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Preliminary characterization of four 2-chlorobenzoate-degrading anaerobic bacterial consortia

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Abstract

Dechlorination was the initial step of 2CB biodegradation in four 2-chlorobenzoate-degrading methanogenic consortia. Selected characteristics of *ortho* reductive dehalogenation were examined in consortia developed from the highest actively dechlorinating dilutions of the original 2CB consortia, designated consortia M34^{-9}, P20^{-9}, P21^{-9} and M50^{-7}. In addition to 2-chlorobenzoate, all four dilution consortia dehalogenated 4 of 32 additional halogenated aromatic substrates tested, including 2-bromobenzoate; 2,6-dichlorobenzoate; 2,4-dichlorobenzoate; and 2-chloro-5-hydroxybenzoate. Dehalogenation occurred exclusively at the *ortho* position. Both *ortho* chlorines were removed from 2,6-dichlorobenzoate. Benzoate was detected from 2-bromobenzoate and 2,6-dichlorobenzoate. 4-Chlorobenzoate and 3-hydroxybenzoate were formed from 2,4-dichlorobenzoate and 2-chloro-5-hydroxybenzoate, respectively. Only benzoate was further degraded. Slightly altering the structure of the parent "benzoate molecule" resulted in observing reductive biotransformations other than dehalogenation. 2-Chlorobenzaldehyde was reduced to 2-chlorobenzyl alcohol by all four consortia. 2-chloroanisole was O-demethoxylated by three of the four consortia forming 2-chlorophenol. GC-MS analysis indicated reduction of the double bond in the propenoic side chain of 2-chlorocinnamate forming 2-chlorohydrocinnamate. None of the reduction products was dechlorinated. The following were not dehalogenated: 3- and 4-bromobenzoate; 3- and 4-chlorobenzoate; 2-, 3-, and 4-fluorobenzoate; 2-, 3-, and 4-iodobenzoate; 2-, 3-, and 4-chlorophenol; 2-chloroaniline; 2-chloro-5-methylbenzoate; 2,3-dichlorobenzoate; 2,5-dichlorobenzoate; 2,4,5-trichlorophenoxyacetic acid; and 2,4-dichlorophenoxyacetic acid. Consortia M34^{-9}, P20^{-9}, P21^{-9}, and M50^{-7} dechlorinated 2-chlorobenzoate at $\leq$4 mm. Dechlorination rates were highest for consortia P20^{-9} followed by those of M50^{-7}with rates declining above 2 and 3 mm 2CB, respectively. The major physiological types of microorganisms in consortia M34^{-9}, P20^{-9}, P21^{-9}, and M50^{-7} were sulfate-reducing and hydrogen-utilizing anaerobes.

Abbreviations: 2CB = 2-chlorobenzoate; 2CHC = 2-chlorohydrocinnamate; 2CPA = 2-chlorophenylacetic acid; BESA = bromoethane sulfonic acid; HPLC = High performance liquid chromatography; MPN = Most probable Number.

Introduction

We previously reported anaerobic biodegradation of 2-chlorobenzoate (2CB) in freshwater sediment enrichments (Sharak Genthner et al. 1989a), and in a methanogenic bacterial consortium derived from one of these enrichments (Sharak Genthner et al. 1989b). The initial step of 2CB biodegradation in this consortium was dechlorination at the *ortho* position to form benzoate that was then degraded to carbon dioxide and methane. Reductive dechlorination at the *ortho* position of multi-chlorinated benzoic acids has been observed in anaerobic enrichments (Gerritse et al. 1992; Van der Woude et al. 1996) and with aerobic bacterial strains (van den Tweel et al. 1987; Romanov & Hausinger 1994; 1996). Aerobic biodegradation of 2CB by *Pseudomonas* sp. via oxygenases with subsequent release of chlorine has also been reported (Engesser & Schulte 1989; Fetzner et al. 1989; Romanov & Hausinger 1994). However, anaerobic biodegradation and/or reductive dechlorination of the mono-chlorinated compound, 2CB, is not usually observed in anaerobic enrichments (Horowitz et al. 1982; Horowitz et al. 1983; Häggblom et al. 1993; Van der Woude et al. 1996; Maloney et al. 1997). If 2CB loss was observed, it had not been accompanied by the detection of benzoate, the product of reductive dehalogenation of 2CB, and stable 2CB-degrading bacterial consortia were not obtained for further study (Häggblom et al. 1993; 1996; Van der Woude et al. 1996).

Thus, our previous studies (Sharak Genthner et al. 1989a; 1989b) in which we detected benzoate as an intermediate of 2CB anaerobic biodegradation have been the only substantiated reports of anaerobic 2CB reductive dechlorination. In the current investigation, we examine characteristics of four stable 2CB-dechlorinating/2CB-degrading anaerobic bacterial consortia obtained during our earlier study, and of four additional consortia derived from the highest actively-dechlorinating dilutions of the original 2CB consortia.

Information gathered on tolerance to 2CB and major physiological types of bacteria present in consortia derived from the highest actively dechlorinating dilution of the original 2CB consortia will be used to aid in the isolation of the bacterial species responsible for 2CB dechlorination in our consortia.

Materials and methods

Cultivation techniques

The anaerobic techniques and medium used were previously described (Sharak Genthner et al. 1989a, 1989b), except that the anaerobic medium was supplemented with 0.02% yeast extract. Consortia were derived from methanogenic enrichments inoculated with freshwater sediment from either Site B of the Escambia River in northwest Florida (consortia M34 and M50), or the Eleven Mile Creek (consortia P20 and P21) in southeast Alabama (Sharak Genthner et al. 1989a). M34 and P20 were originally methanogenic. Stable bacterial consortia were obtained as previously described (Sharak Genthner et al. 1989b). The original M50 and P21 enrichment cultures contained 1 mm and 10 mm, respectively, of bromoethane sulfonic acid (BESA), a potent inhibitor of methanogenesis (Gunsalus, et al. 1978). Since BESA (1 mm) did not enhance biodegradation of 2CB in the first laboratory transfer of M50, but 10 mm BESA inhibited biodegradation of 2CB in P21 (Sharak Genthner et al. 1989a), subsequent laboratory transfers were prepared without BESA. In both cases consortia were methanogenic. Media were routinely inoculated with 10% of a stable consortium.

MPN analyses

MPN analyses were done in serum stopper tubes (18 $\times$ 150 mm) by preparing triplicate dilutions (10^{-2} through 10^{-9}) of a stable consortia in a medium appropriate for the particular MPN. The MPN sets were incubated up to 6 months. Enumeration of 2CB dechlorinators was performed in anaerobic medium containing 800 μm 2CB. Loss of the dechlorination product, benzoate, was the criterion for enumerating benzoate-degrading bacteria. The highest dechlorinating dilution, i.e. 1×10^{-7} or 1×10^{-9}, of each consortium was propagated as described for the stable consortia and were designated $M50^{-7}$, $M34^{-9}$, $P20^{-9}$, and $P21^{-9}$.

The MPN anaerobic medium was modified to enumerate other physiological types of bacteria in consortia $M50^{-7}$, $M34^{-9}$, $P20^{-9}$, and $P21^{-9}$. Benzoate and acetate-utilizing anaerobes were enumerated in medium containing 10 mm sodium benzoate and 50 mm sodium acetate, respectively. Nitrate- and sulfate-reducing anaerobes were enumerated in medium amended with 15 mm KNO_3 and 20 mm Na_2SO_4. Hydrogen-utilizing anaerobes were enumerated in medium containing 2X bicarbonate buffer under two atmospheres of 20/80 CO_2/H_2. Growth in MPN sets was measured as an increase in optical density (600 nm) and monitored until a maximum optical density was obtained after which nitrate, nitrite and sulfate concentrations were determined using ion chromatography (Sharak Genthner et al. 1994).

Ortho-*dechlorination*

The ability of the four original 2CB consortia to dehalogenate 2CB was monitored monthly in fresh and refed laboratory transfers for several years. The effect of yeast extract on 2CB dechlorination was determined by transferring the consortia into medium lacking yeast extract, or to medium in which yeast extract was replaced by pyruvate.

The ability of consortia M50^{-7}, M34^{-9}, P20^{-9}, and P21^{-9} to dehalogenate 2CB and other halogenated aromatic compounds was examined in anaerobic medium containing yeast extract. Test compounds were added from concentrated (100×), anaerobic, sterile stock solutions (pH 7.0). Compounds were tested at 500 μM, except where indicated otherwise in parentheses, in triplicate cultures with duplicate uninoculated controls. Uninoculated controls were prepared to monitor abiotic changes under reducing conditions. Cultures were incubated at 30 °C for up to 9 months.

Loss of the halogenated compound and formation of the non-halogenated product were monitored by High performance Liquid chromatography (HPLC). HPLC samples were prepared and analyzed on a Hewlett-Packard 1050 HPLC as previously described (Kuo & Sharak Genthner 1996) using the acetonitrile/phosphate buffer at a ratio of 10/90 to 50/50 under isocratic conditions. Absorbance wavelengths from 220 to 270 nm were chosen to optimize separation and detection of parent compound and dechlorination product. UV spectral and gas chromatographic-mass spectral (GC-MS) analyses were used, as previously described (Sharak Genthner, et al. 1989c), to identify dehalogenation products.

Results/discussion

2CB-dechlorination/degradation

We have been able to propagate all four 2CB consortia while retaining their ability to dechlorinate and degrade 2CB for several years through numerous laboratory transfers in anaerobic medium containing 0.02% yeast extract. Consortium M50 is currently in its 17th laboratory transfer, while M34 and P20 are in their 11th transfer and P21 is in its 10th. We were unable to propagate 2CB degradation by consortium P20 or P21 in defined medium, lacking yeast extract, but have maintained their activity through a 5th transfer in defined pyruvate (0.2%) medium lacking yeast extract. Although degradation was much slower than in medium containing yeast extract, M34 and M50 have remained active through their 3rd and 5th transfers, respectively, in both defined medium and defined pyruvate medium.

Most Probable Number (MPN) analyses (Rodina 1972) were performed to determine the number of *ortho*-dechlorinating bacteria/ml in the original 2CB consortia. Consortium M50 contained 1 × 10^7 dechlorinating bacteria/ml (Table 1). The other three consortia, designated M50^{-7}, M34^{-9}, P20^{-9}, and P21^{-9}, contained 1 × 10^9 dechlorinating bacteria/ml each. Microscopic examination indicated that these dilution consortia were mixed cultures containing several morphologically distinguishable gram-negative rods. After the 3rd laboratory transfer (10%), the bacterial populations in M50^{-7}, M34^{-9}, P20^{-9}, and P21^{-9} were characterized.

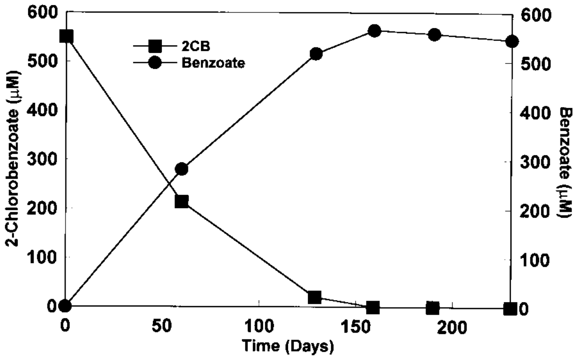

Figure 1. Accumulation of *ortho* dehalogenation product, benzoate, from reductive dechlorination of 2CB by consortium P21^{-9}.

2CB dechlorination rates were determined from the linear portion of the 2CB dechlorination curve. Benzoate, the *ortho* dehalogenation product, accumulated in the highest actively dechlorinating MPN dilution, as shown for consortium P21^{-9} (Figure 1). The rates of 2CB dechlorination by M50^{-7}, M34^{-9}, P20^{-9} and P21^{-9} at increasing concentrations of 2CB (500 μM − 4 mm) are shown in Figure 2. The maximum 2CB dechlorination rate of 29 μm/day was observed with consortium P20^{-9} at 2 mm, followed by M50^{-7} with 19 μm/day at 500 μm 2CB. Consortium P20^{-9} dechlorinated 2CB at rates near maximum from 500 μm up to and including 2 mm, but dechlorination rates abruptly declined to approximately 4 μm/day from 2.5 to 4 mm 2CB. Consortia M34^{-9} and P21^{-9} dechlorinated 2CB at 2–5 μm/day over the range of 2CB concentrations tested with increasing 2CB concentrations having little effect on dechlorination rates. In contrast dechlorination rates increasingly slowed in consortium M50^{-7} above 1 mm. Thus, consortium P20^{-9} was the most efficient in dechlorinating 2CB at $\leq$ 2 mm.

Gerriste et al. (1992) reported that their anaerobic freshwater sediment enrichments removed the *ortho* chlorine from various tri- and dichloroben-

Table 1. Enumeration of physiological cell types in 2CB-dechlorinating[a] anaerobic bacterial consortia

Consortium[b]	Cell types (bt/ml) Benzoate degraders	Acetate utilizers	H_2/CO_2 utilizers	Nitrate reducers	Sulfate-reducing bacteria
$P20^{-9}$	1.6×10^7	1×10^5	1.1×10^7	$<1 \times 10^{2}$ [c]	4.5×10^7
$P21^{-9}$	$<1 \times 10^{2}$ [c]	$<1 \times 10^2$	1.1×10^7	4.5×10^5	2.5×10^6
$M34^{-9}$	$<1 \times 10^{2}$ [c]	1×10^5	1.5×10^6	$<1 \times 10^{2}$ [c]	2.5×10^7
$M50^{-7}$	$<1 \times 10^{2}$ [c]	1×10^3	4.5×10^6	1×10^{5} [d]	9.5×10^6

[a] All consortia dechlorinated 2CB; $P20^{-9}$ also degraded the benzoate produced.

[b] Consortia were the highest dilution of the original consortium to dechlorinate 2CB; dilution is indicated by superscript.

[c] Lowest dilution tested was 1×10^{-2}.

[d] Nitrate was reduced to nitrite.

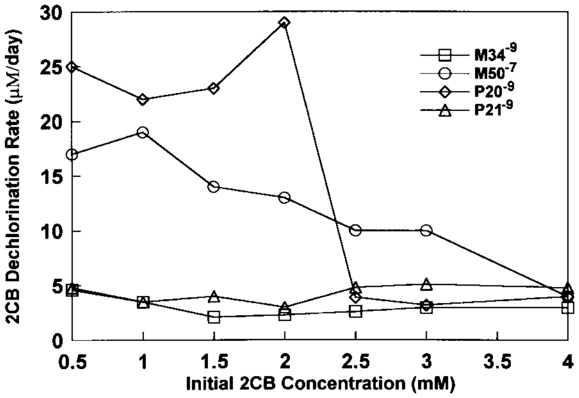

Figure 2. Tolerance of 2CB-dechlorinating consortia, $M50^{-7}$, $M34^{-9}$, $P20^{-9}$, and $P21^{-9}$ toward 2CB as shown by dechlorination rates at increasing concentrations of 2CB.

zoic acids. In contrast to our findings, 2,3- and 2,5-dichlorobenzoate were dechlorinated at the *ortho* position, but 2,6-dichlorobenzoate was not. 2CB dechlorination was not observed in this study. In a follow-up study 2CB was lost from one of six sediment slurry replicates (Van der Woude et al. 1996). Haggblom et al. (1993) reported a similar loss of 2CB in a methanogenic sediment enrichment. Neither research group detected benzoate as a product from 2CB, therefore, neither confirmed reductive dechlorination of 2CB. Apparently neither group studied these 2CB-degrading enrichments further.

Alcaligenes denitrificans NTB-1, an aerobic bacterial species, was reported to remove the *ortho* chlorine from 2,4-dichlorobenzoate by reductive dechlorination forming 4-chlorobenzoate which was metabolized aerobically (van den Tweel et al. 1987). Similarly, *Corynebacterium sepedonicum* KZ-4 (Romanov & Hausinger 1996), also an aerobic bacterial species, reductively dechlorinated 2,4-dichlorobenzoate and 2-chloro-4-fluorobenzoate to form 4-chlorobenzoate and 4-fluorobenzoate, respectively. However, *A. denitrificans* NTB-1 did not reductively dechlorinate 2,6-dichlorobenzoate, and neither species reductively dechlorinated 2CB. These observations indicate that the *ortho*-dechlorinating activity in our consortia was different from the *ortho*-dechlorination of multichlorinated benzoates.

Some *Pseudomonas* sp. have been shown to degrade 2CB (Engesser & Schulte 1989; Fetzner et al. 1989; Romanov & Hausinger 1994), but metabolism is oxidative and chlorine release is fortuitous, i.e. a dehalogenase enzyme is not involved.

Other ortho-dechlorinating activities in consortia $M50^{-7}$, $M34^{-9}$, $P20^{-9}$, and $P21^{-9}$

Of the 32 halogenated aromatic compounds tested, four halogenated benzoic acids, in addition to 2CB, were dehalogenated at the *ortho* position by all four consortia. These included 2-bromobenzoate, 2-chloro-5-hydroxybenzoate, 2,4-dichlorobenzoate, and 2,6-dichlorobenzoate. Both *ortho* chlorines were removed from 2,6-dichlorobenzoate. Benzoate, 4-chlorobenzoate, or 3-hydroxybenzoate were detected where appropriate indicating reductive dechlorination. Of these dechlorination products, only benzoate was further degraded. The consortia did not dehalogenate the following compounds: 3 - and 4-bromobenzoate; 3- and 4-chlorobenzoate; 2-, 3-, and 4-fluorobenzoate; 2-, 3-, and 4-iodobenzoate; 2-, 3-, and 4-chlorophenol; 2-chloroaniline (100 µM); 2-chloro-5-methylbenzoate (50 µM); 2,3-dichlorobenzoate; 2,5-

dichlorobenzoate; 2,4,5-trichlorophenoxyacetic acid (50 μM); and 2,4-dichlorophenoxyacetic acid (50 μM).

These dechlorination data indicate that dehalogenation by our consortia occurred only at the *ortho* position; that benzoate, and some compounds closely related to benzoate, are the preferred substrate; and that the presence and position of additional side groups on the benzoate molecule can affect the ability of these dilution consortia to *ortho*-dehalogenate.

Several halogenated compounds were tested to examine the effect of altering the aromatic carboxyl group on *ortho*-dechlorination in our consortia. These included: 2-chlorobenzaldehyde (100 μm), 2-chlorobenzyl alcohol (100 μM), 2-toluene (50 μM), 2-chloroanisole (50μM), and 2-chloroaniline (50μM). 2-Chlorobenzaldehyde was reduced to 2-chlorobenzyl alcohol (2CBA) by all four consortia. 2CBA was not observed to decline. The reduction of aromatic aldehydes to alcohols has been previously reported (Neilson, et al. 1988). Both 2-chlorotoluene (50 μM) and toluene concentrations declined, but neither was completely degraded in any of the four consortia and toluene was not detected as a product from 2-chlorotoluene. Three consortia demethoxylated 2-chloroanisole to 2-chlorophenol which was not dechlorinated. Only consortium P21 did not degrade or transform 2-chloroanisole. It is apparent that the carboxyl function is important to the particular *ortho*-dechlorination process under study.

Two compounds, including 2-chlorophenylacetic acid (200 μm; 2CPA) and 2-chlorocinnamate (200 μM; 2CC), were chosen to investigate the effect of additional alkyl residues between the aromatic ring and the carboxyl side chain. A small amount (39 μM) of a compound tentatively identified by co-chromatography as phenylacetate was detected in consortium M34 incubated with 2CPA. However, the concentration of the product was too low to obtain an accurate UV spectrum to confirm its identity. Phenylacetate was not detected in the other three consortia. 2CC concentrations declined in all four consortia, but cinnamate was not detected. GC-MS analysis revealed the presence of an aromatic product, 2-chlorohydrocinnamate (2CHC), indicating reduction of the double bond in the propenoic side chain. This result was confirmed by testing 2-chlorohydrocinnamate, cinnamate, and hydrocinnamate. 2CC and cinnamate were biotransformed to 2CHC and hydrocinnamate, respectively, confirming reduction of the propenoic side group and indicating that 2CC was not dechlorinated. Hydrocinnamate was degraded by consortium P21 to non-aromatic end products. Anaerobic biodegradation of hydrocinnamate has been previously reported (Barik, et al. 1985). However, 2CHC was neither dechlorinated nor degraded. Results were similar with all four of our consortia. Again, the data indicate that the carboxyl group of the benzoate molecule is important to the *ortho* dechlorination process under study.

Most Probable Number (MPN) analyses (Rodina 1972) were performed to characterize other physiological types of bacteria present in the original 2CB-degrading consortia and the derived 2CB-dechlorinating consortia, M50^{-7}, M34^{-9}, P20^{-9}, and P21^{-9}. Consortium M50 and M34 contained 1×10^5 benzoate-degrading bacteria/ml, while P20 and P21 contained 1×10^4, and 1×10^9 per ml, respectively. Consortia M50^{-7}, M34^{-9}, P20^{-9}, and P21^{-9} were similar in containing H_2-utilizing anaerobes and sulfate-reducing bacteria as major physiological types of bacteria, i.e. 1×10^6 to 1×10^7 (Table 1). Benzoate-degrading bacteria were below detectable levels ($<1 \times 10^2$bt/ml) in consortia M50^{-7}, M34^{-9}, and P21^{-9}. As expected, benzoate accumulated in these three dilution consortia as shown for P21^{-9} in Figure 1. Benzoate-degrading anaerobes were also among the major physiological types of bacteria in consortium P20^{-9} and, as expected, was also the only dilution consortium to degrade the benzoate resulting from dechlorination of 2CB. After several laboratory transfers, P20^{-9} also lost its ability to degrade benzoate which was likely the result of loss of the bacterial strain(s) responsible for benzoate degradation.

Using the Gibbs free energies of formation (Dolfing & Harrison 1992; Thauer & Morris 1984; van den Tweel et al. 1987; Weast 1987), the thermodynamics for 2CB reductive dechlorination (pH 7.0, 25 °C) were calculated as:

$$\begin{array}{lr} & \Delta G^{0\prime} \\ 2CB^- + H_2 \rightarrow \text{Benzoate}^- + H^+ + Cl^- & -129\ \text{kJ/rx} \end{array}$$

The change in free energy indicates that 2CB could serve as a terminal electron acceptor during reductive dechlorination and provide enough energy for ATP synthesis and, hence, for cell growth.

These thermodynamic facts and our current data will allow us to design laboratory enrichment and isolation schemes to aid in obtaining pure cultures of the bacterial strains responsible for 2CB reductive dehalogenation in our consotia. Having determined the tolerance of our consortia toward 2CB, we will pass the consortia on relatively high concentrations of 2CB

(>2 mm) at intervals determined from our rate studies to take advantage of an initial increase in numbers of 2CB-dechlorinating bacteria during growth on 2CB as the terminal electron acceptor, as well as a likely inhibition of non-dechlorinating bacterial strains that may not tolerate these higher concentrations of 2CB well. This would provide consortia enriched in numbers of 2CB bacteria that would then be plated onto anaerobic 2CB agar medium or into anaerobic 2CB roll tubes containing the same high concentration of 2CB. The colonies that develop would have been selected for their ability to tolerate and grow on high concentrations of 2CB. The ability of the bacterial isolates to dechlorinate 2CB to benzoate would be determined in liquid anaerobic 2CB medium via HPLC.

In addition to the above approach, our current MPN studies revealed that sulfate-reducing and H_2/CO_2-utilizing bacterial species were the major physiological types of bacteria in the highest actively dechlorinating dilutions of the 2CB consortia, despite no enrichment pressure to select for either type of bacteria in the consortia. The first observation is of interest because *Desulfomonile tiedjei*, the only 3-chlorobenzoate-dechlorinating anaerobic bacterial species in pure culture (DeWeerd et al. 1986), was identified as a sulfate-reducing bacterial species after its isolation as a dechlorinating species and with no selective pressure for the isolation of sulfate-reducing bacterial strains.

Sulfate-reducing bacteria will be isolated from the 2CB dilution consortia using our technique that has proven successful in isolating a wide variety of new strains of sulfate-reducing bacterial species from enrichment of a salt marsh rhizosphere (Rooney-Varga et al. in press). In this technique an array of sulfate-reducing media that differ by the electron donors included are incorporated into liquid medium. Dilutions of the consortia, enrichment or culture under study are prepared in these media to obtain the most numerous of a particular physiological type of sulfate-reducing bacteria. Similar to the above approach, isolated sulfate-reducing bacterial strains will be tested for their ability to dechlorinate 2CB in a variety of liquid anaerobic medium containing a array of electron donors with the potential to support for 2CB dechlorination.

H_2/CO_2-utilizing bacterial strains may be present in the dilution consortia due to the formation of hydrogen and interspecies hydrogen transport, but could also be involved in reductive dechlorination. In order to obtain pure cultures of the H_2/CO_2-utilizing anaerobic bacterial strains, which can include both methanogens and acetogens, consortia will be inoculated into anaerobic H_2/CO_2 roll tubes for isolation. Colonies will be selected from roll tubes and inoculated into dechlorinating liquid medium, as described above, to determine if the isolate has the capacity to dechlorinate 2CB, and into liquid medium under a H_2/CO_2 gas phase in which the major metabolic end-product will be identified as either methane or acetate. Thus, we will be able to confirm the identity of the any isolate that dechlorinates 2CB as either a methanogen or acetogen, respectively.

Conclusions

The current study is the first to show reductive dechlorination of 2CB by anaerobic bacterial consortia as shown by the accumulation of benzoate as the dehalogenation product. Dehalogenation by our consortia occurred exclusively at the *ortho* position. Benzoate was the preferred substrate, although compounds closely related to benzoate were also *ortho*-dehalogenated. The carboxyl group of benzoate was important to this *ortho*-dehalogenation process under study. The presence and position of additional side groups on the benzoate molecule as well as the presence of additional alkyl units between the carboxyl group and the aromatic ring affected the ability of our consortia to *ortho*-dehalogenate aromatic compounds related to benzoate. These observations indicate that the *ortho*-dechlorinating activity in our consortia was unique and different from reductive *ortho*-dechlorination of multi-chlorinated benzoates (Gerritse, et al. 1992; Van der Woude et al. 1996).

Information gathered on tolerance to 2CB and major physiological types of bacteria present in consortia derived from the highest actively dechlorinating dilution of the original 2CB consortia will be used to aid in the isolation of the bacterial species responsible for 2CB dechlorination in our consortia.

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