failed.

Nutritional requirements, physiology, and growth conditions. In the presence of H_2-CO_2 and PCE, PER-K23 grew only in mineral medium supplemented with 10 ml of a fermented yeast extract solution per liter. If the fermented yeast extract was omitted, PER-K23 did not grow. In the absence of either H_2 or PCE, no growth was observed when fermented yeast extract was added. The addition of a mixture of different carbon sources and growth factors such as fatty acids (acetate, isobutyrate, isovalerate, methylbutyrate, methylvalerate, propionate, butyrate, and valerate) and additional vitamins (choline chloride, *myo*-inositol, menadione sodium bisulfite, 1,4-naphthoquinone, and hematin) to the medium did not substitute for fermented yeast extract. Attempts to replace fermented yeast extract by culture fluid of a pregrown PER-K23 culture, by supernatant of a culture of the contaminant grown on medium amended with 1% (wt/vol) yeast extract, or by crude or boiled cell extracts of the contaminant grown on Wilkins-Chalgren anaerobe broth failed.

PER-K23 had a very narrow substrate spectrum. The sole electron donors that supported growth were H_2 and formate and then only in the presence of PCE or TCE as an electron acceptor. All other electron and carbon sources tested (i.e., lactate, pyruvate, propionate, butyrate, acetate, succinate, fumarate, glycine, alanine, aspartate, glutamate, methanol, ethanol, propanol, glucose, fructose, xylose, glycerol, acetoin, and CO) were not utilized. PCE or TCE could not be replaced by inorganic or organic electron acceptors used by other anaerobic bacteria (i.e., O_2, NO_3^-, NO_2^-, SO_4^{2-},

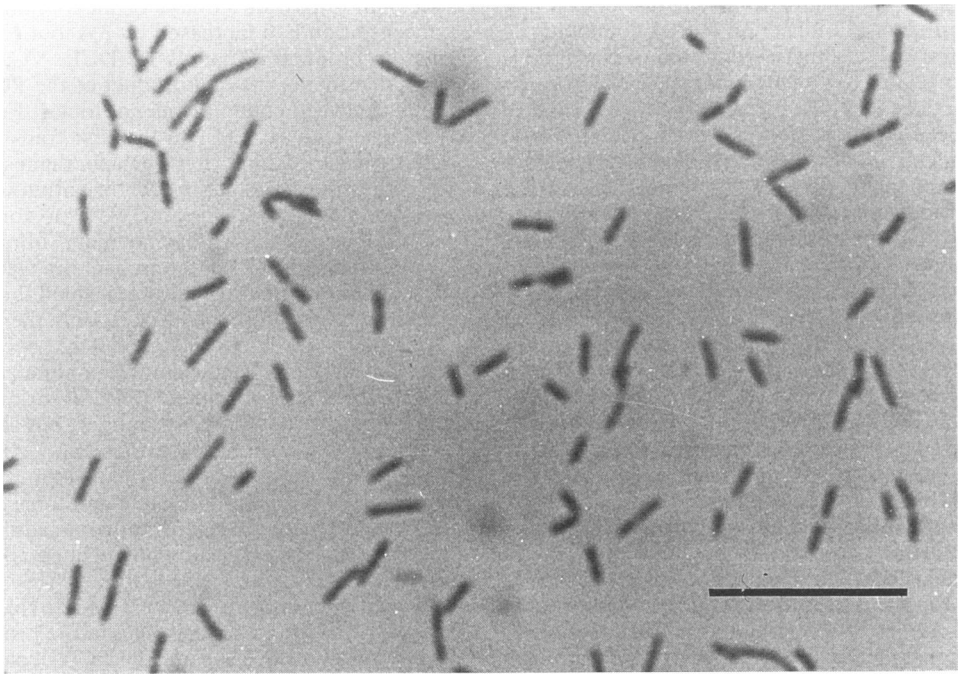

FIG. 1. Bright-light micrograph of methylene blue-colored cells of PER-K23 grown on H_2-CO_2 and PCE. Bar, 10 μm.

SO_3^{2-}, $S_2O_3^{2-}$, S, CO_2, acetoin, acetol, dimethyl sulfoxide, fumarate, glycine, 2-oxoglutarate, pyruvate, and trimethylamine *N*-oxide) and also not by other chlorinated compounds (i.e., all chloroethanes, 1,1-DCE, *trans*- and *cis*-1,2-DCE, VC, and hexachloro-1,3-butadiene).

PER-K23 grew optimally between 25 and 35°C (Fig. 2A). At 37°C, no growth was observed. Cultures incubated at 10°C did not grow. Dechlorination rates remained constant between pH 6.8 and 7.6 (Fig. 2B). No growth was observed below pH 6.4 and above pH 8.0. The maximum PCE concentration for growth in the water phase was 200 μM (data not shown). Higher concentrations were apparently toxic for PER-K23.

Chloride production in stationary incubated cultures became linear after a short exponential phase (Fig. 3). Growth limitation was apparently caused by the too-slow mass transfer of H_2 or PCE. Gently shaking cultures of PER-K23 indeed extended the exponential phase. A maximal specific growth rate of 0.024 h^{-1} (t_d = 29 h) was estimated from the chloride production data.

$^{14}CO_2$ incorporation into biomass. The specific radioactivity of the carbon fraction in the biomass was only about

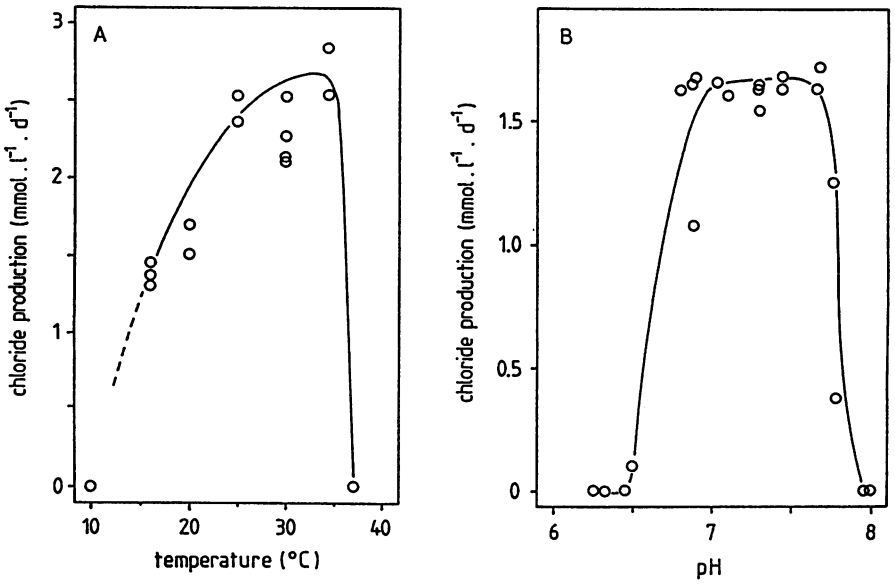

FIG. 2. Temperature (A) and pH (B) dependence of the chloride production rate on PCE.

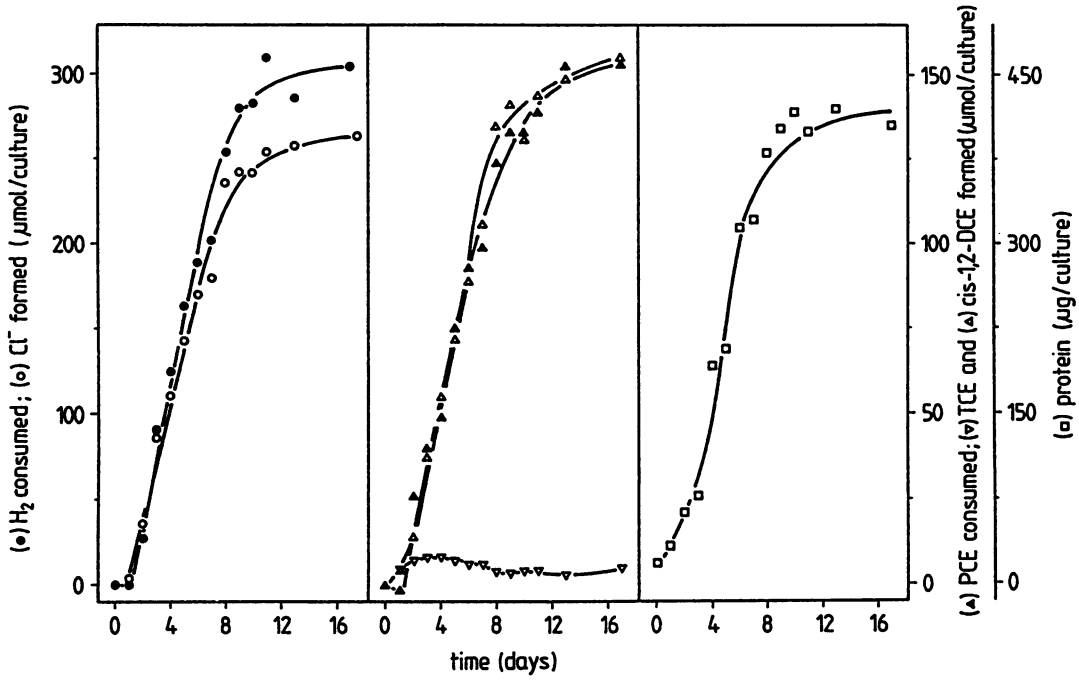

FIG. 3. H_2 and PCE consumption and product formation during growth of PER-K23.

one-third of the specific radioactivity in the CO_2-HCO_3^- pool (Table 1). Therefore, CO_2 has been incorporated into biomass by heterotrophic CO_2 fixation (e.g., pyruvate synthase), and autotrophic growth did not occur in the employed medium. Apparently, enough assimilable organic compounds were present in the fermented yeast extract to account for the rest of the biomass formed.

H_2 and PCE metabolism during growth, electron balances, and growth yields. H_2 consumption was paralleled by chloride production, PCE degradation, *cis*-1,2-DCE formation, and growth of PER-K23 (Fig. 3). TCE was found only in minor amounts and did not accumulate. In electron balances determined from PER-K23 cultures grown on H_2-CO_2 plus PCE or formate plus PCE, all electrons derived from H_2 or formate oxidation were completely recovered in dechlorination products and biomass (Table 2). Other electron-consuming processes apparently did not occur in these cultures. A molar growth yield of 2.1 g of protein per mol of Cl^- was determined. This, however, may be an underestimation since the hexadecane phase was present as an emulsion after growth of PER-K23, indicating that significant amounts of biomass have adsorbed to the organic phase.

DISCUSSION

D. tiedjei was the first organism isolated which couples growth on formate, hydrogen, or acetate to a reductive aryl-dechlorination reaction, namely the reductive dechlorination of 3-chlorobenzoate (12, 23). On the basis of the physiological characterization and 16S rRNA analysis, *D. tiedjei* was classified as a new species of a new genus of sulfate-reducing bacteria (6). The dominating organism in the enrichment culture PER-K23 is the first bacterium described that completely depends on a chlorinated hydrocarbon as an electron acceptor. We were not able to affiliate this bacterium with an already known group of bacteria on the basis of physiological characteristics. A complete purification and further characterization on the basis of cytochemical and molecular biological methods will enable us to clarify the relationship of this bacterium with other bacteria.

TABLE 1. $^{14}CO_2$ incorporation into biomass during growth of PER-K23 on H_2-CO_2 and PCE[a]

Fraction	Carbon content (µmol/culture)	Radioactivity (dpm/culture)	Specific radioactivity	
			dpm/µmol of C	%
CO_2-HCO_3^-	3,164	1.071×10^7	3,385	100
Biomass	90	1.034×10^5	1,149	34

[a] The values given are means of three independent cultures.

A particular feature of PER-K23 is the narrow spectrum of electron donors utilized. Anaerobic H_2-oxidizing eubacteria like sulfate reducers or homoacetogens are more versatile (11, 36). Methanogens are the only group of bacteria with a limited number of substrates comparable to that of PER-K23 (34). However, PER-K23 did not utilize CO_2 as an electron acceptor and did not show a fluorescence when irradiated with UV light. The only other indication for PCE-dechlorinating bacteria with such a restricted substrate spectrum was reported in a study with an anaerobic mixed culture and the inhibitor vancomycin (9). In methanol-fed cultures, acetogenesis and dechlorination were inhibited, whereas in hydrogen-fed cultures dechlorination still occurred.

Most surprisingly, PER-K23 utilized only PCE and TCE as electron acceptors. As far as we know, chlorinated ethenes have no natural origin and were not present in the environment before the start of large-quantity industrial applications about 50 years ago. Reductive dechlorination of PCE to *cis*-1,2-DCE is an exergonic reaction with a $\Delta G^{\circ\prime}$ of -377.5 kJ/mol of PCE. This would be enough energy to synthesize approximately 5 mol of ATP, assuming that an energy difference of 70 kJ is needed for the formation of 1 mol of ATP in an irreversible reaction under physiological conditions (29). Since ATP formation by substrate-level

TABLE 2. Electron balances of the reductive dechlorination of PCE to *cis*-1,2-DCE by PER-K23 with H_2 or formate as electron donor

Electron donor[a]	H_2 or formate consumed (μmol/culture)			
	Measured	Calculated from[b]:		
		Chloride + biomass	DCE + TCE + biomass	PCE − TCE + biomass
H_2	282	262 (93 ± 3)[c]	287 (99 ± 7)	299 (107 ± 10)
Formate	600	536 (91 ± 11)	641 (108 ± 5)	619 (95 ± 8)

[a] Five parallel cultures per electron donor were done. However, the electron balances of only one of the cultures are given.
[b] Calculated from measured data. It was assumed that the production of one chloride or TCE consumed one equivalent H_2 or formate and that the production of DCE or consumption of PCE consumed two equivalents of H_2 or formate. The assumptions made for the calculation of the amount of electrons consumed by biomass production are described in Materials and Methods.
[c] Values given in parentheses are percentages of the electrons derived from electron donor consumption recovered in dechlorination products and biomass and are means of five independent cultures ± standard deviations.

phosphorylation is unlikely to occur upon H_2 oxidation, electron transport phosphorylation might be the mechanism of ATP synthesis in PER-K23. The electron acceptor that was utilized by PER-K23 before the presence of PCE as pollutant, the exact process of energy conservation and the efficiency of this process remain to be elucidated.

The affinity of PER-K23 for PCE is apparently high. Initial enrichment and subsequent subculturing occurred with 40 and 200 μM PCE in the water phase, respectively. From an energetic point of view, reductive dechlorination is more favorable than acetogenesis or methanogenesis, two processes competing for the electron donor in enrichment cultures with H_2-CO_2. However, nothing is known about the affinity of PER-K23 for H_2. To predict the competitiveness of the PCE-dechlorinating bacterium with other H_2- or formate-consuming bacteria in natural environments, these kinetic data are needed. Results obtained with initial enrichments in this study and in enrichments from digested sludge (8) suggested that PCE-dechlorinating organisms can outcompete methanogens but not acetogens. It is not known whether winning the competition over methanogens is based on a higher substrate affinity or on the toxic effects of PCE. Investigations showing the inhibitory effect of chlorinated ethenes on methanogens suggest that the latter is true (2).

Experiments with $^{14}CO_2$ showed that only one-third of the assimilated carbon originated from CO_2, which indicated heterotrophic growth. PER-K23 completely depended on the addition of fermented yeast extract, and, therefore, it was impossible to investigate the possibility of autotrophic growth. The nature of the essential growth factor present in fermented yeast extract is not known. A similar nutritional dependency of hydrogen-utilizing dechlorinators was also observed in cultures enriched from a methanol-fed, PCE-dechlorinating system (9). The addition of 1,4-naphthoquinone, a growth factor that enabled the cultivation of *D. tiedjei* on a defined medium (6), did not replace fermented yeast extract. The use of fermented yeast extract probably also did not allow purification of PER-K23. The contaminant was found in all dilutions where growth occurred. On the basis of this observation, one might even postulate a certain symbiotic relationship between the PCE-dechlorinating bacterium and the contaminant.

The temperature experiment revealed that, at 10°C, no growth occurred. This is in contrast with the results obtained with the anaerobic packed-bed column (5) from which PER-K23 was isolated. In this column, PCE was efficiently dechlorinated to ethene and further transformed to ethane at 10°C. No residual PCE was found, and transformation rates of 3.7 μmol/liter/h were achieved. The experimental system of the batch incubations could explain the failure of PER-K23 to be active at 10°C. At such low temperatures, hexadecane became solid, which probably made the exchange of PCE between the organic and water phases too slow to allow growth.

It is not yet known what the physiological properties of the PCE-dechlorinating bacterium were before PCE was present as a contaminant in the environment. The high electron donor and acceptor specificities of the enrichment culture PER-K23 indicates that, within a few decades, a physiologically new type of bacterium has evolved. Further, it demonstrates the tremendous capacity of the bacterial world to adapt to new anthropogenically altered environmental conditions.

ACKNOWLEDGMENTS

This research was supported by a grant from the Netherlands Integrated Soil Research Programme and by a grant from the Innovative Research Program on Environmental Biotechnology (IOP-Milieubiotechnologie) of the Ministries of Economic Affairs and Housing, Physical Planning, and Environment.

We thank Wil de Bruin for the material from the PCE-degrading packed-bed column. We are grateful to Henk Duits and Eef Veldhorst for the help with the total organic carbon measurements and Nees Slotboom for the drawings.

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