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The effect of varying levels of sodium bicarbonate on polychlorinated biphenyl dechlorination in Hudson River sediment cultures

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Summary

The addition of different concentrations of sodium bicarbonate had a profound effect on 2,3,4,5-chlorobiphenyl (2,3,4,5-CB) dechlorination in Hudson River sediment cultures. The most extensive dechlorination was observed in cultures to which 100 mg l^{-1} bicarbonate was added. Cultures amended with 1000 mg l^{-1} bicarbonate had the least extensive dechlorination, with 2,4-CB and 2,5-CB as predominant end-products. A significant loss of total chlorinated biphenyl mass was observed in cultures to which ≤500 mg l^{-1} bicarbonate was added, suggesting that degradation beyond chlorinated biphenyls occurred. The dynamics of acetate formation were different among the treatments, with high acetate concentrations detected throughout the 303-day experiment in cultures to which 1000 mg l^{-1} bicarbonate had been added. Sodium bicarbonate addition also had a significant impact on bacterial community structure as detected by polymerase chain reaction-denaturant gradient gel electrophoresis (PCR-DGGE) of 16S rRNA gene fragments. Three putative polychlorinated biphenyl (PCB) dechlorinators were identified; one *Dehalococcoides*-like population was detected in all enrichment cultures, whereas two *Dehalobacter*-like populations were only detected in the enrichment cultures with the most extensive dechlorination. These results suggest that the availability of bicarbonate, and potentially sodium, may affect PCB dechlorination in Hudson River sediment and thus need to be taken into consideration when assessing the fate of PCBs or implementing bioremediation.

Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous contaminants in soils and sediments. For example, from the late 1940s until 1977, an estimated 0.2–1.3 million pounds of PCBs were discharged into the Hudson River (US Environmental Protection Agency, 2000). The stable and hydrophobic nature of PCBs causes them to bioaccumulate and biomagnify through the food chain (Safe *et al.*, 1987). Because of the risk that PCBs pose to human and ecosystem health, remediation technologies have been sought for the clean-up of PCB-contaminated sediments.

Anaerobic PCB dechlorination was first discovered in Hudson River sediment based on altered congener distribution patterns *in situ* (Brown *et al.*, 1987); PCB dechlorination was subsequently validated in laboratory experiments (Quensen *et al.*, 1988). Although little is known about the physiology of the organisms that anaerobically dechlorinate PCBs, it is believed that PCBs are used as electron acceptors during dehalorespiration (Quensen *et al.*, 1988). Dehalorespiration of PCBs may therefore be similar to that observed with other chlorinated compounds, such as tetrachloroethylene (Maymó-Gatell *et al.*, 1997), 1,1,1-

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trichloroethane (Sun *et al.*, 2002) and chlorobenzenes (Adrian *et al.*, 2000). Two putative PCB dechlorinators, detected in highly enriched cultures actively dechlorinating PCBs, were phylogenetically related to other dehalorespirers (Cutter *et al.*, 2001;Wu *et al.*, 2002), providing additional support for this hypothesis.

Although the addition of H_2 (Sokol *et al.*, 1994) and organic electron donors (Nies and Vogel, 1990;Alder *et al.*, 1993) can stimulate PCB dechlorination, no research has specifically investigated the effect of inorganic carbon on dechlorination. Indeed, bicarbonate has been typically added to the medium at concentrations of 1.2–2.5 g l^{-1} in studies investigating PCB dechlorination (Shelton and Tiedje, 1984;van Dort *et al.*, 1997;Adrian *et al.*, 1998;Zwiernik *et al.*, 1998;Magar *et al.*, 1999;Zwiernik *et al.*, 1999). Nevertheless, its concentration during incubation has, to our knowledge, not been monitored or replenished. Most hydrogenotrophs utilize aqueous CO_2 as their carbon source (Madigan *et al.*, 2003), although *Dehalococcoides ethenogenes* strain 195 respires tetrachloroethylene while utilizing acetate as a carbon source (Maymó-Gatell *et al.*, 1997). The carbon source for PCB dechlorinators is currently unknown, although H_2 appears to be an electron donor for PCB dechlorinators (Sokol *et al.*, 1994;Rysavy *et al.*, 2005), suggesting that aqueous CO_2 may be important for the growth of these organisms. Although it is generally assumed that inorganic carbon has little effect on the growth of bacteria, a recent study demonstrated that higher-than-ambient partial pressures of CO_2 (5%) improved the growth of *Acidobacteria* (Stevenson *et al.*, 2004). Whether or not PCB dechlorinators are autotrophs, the presence of elevated concentrations of CO_2 may improve the growth and subsequent activity of these organisms because they live in sediments that contain regions of higher-than-ambient levels of CO_2. It is therefore important to determine whether CO_2 can in fact affect the growth of PCB dechlorinators so that this information can be exploited for either bioremediation or the isolation of these organisms.

In this study, the impact of aqueous CO_2 on the reductive dehalogenation of 2,3,4,5-chlorobiphenyl (2,3,4,5-CB) was investigated in Hudson River sediment cultures. Our hypothesis was that the amendment of bicarbonate to the cultures could directly stimulate reductive dechlorination by providing additional inorganic carbon for the growth of PCB dechlorinators. Alternatively, the addition of bicarbonate could stimulate homoacetogenesis, which would generate acetate (another possible carbon source). Because it may be possible to alter the inorganic and organic carbon concentrations in sediment or in an active sediment cap, this research could lead to substantially improved knowledge-based designs for sediment bioremediation. In addition, if the effect of inorganic carbon on PCB dechlorinators is known, then aqueous CO_2 concentrations could be optimized to enrich and eventually isolate these important organisms.

Results

2,3,4,5-CB dechlorination

Dechlorination of 2,3,4,5-CB was observed in all live microcosms after 136 days of incubation (Fig. 1); no dechlorination was observed in the sterile controls (data not shown). The use of a single congener (2,3,4,5-CB) enabled us to identify the active dechlorination processes under different conditions. Because of the extremely low solubility of PCBs, evenly distributing them in sediment cultures was a challenge, initially resulting in poor recoveries of 2,3,4,5-CB and high variability in the beginning of the experiment (Fig. 1). This problem, however, was solved by continued incubation and homogenization with time (Fig. 1).

Dechlorination began with the removal of doubly flanked (DF) *meta* and *para* chlorines (forming 2,3,5-CB and 2,4,5-CB). This was followed by the removal of singly flanked (SF) *meta* or *para* chlorines (forming 2,4-CB and 2,5-CB). The concentration dynamics of 2,3,5-CB, 2,5-CB and 2-CB (Fig. 1) suggest that the majority of 2,5-CB was formed from 2,3,5-CB

dechlorination, and that the majority of 2-CB was formed from 2,5-CB dechlorination. In treatments to which ≤ 500 mg l^{-1} bicarbonate was added, the increase of 2,3,5-CB dechlorination products was not proportional to the decrease of 2,3,5-CB. This suggests the concomitant production and degradation of 2,3-CB, 2,5-CB and 2-CB.

There were substantial differences in the dechlorination pattern of 2,3,4,5-CB (Fig. 1) and the resulting product distributions (Fig. 2). In the microcosms to which 500 or 1000 mg l^{-1} bicarbonate was added, 2,3,5-CB dechlorinated either slowly or not at all (Fig. 1C and D). In contrast, 2,3,5-CB was formed and dechlorinated rapidly in the microcosms fed with 100 mg l^{-1} bicarbonate (Fig. 1B). The majority of the other observed dechlorination products (2,4,5-CB, 2,5-CB, 2,3-CB) were subsequently dechlorinated, with only 2,4-CB and 2-CB forming and persisting at very low concentrations (approximately 7 and 2 M respectively) (Fig. 1B). In the microcosms fed with no additional bicarbonate (Fig. 1A), 2,3,4,5-CB appeared to be dechlorinated more slowly, but all of the observed dechlorination products appeared to be formed and dechlorinated simultaneously, as they did not accumulate to a great extent. The dechlorination of 2,3,5-CB, 2,5-CB and 2,4-CB appeared to be slower than in the microcosms amended with 100 mg l^{-1} bicarbonate (Fig. 1A and B).

The total quantity of chlorinated biphenyls varied substantially over time (averaged over triplicate microcosms) in all of the treatments (Fig. 3). Initially, the mass of total chlorinated biphenyls (present as 100% 2,3,4,5-CB on day 0) measured in all of the microcosms, including the sterile control, was low (6.8 ± 1.4 mol) compared with the quantity added (12 mol). This is likely a result of the hydrophobicity of PCBs and the fact that they are very difficult to homogenize upon addition to sediment microcosms. Over time, however, the total mass of chlorinated biphenyls increased as the PCBs homogenized more completely and more representative slurry samples were taken.

By day 99, the total quantity of chlorinated biphenyls was much higher and closer to 12 mol as expected (10.5 ± 2.7 mol). From day 0 to day 136, the quantity of total chlorinated biphenyls measured in all of the microcosms at a given time point was relatively consistent, with an average standard deviation over all of the microcosms of 15.6%. Therefore, although the total mass measured was lower than expected until around day 100, all of the microcosms behaved similarly with respect to the total quantity of chlorinated biphenyl present and extractable/measurable.

On day 225, however, the total measured mass of chlorinated biphenyls began to significantly decrease in the microcosms to which 500 mg l^{-1}, 100 mg l^{-1} and no bicarbonate were added (Fig. 3) and began to deviate significantly from the calculated total molar mass (12 mol). The total measured chlorinated biphenyls in the microcosms amended with 1000 mg l^{-1} bicarbonate [9.1 ± 0.4 mol (day 225), 11.7 ± 1.0 mol (day 267) and 11.9 ± 0.9 mol (day 303)], and in the sterile control on day 303 (13.1 ± 0.7 mol) remained relatively constant over days 225–303 (11.4 ± 1.7 mol) and close in value to the calculated total molar mass of chlorinated biphenyl added to the microcosms (12 mol). The mass of chlorinated biphenyls measured on day 303 in the microcosms receiving ≤ 500 mg l^{-1} bicarbonate was significantly lower ($P < 0.05$) than that in the microcosms receiving 1000 mg l^{-1} bicarbonate. The greatest mass balance deficit was observed in microcosms receiving 100 mg l^{-1} bicarbonate; the differences in the quantity of total chlorinated biphenyls present on day 303 among all live treatments were statistically significant.

Acetogenesis and methanogenesis

All live microcosms produced similar quantities of methane, whereas no methane was produced by the sterile control (data not shown). The production of acetate, however, varied substantially between the different treatments in the live microcosms (Fig. 4). The initial acetate

concentration in all microcosms on day 0 was very low (0.077 ± 0.018 mM). On day 69, the acetate concentration increased to about 2 mM in all microcosms, presumably as a result of the fermentation of sediment organic matter and subsequent acetogenesis. After day 197, the acetate concentrations declined and remained low except in the microcosms receiving 1000 mg l^{-1} bicarbonate, in which acetate concentrations were significantly higher than those in all of the other microcosms.

Community analysis

Polymerase chain reaction-denaturant gradient gel electrophoresis (PCR-DGGE) was used to fingerprint the bacterial community structures of the microcosms receiving different amounts of bicarbonate on day 225, when different dechlorination patterns were first observed (Fig. 5A). No defined bands were detectable in a sample of Hudson River sediment that was used to inoculate the cultures, indicating the presence of numerous bacterial populations of low densities (Nakatsu *et al.*, 2000). Numerous defined bands were discernible, however, in the PCB-amended enrichment cultures. Four bands (Bands A, B, H and J) were common to all of the experimental microcosms, whereas several populations were found in only a few of the enrichments. For example, Band E was only present at a discernible density in microcosms containing bicarbonate ≤ 500 mg l^{-1}. Principal component analysis demonstrated that different concentrations of bicarbonate addition had a significant impact on bacterial community structure (Fig. 5B).

Numerous prominent bands were excised from this gel and their nucleotide sequences were determined (Table 1). Phylogenetic analysis of the nucleotide sequences suggested three putative dechlorinating populations. Band B was phylogenetically related to *Dehalococcoides* strain CBDB1, which can dehalogenate chlorinated benzenes (Adrian *et al.*, 2000). This band was present in all microcosms. Bands C and E were phylogenetically related to *Dehalobacter restrictus*, which can. dehalogenate chlorinated aliphatic compounds (Wild *et al.*, 1996;Holliger *et al.*, 1998;Sun *et al.*, 2002). Both of these *Dehalobacter*-like populations were more prominent in the microcosms amended with 100 mg l^{-1} bicarbonate compared with the other microcosms (Fig. 5A).

Because PCR-DGGE provides only limited phylogenetic information of the detected populations, the bacterial community in one of the microcosms receiving 100 mg l−1 bicarbonate (day 225) was also investigated by PCR cloning of nearly complete 16S rRNA genes. A total of 65 clones were screened by PCR-DGGE (data not shown) to give 19 different 16S rRNA gene sequences (Table 2). The population with the highest frequency of appearance in the clone library (clone Z42) was closely related to *Dehalococcoides* strain CBDB1, and was identical in sequence to Band B. Two other populations (clones Z29 and Z40) were phylogenetically related to *Dehalobacter restrictus* and were identical in sequence to Bands C and E respectively.

Discussion

This study demonstrates that the addition of exogenous sodium bicarbonate to Hudson River sediment cultures had a profound effect on PCB dechlorination. Microcosms amended with an intermediate amount of sodium bicarbonate (100 mg l^{-1} as HCO_3^-) appeared to exhibit more extensive dechlorination of 2,3,4,5-CB (Figs 2 and 3) and more rapid dechlorination of its daughter products (Fig. 1) than either the control microcosms to which no additional sodium bicarbonate was added or the microcosms to which higher amounts of sodium bicarbonate (500 or 1000 mg l^{-1} as HCO_3^-) were added. The microcosms to which ≤ 500 mg l^{-1} bicarbonate was added also contained three putative dechlorinating populations. The two *Dehalobacter*-like populations were more prominent in the microcosms to which 100 mg l^{-1} bicarbonate was added (Fig. 5A) and in which more rapid and extensive dechlorination occurred. These results

suggest that the addition of a small quantity of sodium bicarbonate can stimulate reductive dehalogenation of PCBs and help select for a more diverse dehalogenating bacterial community, whereas the addition of excessive sodium bicarbonate can adversely affect dechlorination processes.

Although both the sodium and bicarbonate concentrations changed in the various treatments (from approximately 2.5–18.9 mM sodium and from approximately 0–16.4 mM bicarbonate), we believe that it was the changing bicarbonate, rather than sodium concentrations, that altered the observed dechlorination. Sodium is known to be important for acetogenesis (Heise *et al.*, 1989) and methanogenesis (Blaut *et al.*, 1985), and if limiting in these experiments, varying sodium concentrations would have been expected to affect the rates of methanogenesis and acetogenesis in the microcosms. This was not observed, suggesting that bicarbonate was indeed the critical variable.

The results presented herein also suggest that a substantial fraction (> 50%) of the originally added 2,3,4,5-CB was degraded beyond chlorinated biphenyls (Fig. 3). This interpretation is supported by a mass balance deficit that occurred only in microcosms where 2-CB was produced and in which substantial sequential dechlorination past the trichlorobiphenyls was observed. In previous studies of PCB dechlorination, either similar mass balance deficits or anaerobic dechlorination of PCB congeners to biphenyl were also observed (Rhee *et al.*, 1993a,b;Williams, 1994;Natarajan *et al.*, 1996). Our results suggest that the addition of a small quantity of sodium bicarbonate enhanced the extent of anaerobic 2,3,4,5-CB degradation beyond chlorinated biphenyls.

The bacterial community structures of the sediment cultures were also substantially affected by sodium bicarbonate concentration. Of particular importance is the selection of several putative dechlorinating populations as a result of the changes in sodium bicarbonate concentration. Although populations with subtly different or even identical 16S rRNA gene sequences can have markedly different physiologies (Jaspers and Overmann, 2004), numerous researchers have isolated *Dehalococcoides*-like populations capable of the dehalorespiration of both aliphatic (Maymó-Gatell *et al.*, 1997;He *et al.*, 2003) and aromatic compounds (Adrian *et al.*, 2000;Fennell *et al.*, 2004), or enriched for *Dehalococcoides*-like populations that appeared to be associated with PCB dechlorination (Cutter *et al.*, 2001;Wu *et al.*, 2002). In addition, work in our laboratory has shown that in paired microcosm experiments in which one set of microcosms received PCBs and another did not, *Dehalococcoides*-like populations developed in microcosms to which PCBs were added, but were absent in microcosms to which no PCBs were added (Yan *et al.*, 2006), suggesting that these organisms were associated with PCB dechlorination. Previous researchers have also characterized *Dehalobacter* spp. as obligate hydrogen-oxidizing, dehalorespiring organisms (Wild *et al.*, 1996;Holliger *et al.*, 1998;Sun *et al.*, 2002); *Dehalobacter*-like populations have also been detected in a 1,2-dichloropropane-dechlorinating bioreactor (Schlotelburg *et al.*, 2002). The *Dehalococcoides*-like populations in these microcosms did not appear to be affected by the changing sodium bicarbonate concentrations. This is consistent with current evidence that suggests that these organisms use acetate as a carbon source, rather than fixing inorganic carbon (Maymó-Gatell *et al.*, 1995;Holliger *et al.*, 1998). The presence of *Dehalobacter*-like populations, however, was affected by the different sodium bicarbonate concentrations. Our PCB-dechlorinating sediment cultures contained as many as two *Dehalobacter*-like populations, which were only present when 2-CB was formed and the bicarbonate concentration was ≤ 500 mg l^{-1}. In addition, these populations appeared to be more prominent in the microcosms to which 100 mg l^{-1} sodium bicarbonate (as HCO_3^-) was added and in which the most rapid and extensive dechlorination occurred. To the knowledge of the authors, the present study is the first to detect *Dehalobacter*-like populations in association with PCB dechlorination and is also the first to

simultaneously detect both *Dehaloccoides*-like and *Dehalobacter*-like populations in PCB-dechlorinating enrichment cultures.

The addition of very high concentrations of sodium bicarbonate resulted in increased acetate concentrations and a reduced rate and extent of dechlorination. We speculate that the high sodium bicarbonate concentrations either decreased the consumption of acetate, which had some negative effect on the development of a niche for PCB dechlorinators, or stimulated homoacetogenesis, which altered the flow of nutrients and electron donor away from dehalorespiration and specifically created an environment unfavourable to the enrichment of *Dehalobacter*-like populations.

In conclusion, considerable attention has focused on the use of appropriate electron donors to stimulate the dechlorination of environmental contaminants (e.g. Fennell *et al.*, 1997;Yang and McCarty, 1998). Little work, however, has been conducted on the effect of carbon dioxide or bicarbonate concentrations on such processes, which may provide information that can be exploited for the bioremediation of contaminated sediments and is important for understanding the physiology of PCB dechlorinators and for use in their isolation (Stevenson *et al.*, 2004). In this research we observed that 2,3,4,5-CB dechlorination in Hudson River sediment could be stimulated by the amendment of low levels of sodium bicarbonate. Because the amendment of higher quantities of sodium bicarbonate adversely affected dechlorination, however, it is critical to continue to probe the mechanisms behind this phenomenon so that appropriate enrichment techniques or *in situ* biostimulation techniques can be developed. This research has practical implications in that it may be possible to alter the inorganic and organic carbon concentrations in contaminated sediments or active caps to stimulate PCB bioremediation. The control of inorganic carbon concentrations could also allow one to better enrich and potentially isolate PCB dechlorinators.

Experimental procedures

Experimental set-up

Sediment samples collected from the Hudson River near Moreau, New York [North American Datum of 1983 (NAD83) Northing Coordinate (ft) 1609914.52 and NAD83 Easting Coordinate (ft) 733570.10] were transported under anaerobic conditions and stored in a glovebag (COY labs, MI) upon arrival. Reduced anaerobic mineral medium was prepared as described by Shelton and Tiedje (1984), except that 2 mM L-cysteine was used as a reducing agent instead of Na_2S and no bicarbonate was added to the medium. The pH of the medium was adjusted to 7.0 with approximately 2.5 mM NaOH. The medium was sterilized by autoclaving and stored in the glovebag before use.

Microcosms were set up in 160 ml serum bottles containing 100 ml medium. Because sediment has been shown to be important for the development of diverse PCB-dechlorinating activities (e.g. Morris *et al.*, 1990;Wu and Wiegel, 1997), Hudson River sediment (5 g dry weight equivalent) was used as the inoculum. Sediment that had been stored under anaerobic conditions was weighed and transferred into the bottles under strict anaerobic conditions. The sediment inorganic carbon was below the detection limit (0.15% by mass) and the organic carbon was 2.12% (by mass). The congener 2,3,4,5-CB (12.5 moles in 0.4 ml of hexane) was added to each bottle. The use of 2,3,4,5-CB as the model congener prevented interference from any naturally present PCBs, as 2,3,4,5-CB and its daughter products are unlikely to be present as a result of industrial contamination. Hexane was allowed to evaporate by keeping the bottles uncapped in the glovebag (gas composition: 4% H_2 and 96% N_2) for about 10 min until the added hexane was no longer visible as a separate layer. Bottles were then capped with Teflon-lined rubber stoppers, sealed with aluminum crimps and flushed with N_2 to remove H_2 from the headspace. Bottles were shaken on a wrist-action shaker for 2 h for PCB distribution, after

which they were incubated on a rotating shaker (120 r.p.m.). Initial samples were collected in the glovebag for PCB analysis 24 h after 2,3,4,5-CB addition.

After initial samples were taken, bottles were resealed and again flushed with N_2. At this time, bottles were amended with a pre-reduced (with 20 mM L-cysteine) bicarbonate stock solution containing 50 g l^{-1} $NaHCO_3$ and 20 mM phosphate at a pH of 7.2 to generate the following treatments: 0, 100, 500 and 1000 mg l^{-1} bicarbonate. This added a small additional quantity of cysteine (0–0.4 mM) to the microcosms, but this was not thought to be significant. An additional treatment containing 1000 mg l^{-1} bicarbonate was autoclaved for 50 min on two consecutive days to serve as a sterile control. Each treatment consisted of triplicate microcosms. H_2 (0.06 ml) was injected into the headspace (60 ml) of each bottle to reach a headspace H_2 partial pressure of 0.001 atm (0.1% H_2). Additional H_2 (0.06 ml) was injected into all of the microcosms weekly. The concentration of H_2 was not monitored between weekly additions; nevertheless, no accumulation of H_2 was observed in the live microcosms when the headspace gas was analysed monthly.

Microcosms were incubated at 25°C in the dark on a rotating shaker (120 r.p.m.). The pH of the culture was measured and adjusted to 7.0 when bottles were opened for sediment sampling. The pH values in all microcosms before adjustment ranged from 6.76 to 7.53 and no distinct trends were observed among the different treatments (data not shown). The concentration of CO_2 in the headspace was measured; the bicarbonate concentration in the medium was calculated assuming equilibrium; and if needed, additional bicarbonate was added to maintain the desired bicarbonate concentration after resealing the microcosms. All gases used in these experiments (N_2, H_2 and He) were ultra-high purity grade.

Sample collection

Microcosms were completely mixed for 2 min before the bottles were opened in the glovebag. Approximately 1.5 ml of the sediment slurry was withdrawn from the bottles using a glass pipette. The bottles were opened and resealed quickly to limit gas exchange. Samples were placed in 20 ml serum vials and the exact weight of the samples was determined. For bacterial community analysis, sediment slurry samples were collected in sterile 1.6 ml microcentrifuge tubes, centrifuged at 19 000 *g* for 5 min, and the pellet was stored at −70°C until DNA extraction. Supernatant samples (2 ml) were also withdrawn from the microcosms and filtered (0.45 m) for immediate acetate analysis.

Chemical analysis

Headspace concentrations of CH_4 and CO_2 were measured before the microcosms were opened for withdrawal of the sediment slurry. Headspace gas samples (200 l) were analysed using a gas chromatograph (GC) [Hewlett Packard (HP) 6890 Series] equipped with a thermal conductivity detector. Separation of the components in the gas sample was accomplished on a packed column (8 ft Hayesep Q, 8 ft × 0.125 in) with He as the carrier gas (flow rate: 20 ml min^{-1}). Acetate was analysed on an ion chromatograph (761 compact IC, Metrohm) with an anion separation column (Metrosep A supp5, Metrohm).

The sample extraction method for PCB analysis was based on the method of Quensen and colleagues (1988) and is described elsewhere (Rysavy *et al.*, 2005). Briefly, sediment slurry samples were weighed and then extracted, first with acetone (10 ml) for 2 min, followed by two hexane-acetone (1:1) (10 ml) extractions, each for 2 min. The pooled extracts (30 ml) were mixed with 10 ml of a 2% (v/v) NaCl solution for 2 min and the hexane layer was removed. The hexane layer was then mixed with 4 ml of a 30% (v/v) sulfuric acid solution for 2 min, after which the hexane layer was removed. This layer was again mixed with 10 ml of a 2% (v/v) NaCl solution, after which the hexane layer was removed, dried with Na_2SO_4 and filtered

through a florisil-copper (25% copper w/w) column. The hexane extract was adjusted to a volume of 25 ml. The extracted PCBs were analysed on a GC equipped with an electron capture detector (ECD). An HP-1 capillary column (25 m × 0.200 mm × 0.11 m film thickness) was used for congener separation. The identification of 2,3,4,5-CB and its dechlorination products was primarily accomplished by comparing retention times with those of authentic standards (AccuStandard) on GC-ECD. Congeners 2,4-CB and 2,5-CB have very close retention times; therefore, identification was accomplished by analysing replicate GC samples and standards. Products were verified using a GC (HP 5890 Series II) equipped with an HP 5972 mass selective detector.

Characterization of sediment carbon

Sediment characterization was performed by the Research Analytical Laboratory in the Department of Soil, Water and Climate at the University of Minnesota. The total organic carbon was determined by dry combustion and subsequent measurement of CO_2 by infrared (IR) spectrum absorption using a Skalar Primacs carbon furnace. The inorganic carbon was converted to CO_2 with phosphoric acid and then measured by IR spectrum absorption. The detection limits were 0.10% and 0.15% for total organic carbon and inorganic carbon respectively.

DNA extraction

Cell lysis was accomplished by a combination of chemical and physical approaches. Briefly, 450 l of phosphate buffer (100 mM, pH = 8) and 450 l of lysis buffer [100 mM NaCl, 500 mM Tris (pH = 8), 10% (w/v) SDS] were added to the sediment pellets. Samples were incubated at 70°C for 90 min and then subjected to bead-beating to help ensure complete cell lysis. Genomic DNA was purified using a FastDNA kit for soil (Q-BIOgene; Vista, CA).

Polymerase chain reaction-denaturant gradient gel electrophoresis (PCR-DGGE)

Polymerase chain reaction was performed using a PTC 100 thermal cycler (MJ Research; Watertown, MA). Partial 16S rRNA genes were amplified from the extracted genomic DNA using primers 338F (5′-ACT CCT ACG GGA GGC AGC AG-3′) (Lane, 1991) and 518R (5′-ATT ACC GCG GCT GCT GCT GG-3′) (Muyzer *et al.*, 1993) with a GC clamp attached to the forward primer (Muyzer *et al.*, 1993). The final 50 l of reaction mixture contained: 1× PCR buffer (Promega; Madison, WI), 175 mol $MgCl_2$, 4 nmol deoxynucleoside triphosphates, 2% bovine serum albumin, 25 pmol forward and reverse primers, 1.25 units of Taq polymerase (Promega) and ~1 ng of template DNA. The PCR protocol included a 5 min initial denaturation at 94°C, 30 cycles of 92°C for 30 s, 55°C for 30 s and 72°C for 30 s, and a final extension for 10 min at 72°C.

Polymerase chain reaction products were loaded onto 8% (w/v) polyacrylamide gels with the denaturing gradient ranging from 30% to 55% [100% denaturant contains 7 M urea and 40% formamide in 0.5× Tris-acetate-EDTA (TAE) buffer]. Electrophoresis was performed on a D-Code apparatus (Bio-Rad; Hercules, CA) in 0.5× TAE buffer at 60°C, initially at 20 V for 20 min followed by 200 V for 270 min. Gels were stained with SYBR Green I (Molecular Probes; diluted 1:5000 in 0.5× TAE), visualized on a UV transilluminator and photographed with a digital CCD camera (BioChemi System; UVP; Upland, CA).

Prominent bands were excised and further purified by repeated PCR-DGGE until only a single band was detectable. A final PCR was performed using primers 338F (without the GC clamp) and 518R. These PCR products were purified using a Geneclean Kit (Q-Biogene) before nucleotide sequence determination. In several cases, vertically co-migrating bands were analysed from different gel lanes for quality assurance.

Polymerase chain reaction cloning

Nearly complete 16S rRNA genes were amplified by PCR using primers 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) (Edwards *et al.*, 1989) and 1522R (5′-AAG GAG GTG ATC CAN CCR CA-3′) (Johnson, 1994). The composition of the PCR mixture was the same as that described above. The PCR protocol included a 5 min initial denaturation at 94°C, 35 cycles of 94°C for 1 min, 55°C for 1 min and 72°C for 2 min, and a final extension for 10 min at 72°C. Polymerase chain reaction amplicons were purified, ligated into the pGEM-T Easy cloning vector (Promega), and transformed into competent *Escherichia coli* DH5α cells. Plasmids were extracted by the alkaline lysis method (Sambrook *et al.*, 1989). Plasmids were then analysed from unique inserts by PCR-DGGE as described previously (LaPara *et al.*, 2000).

Nucleotide sequence analysis

Nucleotide sequences were determined at the Advanced Genetic Analysis Center (University of Minnesota) using an ABI 3100 Genetic Analyser (Applied Biosystems; Foster City, CA). Polymerase chain reaction-DGGE bands were sequenced using primers 338F and 518R. Plasmids were sequenced using primers 27F, 907R, 907F (Muyzer *et al.*, 1995) and 1522R. Reported nucleotide sequences are the consensus of bi-directional sequence information and do not include the original PCR primer sequences.

Data analysis

A two-sided Student's *t*-test was performed at the 95% confidence interval to determine whether observed differences between experimental treatments were statistically significant. Principal component analysis was performed using StatistiXL ver. 1.4 (Kalamunda, Australia).

Sequences were compared with sequences in the Gen-Bank database (Benson *et al.*, 2000) using the BLASTN program (Altschul *et al.*, 1997) to search for their closest phylogenetic relatives. DNA sequences were screened for potential chimera using Chimera-Check from the Ribosomal Database Project II (Maidak *et al.*, 2001). Putative chimeric sequences were manually split into three different components and re-submitted to GenBank to determine whether these segments were from the same phylogenetic group.

Nucleotide sequence accession number

Sequences were deposited in the GenBank database under Accession No. AY754828 to AY754856.

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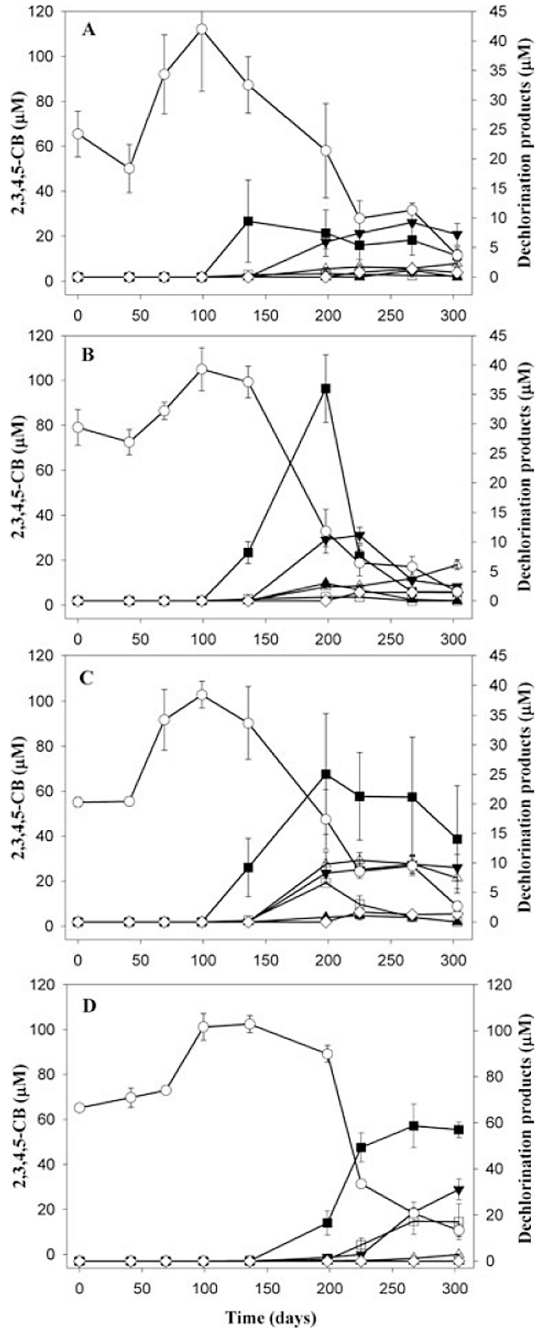

Fig 1.

Patterns of 2,3,4,5-CB dechlorination in microcosms amended with 0 mg l^{-1} (A), 100 mg l^{-1} (B), 500 mg l^{-1} (C) and 1000 mg l^{-1} (D) bicarbonate. Error bars represent the standard deviation of triplicate microcosms. ○, 2,3,4,5-CB; ■, 2,3,5-CB; □, 2,4,5-CB; ▲ = 2,3-CB; △, 2,4-CB; ▼, 2,5-CB; ◇, 2-CB.

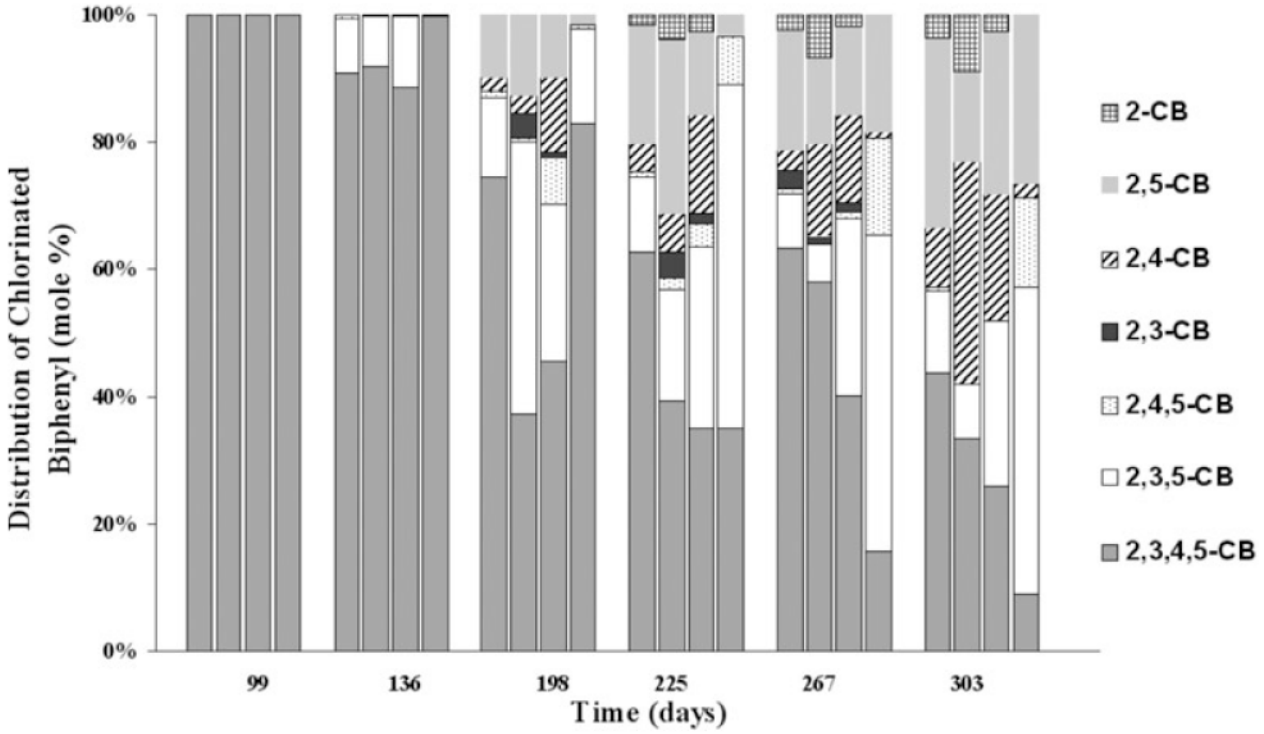

Fig 2.

Molar distribution of 2,3,4,5-CB and its dechlorination products. For each four-column group at a certain time point, the first to fourth columns represent microcosms with 0, 100, 500 and 1000 mg l^{-1} bicarbonate added respectively. The molar percentages presented here are average values of the triplicate microcosms.

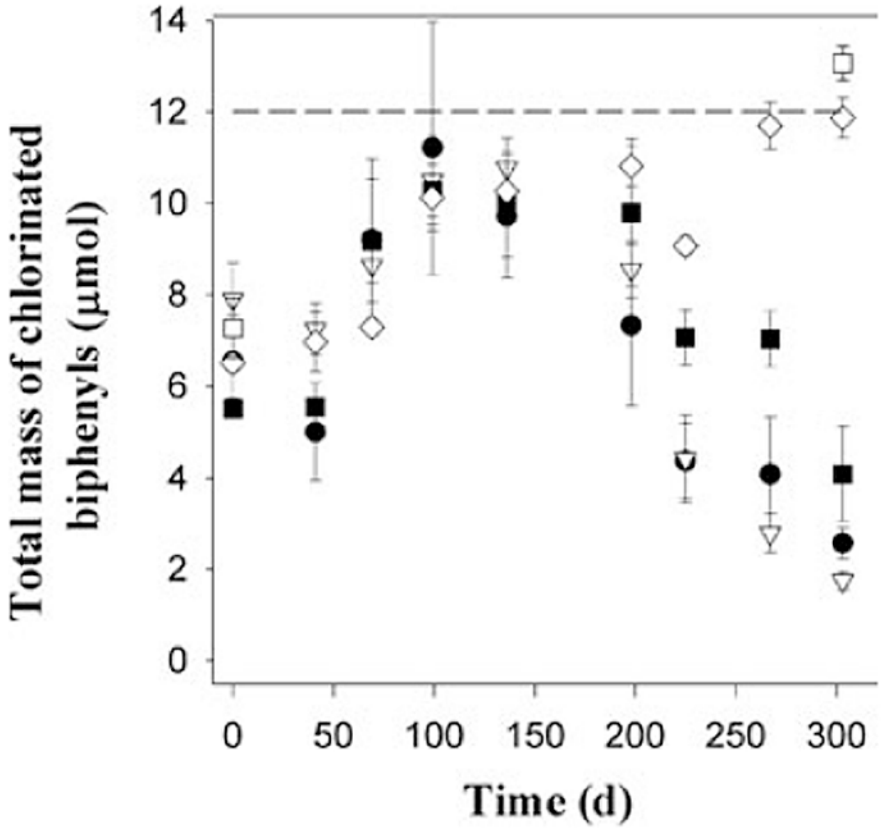

Fig 3.

Total measured mass of 2,3,4,5-CB and its identified dechlorination products (2,3,5-CB, 2,4,5-CB, 2,3-CB, 2,4-CB, 2,5-CB and 2-CB). Treatments are: ●, 0 mg l^{-1} bicarbonate; ▽, 100 mg l^{-1} bicarbonate; ■, 500 mg l^{-1} bicarbonate; ◇, 1000 mg l^{-1} bicarbonate, □, sterile control. The dashed line represents the calculated mass of 2,3,4,5-CB added to the microcosms.

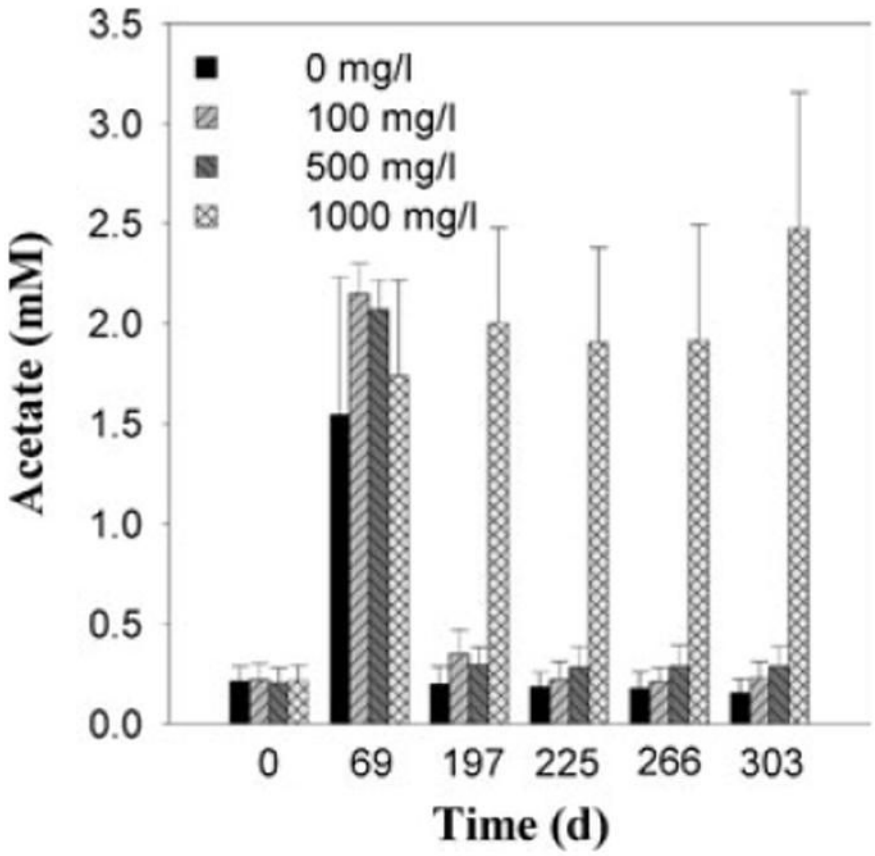

Fig 4.

Acetate concentrations in the microcosms amended with 2,3,4,5-CB as a function of time. Results are the mean ± standard deviation of triplicate microcosms.

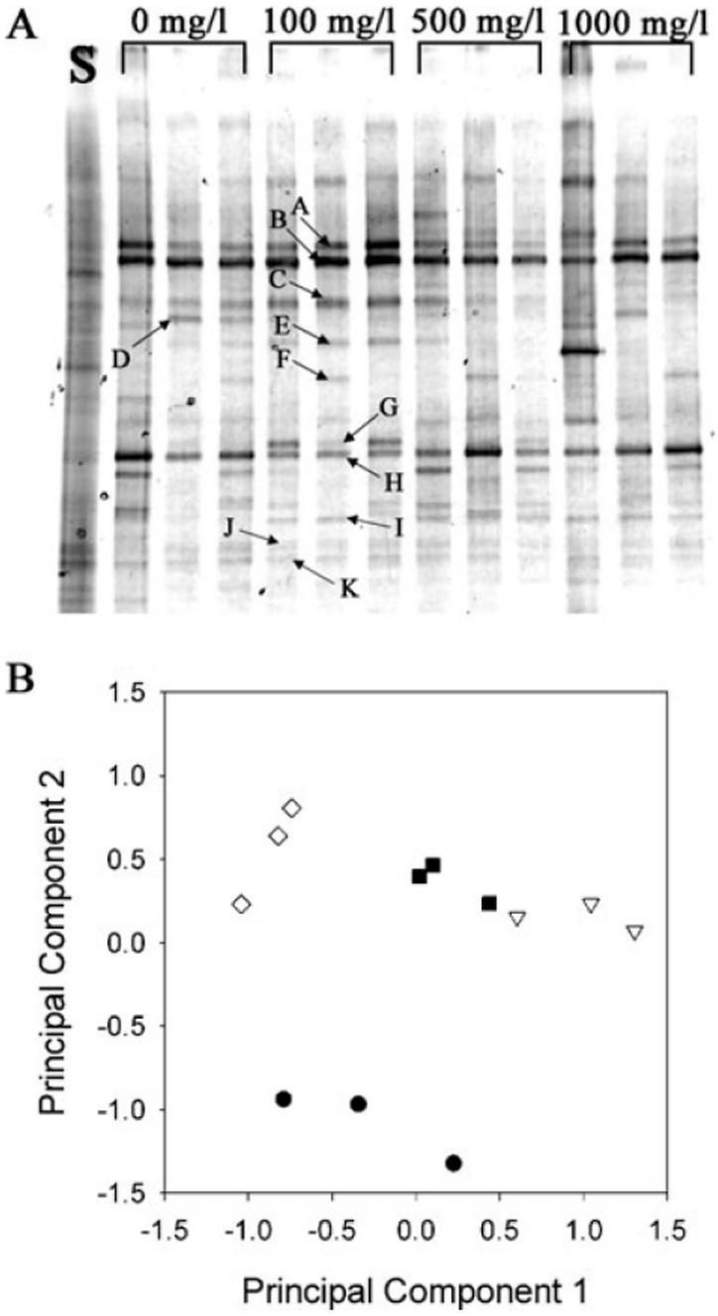

Fig 5.

A. Fingerprints of the bacterial community structures of microcosms (day 225) amended with 2,3,4,5-CB and different concentrations of bicarbonate as determined by PCR-DGGE of PCR-amplified 16S rRNA gene fragments. Lane S is the initial community profile for Hudson River sediment before amendment with 2,3,4,5-CB. All other lanes are identified by the concentration of bicarbonate amended to the microcosm. Letters and arrows identify specific bands that were excised from the gel and sequenced. The results of the nucleotide sequence analysis for these PCR-DGGE bands are shown inTable 1.

B. Principal component analysis of PCR-DGGE fingerprints. Principal components 1 and 2 represent 52% of the total variance in the community fingerprints. ●, 0 mg l^{-1} bicarbonate; ▽, 100 mg l^{-1} bicarbonate; ■, 500 mg l^{-1} bicarbonate; ◇, 1000 mg l^{-1} bicarbonate.

Table 1

The phylogenetic affiliations of bacterial populations detected by PCR-DGGE (Fig. 5A).

Band	Sequence length	Band intensity (%)	Phylogenetic affiliation		
			Organism (accession No.)	% Identity	Phylum
A	154	12.3	Environmental clone WCHB1-29 (AF050544)	96.1	*Bacteroidetes*
B	136	32.4	*Dehalococcoides* strain CBDB1 (AF230641)	100	*Chloroflexi*
C	161	20.3	*Dehalobacter restrictus* (Y10164)	100	*Firmicutes*
D	135		Environmental clone WCHB1-80 (AF050563)	99.2	*Chloroflexi*
E	161	7.8	*Dehalobacter restrictus* (Y10164)	99.3	*Firmicutes*
F	138	5.3	*Bdellovibrio bacteriovorus* (AF148941)	92.7	*δ-Proteobacteria*
G	135	1.5	*Clostridium* sp. 13A1 (AY554421)	100	*Firmicutes*
H	135	9.8	*Sedimentibacter* sp. BRS2 (AY221992)	94.8	*Firmicutes*
I	138	4.8	Environmental clone G18 (AF407701)	100	*Firmicutes*
J	135	2.6	Environmental clone LBS18 (AF445645)	90.3	Not known
K	136	3.3	Environmental clone LBS18 (AF445645)	88.9	*Planctomycetales*

Table 2

The phylogenetic affiliations of the bacterial populations detected by PCR cloning from a Hudson River sediment microcosm (day 225) amended with 2,3,4,5-CB and 100 mg l^{-1} of bicarbonate.

Clone	Sequence length	Clone frequency (%)	Phylogenetic relationship		
			Species (accession No.)	% Identity	Phylum
Z9	852	3.7	*Clostridium cylindrosporum* (Y18179)	91.6	*Firmicutes*
Z17	1476	7.4	Environmental clone KD3-5 (AY188305)	97.2	*Bacteroidetes*
Z20	1415	3.7	Chimeric		
Z29	1447	11.1	*Dehalobacter restrictus* (Y10164)	99.5	*Firmicutes*
Z30	815	3.7	Environmental clone GR25 (AY150887)	97.2	Ambiguous
Z35	1456	7.4	*Sedimentibacter hydroxybenzoicus* (L11305)	93.7	*Firmicutes*
Z39	873	3.7	*Pelospora glutarica* (AJ251214)	93.6	*Firmicutes*
Z40	1446	3.7	*Dehalobacter restrictus* (Y10164)	99.0	*Firmicutes*
Z42	1449	14.8	*Dehalococcoides* strain CBDB1 (AF230641)	99.8	*Chloroflexi*
Z43	824	3.7	Environmental clone DEL55 (AJ616285)	97.9	*Planctomycetales*
Z44	838	3.7	Environmental clone NMW3.210WL (AY043958)	87.5	Ambiguous
Z45	853	3.7	Environmental clone BIOEST-22 (AJ548911)	98.3	*Firmicutes*
Z52	849	3.7	*Sedimentibacter hydroxybenzoicus* (L11305)	92.1	*Firmicutes*
Z54	862	11.1	*Clostridium* sp. 9B4 (AY554416)	96.8	*Firmicutes*
Z57	820	3.7	Environmental clone WCHB1-40 (AF050549)	96.2	*Spirochaetes*
Z60	853	3.7	*Arthrobacter globiformis* (AB098573)	99.5	*Actinobacteria*
Z62	1470	3.7	Environmental clone BSA1B-04 (AB175358)	98.5	*Firmicutes*
Z63	818	3.7	Environmental clone BIOEST-22 (AJ548911)	97.0	*Firmicutes*