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Isolation and Characterization of a Facultatively Aerobic Bacterium That Reductively Dehalogenates Tetrachloroethene to *cis*-1,2-Dichloroethene

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A rapidly-growing facultatively aerobic bacterium that transforms tetrachloroethene (PCE) via trichloroethene (TCE) to *cis*-1,2-dichloroethene (*cis*-1,2-DCE) at high rates in a defined medium was isolated from a contaminated site. Metabolic characterization, cellular fatty acid analysis, and partial sequence analysis of 16S rRNA showed that the new isolate, strain MS-1, has characteristics matching those of the members of the family *Enterobacteriaceae*. Strain MS-1 can oxidize about 58 substrates including many carbohydrates, short-chain fatty acids, amino acids, purines, and pyrimidines. It can transform up to 1 mM PCE (aqueous) at a rate of about 0.5 μmol of PCE · h^{-1} · mg (dry weight) of $cell^{-1}$. PCE transformation occurs following growth on or with the addition of single carbon sources such as glucose, pyruvate, formate, lactate, or acetate or with complex nutrient sources such as yeast extract or a mixture of amino acids. PCE dehalogenation requires the absence of oxygen, nitrate, and high concentrations of fermentable compounds such as glucose. *Enterobacter agglomerans* biogroup 5 (ATCC 27993), a known facultative bacterium that is closely related to strain MS-1, also reductively dehalogenated PCE to *cis*-1,2-DCE. To our knowledge, this is the first report on isolation of a facultative bacterium that can reductively transform PCE to *cis*-1,2-DCE under defined physiological conditions. Also, this is the first report of the ability of *E. agglomerans* to dehalogenate PCE.

Reductive dehalogenation is the main known mechanism for the anaerobic degradation of tetrachloroethene (PCE) and trichloroethene (TCE), with isomers of dichloroethene (*cis*-1,2-DCE, *trans*-1,2-DCE, and 1,1-DCE), vinyl chloride (VC), ethene, and ethane being the major intermediates and end products. Generally, the reductive dehalogenation of PCE was thought to require initiation by anaerobic bacteria.

Several known pure cultures of anaerobic bacteria can reductively dehalogenate PCE to TCE (12, 13, 15, 16). Holliger et al. (20) obtained an anaerobic enrichment consisting of one predominant bacterial species that can transform PCE to *cis*-1,2-DCE at high rates. The culture grows only in the presence of H_2, PCE, and yeast extract under strictly anaerobic conditions (20). Scholz-Muramatsu et al. (27) have isolated a strict anaerobe, *Dehalospirillum multivorans*, that can dechlorinate PCE to *cis*-1,2-DCE as part of its energy metabolism under defined growth conditions. Krumholz (23) reported on a strict anaerobe that can reduce PCE to *cis*-1,2-DCE via acetate oxidation.

Here, we report on the isolation of a new, rapidly growing facultatively aerobic bacterium, strain MS-1, that can transform near saturation levels of PCE via TCE to *cis*-1,2-DCE at high rates in a defined growth medium. To our knowledge, this is the first report on isolation of a facultative bacterium with this capability.

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MATERIALS AND METHODS

Isolation of strain MS-1. Strain MS-1 was isolated from aquifer material taken from a PCE-contaminated site in Victoria, Tex. (2). Enrichments were developed by addition of the aquifer material to a basal medium containing sodium benzoate, PCE, and sodium sulfate (19). The liquid portion of the enrichment was transferred to fresh medium to obtain an aquifer material-free enrichment. PCE was converted to ethene in both enrichments (19). After 3 months of incubation, aquifer material-free enrichment was subcultured in Tanner's basal medium (pH 7.0) (29) containing benzoate (60 mg/liter) and yeast extract (50 mg/liter) and subsequently was serially diluted in the same medium. A low-dilution culture thus obtained was serially diluted again. Further isolation and confirmation of purity were done with thioglycolate medium plus 2% (wt/vol) agar. Well-isolated colonies on agar plates were picked and restreaked to obtain a pure culture of strain MS-1. All manipulations were done under strictly anaerobic conditions. Once a pure culture was obtained, physiological or biochemical characteristics of strain MS-1 were determined under aerobic and anaerobic conditions.

Composition and preparation of growth medium. Tanner's basal medium (29) was supplemented with 10 ml of vitamin solution (1) per liter except when yeast extract was the growth substrate. Sulfur-free basal medium was prepared by replacing sulfate salts with chloride salts. Yeast extract, a defined mixture of amino acids (Grace's amino acids solution modified for TC-100; Sigma Chemical Co., St. Louis, Mo.), or single carbon sources such as glucose, pyruvate, formate, acetate, lactate, succinate, or glutamate were added to the basal medium as indicated. The PCE (neat) concentration ranged from 10 μM to 10 mM. Methods for the preparation and use of anaerobic media were those of Bryant (4) and Balch and Wolfe (1). The gas phase for all anaerobically prepared media was 100% N_2 unless specified otherwise.

Physiological and metabolic characterization. Transmission electron micrographs of cells grown on thioglycolate liquid medium, spread onto Formvar-coated carbon-reinforced copper grids, and negatively stained with 1% phosphotungstic acid (pH 6.5) were obtained with a Philips 410 transmission electron microscope. Biolog Inc., Hayward, Calif., performed the carbon utilization test with the microplate system. Cellular fatty acid composition was determined by Microcheck, Inc., Northfield, Vt.

16S rRNA sequence analysis. 16S rRNA sequence analysis was performed by the Center for Microbial Ecology, Michigan State University. The 16S gene was amplified with primers targeted to the 5′ and 3′ ends of the molecule. The partial sequence analysis was done on an Applied Biosystems sequencer with a reserve primer targeted to the conserved region located at approximately position 529 (*Escherichia coli* numbering). Partial sequence was obtained from only one strand, resulting in a 471-nucleotide segment corresponding to nucleotide positions 29 to 501 in *Escherichia coli* numbering.

REP-PCR. Genomes of strain MS-1, the enteric bacteria most closely related

to strain MS-1 (based on metabolic characterization, fatty acid analysis, and 16S rRNA sequence analysis), and some representative enteric bacteria were analyzed by the repetitive extragenic palindromic (REP)-PCR method (31). Closely related bacterial strains and representative enteric bacteria were obtained from the American Type Culture Collection, Rockville, Md., and included *Enterobacter agglomerans* biogroup 1 (ATCC 27155), *E. agglomerans* biogroup 5 (ATCC 27993), *E. agglomerans* biogroup 5 (ATCC 27994), *E. cloacae* (ATCC 13047), *Citrobacter freundii* (ATCC 33421), *Serratia marcescens* (ATCC 8100), *S. grimesii* (ATCC 14461), *S. proteamaculans* (ATCC 19323), *S. odorifera* (ATCC 33077), *Leclercia adecarboxylata* (ATCC 23216), *Escherichia coli* (ATCC 9637), and *Hafnia alvei* (ATCC 29926).

PCE dehalogenation by closely related known bacterial strains. Closely related enteric bacteria and some representative enteric bacteria (mentioned above; they also included *E. intermedius* [ATCC 33421]) were tested for their ability to reductively dehalogenate PCE in Tanner's basal medium containing 260 mg of acetate per liter, 10 mg of yeast extract per liter, and 100 μM PCE, with 100% N_2 as the gas phase.

Compound analysis. For most experiments, PCE and its chlorinated products were identified and quantitated by static-headspace analysis with a gas chromatograph coupled to a photoionization detector with a 10.2-eV lamp. Analyses were isothermal with splitless injection. The liquid concentration of gaseous compounds was determined by comparing peak areas with those of external standards and by using Henry's law constants reported by Gossett (18) and verified in our laboratory. The identity of PCE and its chlorinated products in liquid samples was confirmed with a purge-and-trap system equipped with a 700-A HALL electrolytic conductivity detector (Tacor Instruments Austin, Inc., Austin, Tex.). Acetate, benzoate, formate, nitrate, and sulfate were monitored with an ion chromatograph. Acetylene, ethane, ethene, and methane were measured with a gas chromatograph coupled to a flame ionization detector.

Nucleotide sequence accession number. The 16S rRNA sequence of strain MS-1 has been deposited in GenBank under accession number L43508.

RESULTS

Cellular and colonial morphology. Strain MS-1 is a motile, gram-negative, capsule-forming, nonsporeforming, rod-shaped bacterium with peritrichous fimbriae. The cells characteristically appear singly, in pairs, or occasionally as long chains and measure 0.8 to 1.0 by 2.8 to 3.5 μm. Colonies on anaerobically prepared thioglycolate medium are circular, entire, opaque, white, and 1 to 2 mm in diameter. Under aerobic conditions, colonies are white on nutrient agar, purple on eosine-methylene blue agar, and pink on MacConkey agar.

Physiological and metabolic characteristics. Under optimum anaerobic conditions (pH 7.0; temperature, 37°C) in medium containing 1 g of yeast extract per liter, strain MS-1 had a doubling time of about 42 min. The isolate is catalase positive, oxidase negative, and Voges-Proskauer negative. It can reduce nitrate to nitrite but does not use sulfate or produce H_2S. Major (>25%) fatty acids present in strain MS-1 were $C_{16:0}$ and $C_{16:1}$.

Under aerobic conditions, strain MS-1 used substrates including polymers such as dextrin, glycogen, Tween 40, and Tween 80; carbohydrates such as *N*-acetyl-D-glucosamine, arabinose, cellobiose, D-fructose, D-galactose, gentibiose, α-D-glucose, α-D-lactose, maltose, D-mannitol, D-mannose, D-melibiose, β-methyl-D-glucoside, D-psicose, raffinose, L-rhamnose, D-sorbitol, and D-trehalose; esters such as methyl pyruvate and monomethyl-succinate; carboxylic acids such as acetic acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucuronic acid, α-hydroxy-butyric acid, *p*-hydroxy-phenylacetic acid, D,L-lactic acid, malonic acid, saccharic acid, succinic acid, and bromosuccinic acid; amides such as glucuronamide and alaniamide; amino acids such as D- and L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, L-proline, D-serine, L-serine, and L-threonine; aromatics such as uronic acid, inosine, uridine, and thymidine; alcohols such as glycerol; and phosphorylated compounds such as D,L-α-glycerol phosphate and glucose-1-phosphate.

Strain identification. Strain MS-1 has metabolic characteristics that closely match those of *E. agglomerans* biogroup 5. The fatty acid composition is consistent with that of *E. agglomerans* (ATCC 27155) (closest match), *Erwinia herbicola*, and some members of the genus *Serratia*. Analysis of the 16S rRNA showed that strain MS-1 was specifically related to *C. freundii* and that *Serratia marcesens* was a member of the same line of descent. However, since strain MS-1 cannot use citrate as the sole carbon source for growth, it may not be placed in the genus *Citrobacter*. The sequence of *E. agglomerans* was not available in three databases (RDP, GenBank, and EMBL) checked, but the REP products of *E. agglomerans* biogroup 1 (ATCC 27155), and *E. agglomerans* biogroup 5 (ATCC 27993 and 27994) were different from those of strain MS-1. The REP products of other related and representative enteric bacteria were also different from those of strain MS-1. The REP products of *Erwinia herbicola* were not analyzed, but previous DNA hybridization studies (3) show a close DNA relatedness between some type strains of *Erwinia herbicola* and *E. agglomerans*. These data show that strain MS-1 belongs to the family *Enterobacteriaceae*, but it cannot be placed in a particular genus at this stage.

PCE dehalogenation by strain MS-1. Strain MS-1 dehalogenates PCE via TCE to *cis*-1,2-DCE within 4 days at room temperature when grown anaerobically in yeast extract-containing medium with 100% N_2 as the gas phase (Fig. 1). The growth medium did not contain any reductant other than those present in yeast extract. A change in headspace gas from N_2 (100%) to N_2-CO_2 (80:20) or H_2/CO_2 (70:30) did not affect either growth or PCE dehalogenation. *cis*-1,2-DCE was the major end product of PCE, while yeast extract was metabolized mainly to acetate (0.65 g/g of yeast extract) with a growth yield of about 0.34 g (dry weight) per g of yeast extract. VC or other isomers of DCE were not produced by the isolate. When cultures were fed with TCE, *cis*-1,2-DCE, or VC, TCE was converted stoichiometrically to *cis*-1,2-DCE within 3 days whereas *cis*-1,2-DCE and VC were not degraded even after 6 months of incubation. Ethene, ethane, methane, or acetylene production was never observed. Although strain MS-1 could grow when an equivalent of 2, 5, or 10 mM PCE was added to the medium, PCE dehalogenation did not occur in such cultures even after 12 days of incubation. However, strain MS-1 dehalogenated PCE at concentrations up to 1 mM at a rate of about 0.5 μmol of PCE · h^{-1} · mg (dry weight) of $cells^{-1}$. Production of TCE or *cis*-1,2-DCE from PCE was not observed in either uninoculated or autoclaved controls even after 6 months.

The apparent lag phase for PCE dehalogenation was longer by 1 day in aerobic medium than in anaerobic medium (Fig. 1). However, when aerobic medium turned anaerobic by cellular growth or metabolism, strain MS-1 resumed PCE dehalogenation (Fig. 1). Addition of nitrate (1 mM) inhibited PCE dehalogenation completely, while sulfate or fumarate (1 mM each) had little or no effect on PCE reduction. Metabolic inhibitors of sulfate reduction or methanogenesis, molybdate(VI) sodium salt (500 μM), or 2-bromoethane sulfonic acid sodium salt (500 μM), did not affect either metabolic or dehalogenation activity. These data suggest that PCE dehalogenation by strain MS-1 requires the absence of thermodynamically more favorable electron acceptors such as oxygen or nitrate (32), while redox conditions associated with sulfate reduction or methanogenesis are not required for such transformation.

PCE dehalogenation occurred in medium with single carbon sources such as glucose, pyruvate, formate, lactate, or acetate, or with complex carbon sources such as an amino acid mixture. Succinate or glutamate did not support PCE dehalogenation. Some cultures were incubated inside an anaerobic glove box to allow frequent sampling under anaerobic conditions. Since the glove box contained H_2 and since H_2 may act as an electron

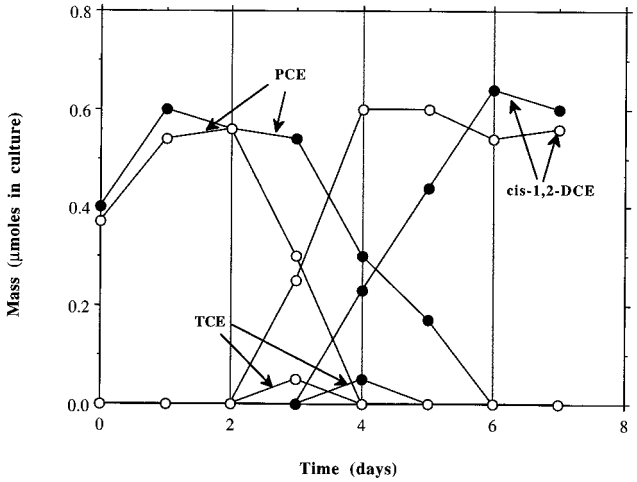

FIG. 1. PCE dehalogenation by strain MS-1 under initial anaerobic (open circles) and aerobic (solid circles) conditions. Yeast extract was the external electron donor (1 g/liter). The initial concentration of PCE was about 50 μM. Cultures were incubated in the dark at room temperature inside an anaerobic glove box. After 3 days of incubation, the initial aerobic medium turned anaerobic, as indicated by the change in color of resazurin. Values are means; ranges were insignificant.

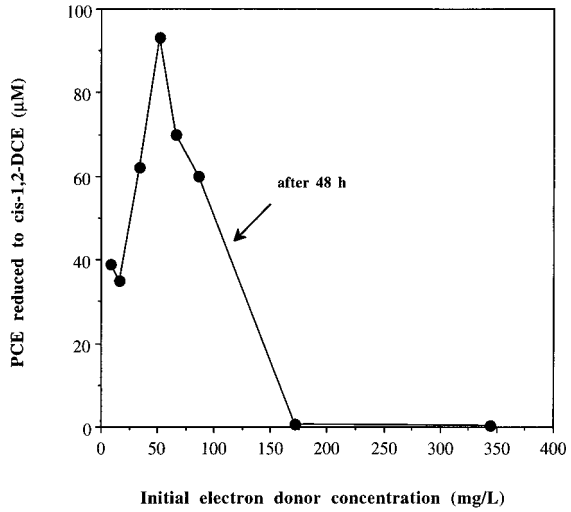

FIG. 2. Effect of initial electron donor concentration on PCE dehalogenation by strain MS-1 after 48 h. The concentration of amino acids ranged from 9 to 345 mg/liter. The initial PCE concentration was about 130 μM. Values are means; ranges were insignificant.

donor for PCE dehalogenation (11, 27), parallel experiments outside the glove box with 100% N_2 as the gas phase were also conducted. The pattern of PCE dehalogenation was the same under both conditions, showing that exogenous H_2 had little or no influence on PCE dehalogenation by strain MS-1.

Although PCE was converted to *cis*-1,2-DCE in all cultures with a low initial concentration of glucose, pyruvate, or formate (50 mg/liter each), the rate of PCE dehalogenation was different with different electron donors. With higher concentrations of these electron donors (1 g/liter each), PCE dehalogenation was not observed with glucose even after 12 days. The effect of initial electron donor concentration on PCE dehalogenation was studied further. Strain MS-1 was grown in medium containing amino acids with an initial concentration ranging from 9 to 345 mg/liter (Fig. 2). After 48 h, the mass of PCE reduced to *cis*-1,2-DCE was linearly related to the mass of amino acids present up to an amino acid concentration of 52 mg/liter. However, a further increase in initial electron donor concentration resulted in reduced PCE reduction, and no PCE reduction occurred within the 48-h period in cultures with amino acid concentrations of 172 mg/liter or more. With extended periods of incubation, all of the PCE was reduced in all cultures. These results are consistent with the adverse effect of high glucose concentrations.

A higher initial cell concentration resulted in a higher PCE utilization rate. However, regardless of the initial PCE concentration used, PCE dehalogenation did not occur during the exponential phase of growth. The possibility that strain MS-1 could derive energy for cellular growth through PCE use was tested by using cultures grown in medium with external electron donors such as acetate, lactate, succinate (1 mM each), or yeast extract (10 mg/liter) and about 1 mM PCE [medium reduced with titanium(III) citrate]. Most of the added PCE was reduced to *cis*-1,2-DCE after 21 days in cultures with yeast extract, acetate, or lactate. About 162 μM PCE was converted to *cis*-1,2-DCE in cultures without an external electron donor. The viable-cell concentration in all PCE-using cultures was higher ($\sim 10^5$ cells per ml) than in cultures in which no PCE was added or used ($\sim 10^4$ cells per ml).

PCE dehalogenation by closely related known bacterial strains. Of 13 closely related bacterial strains tested, only *E. agglomerans* biogroup 5 (ATCC 27993) transformed the added PCE (100 μM) to *cis*-1,2-DCE within 12 days. Thus, at least two facultative bacterial species from the family *Enterobacteriaceae* appear capable of PCE dehalogenation.

DISCUSSION

Characteristics that differentiate strain MS-1 from other known PCE-dehalogenating bacterial strains. Other reported bacterial strains that can dehalogenate PCE are all strict anaerobes (Table 1). In most cases (12, 13, 15, 16), previously isolated strains of sulfate-reducing and methanogenic bacteria were tested and found to dehalogenate PCE to TCE at very low rates (<0.001 μmol of PCE · h^{-1} · mg [dry weight] of $cells^{-1}$). Holliger et al. (20) were the first to report a microscopically pure culture that can dehalogenate PCE to *cis*-1,2-DCE at high rates. This required strictly anaerobic conditions, and their culture could grow and transform PCE only in the presence of H_2 and yeast extract. The role here of yeast extract in cellular growth or PCE dehalogenation is not clear. PCE concentrations higher than 200 μM were toxic to the organism. Recently, Scholz-Muramatsu et al. (27) reported isolating a strictly anaerobic bacterium, *Dehalospirillum multivorans*, that can dehalogenate up to 160 μM PCE to *cis*-1,2-DCE as a part of its energy metabolism at a rate of 3 μmol of PCE · h^{-1} · mg of cell $protein^{-1}$. In addition to H_2, *D. multivorans* can use a variety of electron donors such as pyruvate, lactate, formate, ethanol, or glycerol. *D. multivorans* can also use nitrate or fumarate as electron acceptors for growth. However, the presence of fumurate in the medium inhibited PCE dehalogenation. Krumholz (23) isolated a strictly anaerobic bacterium that can grow only in medium containing PCE (tolerance level 100 μM) and acetate (or pyruvate). Strain MS-1 is very distinct from all of these PCE-dehalogenating bacterial cultures in that it is facultative and can grow on a number of carbohydrates, fatty acids, amino acids, purines, and pyrimidines fermentatively or with O_2 or nitrate as the electron acceptor. Also, it can tolerate PCE levels as high as 10 mM and can transform up to 1 mM PCE to *cis*-1,2-DCE at rates of about 0.5 μmol of

TABLE 1. Comparison of strain MS-1 with other known PCE-dehalogenating bacterial pure cultures

Organism	Metabolic group	Reaction(s)	Electron donor(s)	Reference(s)
Methanosarcina sp.	Methanogen	PCE → TCE	Methanol	15, 16
Methanosarcina mazei	Methanogen	PCE → TCE	Methanol	15, 16
Desulfomonile tiedje	Sulfate reducer	PCE → TCE	Pyruvate	16
		PCE → *cis*-1,2-DCE	H_2 in cell extracts	30
Desulfobacterium sp.	Sulfate reducer	PCE → TCE	H_2, lactate	13
Acetobacterium woodii	(Anaerobe)	PCE → TCE	H_2, fructose	12
Strain PER-K23	(Anaerobe)	PCE → *cis*-1,2-DCE	H_2/formate plus yeast extract	20
Dehalospirillum multivorans	(Anaerobe)	PCE → *cis*-1,2-DCE	H_2, formate, pyruvate, lactate, ethanol, glycerol	27
Strain TT4B	Anaerobe	PCE → *cis*-1,2-DCE	Acetate, pyruvate	23
Strain MS-1	Facultative	PCE → *cis*-1,2-DCE	Acetate, lactate, pyruvate, formate, glucose, yeast extract, amino acids	This study
Enterobacter agglomerans	Facultative	PCE → *cis*-1,2-DCE	Acetate (and yeast extract)	This study
Pseudomonas sp.	Facultative	PCE → ?	PCE ?	10

$PCE \cdot h^{-1}$ mg (dry weight) of $cells^{-1}$. This compares favorably with the PCE dehalogenation rate reported for *D. multivorans* (23) and is much higher than rates reported for mixed cultures (5, 17, 28).

In addition to the two bacterial strains reported here (strain MS-1 and *E. agglomerans* biogroup 5), the only other facultative bacterium reported to degrade PCE was that described by Deckard et al. (10) (Table 1). The data here were limited, and no intermediates were found; however, chloride was produced. These investigators suggested that PCE acted here as the sole carbon source for growth whereas oxygen served as the electron acceptor.

Role of facultative bacteria in PCE dehalogenation. Facultative bacterial strains belonging to the genera *Escherichia* and *Pseudomonas* reductively dehalogenate DDT and some halomethanes (6–9, 12, 24, 25). This is the first report on facultative bacteria (strain MS-1 and *E. agglomerans* biogroup 5) that can perform reductive dehalogenation of PCE to *cis*-1,2-DCE. Prior to isolation of strain MS-1, the involvement of facultative bacteria in such reductive dehalogenation has been speculated about on the basis of results of mixed-culture studies (14, 22). Kastner (22) showed that PCE or TCE was reductively and stoichiometrically converted to *cis*-1,2-DCE within 4 days in an aerobic enrichment culture but only after the transition from aerobic to anaerobic conditions, which was accompanied by production of sulfide, and a decrease in redox potential from 0 to −150 mV. PCE dehalogenation in this case required the absence of oxygen and nitrate. Kastner suggested that this reductive PCE dechlorination may have been carried by facultative bacteria. Enzien et al. (14) also found that PCE and TCE can be reductively dehalogenated in a sediment column with bulk aerobic conditions. They proposed that facultative bacteria in the anaerobic microsites may have been at least partially responsible for reductive dechlorination of PCE and TCE. These observations are consistent with our observations that strain MS-1 reduces the redox potential of an initially aerobic medium by cellular growth or metabolism and then converts PCE to *cis*-1,2-DCE in the reduced medium. Transformation of PCE by strain MS-1 does require the absence of oxygen and nitrate.

The role of facultative bacteria in PCE dehalogenation has probably not been fully appreciated, since strictly anaerobic bacteria such as fermentors, acetogens, sulfate reducers, and methanogens were originally implicated in reductive dehalogenation of PCE. Another reason may be related to the fact that facultative bacteria with the properties of strain MS-1 are often grown fermentatively on high concentrations of carbohydrates (in the range of grams per liter) and PCE dehalogenation under such conditions does not readily occur.

PCE dehalogenation by strain MS-1. The results for PCE dehalogenation are consistent with a hypothesis that strain MS-1 uses electron donors and acceptors in an order of preference that provides maximum energy for growth. Thus, PCE was not used until electron acceptors yielding more energy to the organism, such as oxygen and nitrate, were absent. Also, PCE was not used as an electron acceptor while organic substrates such as glucose and amino acids that are capable of relatively high energy production through fermentation were present in high initial concentrations. Following such fermentation, the organic fermentation products produced, such as acetate, which cannot be fermented or used for energy without an acceptable external electron acceptor, then served as electron donors for PCE dehalogenation. The good PCE dehalogenation obtained with acetate as the sole external electron donor is consistent with this hypothesis. Another hypothesis is that PCE dehalogenation occurs only when redox conditions are sufficiently reducing. Thus, external electron acceptors that create more highly oxidizing environments must be absent for PCE dehalogenation to occur. This alone, however, does not explain why PCE dehalogenation did not occur when oxygen and nitrate were absent but glucose, a strongly reducing sugar, was present. In any event, the results obtained raise the important question of the regulatory mechanism for PCE dehalogenation by MS-1.

An experiment with growth medium that contained PCE as the electron acceptor and acetate as the only external electron donor resulted in a small increase in cell numbers and dehalogenation of PCE. Since little PCE reduction occurred in the absence of an exogenous electron donor, it appears that PCE reduction was coupled in some manner with the external electron donor. Whether such coupling involved energy production for the cell is not clear, because the resulting increase in cell numbers was so very small. Either energy production was not involved or energy utilization from the reaction was quite inefficient.

PCE dehalogenation by strain MS-1 in its natural environment. Sediment columns, microcosms, and enrichment cultures developed from aquifer material from which strain MS-1 was isolated converted PCE completely to ethene when benzoate and sulfate were added to these cultures (19). Strain MS-1 cannot use benzoate or sulfate, but it can use the benzoate oxidation products acetate and formate for PCE dehalogenation to *cis*-1,2-DCE, suggesting that interspecies transfer of benzoate oxidation products may have enabled strain MS-1

to carry out the dehalogenation reaction in its natural environment.

Enhanced in situ bioremediation and use of strain MS-1 for bioaugmentation processes. Strain MS-1 may be stimulated for growth to obtain PCE reduction by the addition of a variety of nonhazardous substrates. However, a complex nutrient source such as yeast extract may be used for initial stimulation. Since yeast extract contains many different electron donors and growth factors, its presence may ensure the growth of strain MS-1 under anaerobic conditions. Carbohydrates such as glucose may not be as useful, since at least at high concentrations, glucose inhibits PCE dehalogenation by strain MS-1.

Since strain MS-1 can grow rapidly on a variety of substrates and has a good tolerance for oxygen, it is an excellent candidate for bioaugmentation of sites where necessary bacteria are absent. PCE can be converted to *cis*-1,2-DCE rapidly and without causing sulfate reduction or methanogenesis, processes that could lead to VC and sulfide formation or iron and manganese dissolution. Such transformation does reduce the environmental hazard posed because, unlike PCE and TCE, *cis*-1,2-DCE is believed to be noncarcinogenic and has a higher U.S. Environmental Protection Agency drinking water maximum contaminant level (26) (75 μg/liter for *cis*-1,2-DCE versus 5 μg/liter for PCE and TCE). In addition, *cis*-1,2-DCE can be readily mineralized through aerobic cometabolism (21). The initial partial dehalogenation of PCE and TCE, followed by aerobic cometabolism of the resulting *cis*-1,2-DCE, may help restore contaminated groundwater to drinking water quality without producing other harmful contaminants in an aquifer.

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