Minireview

The little bacteria that can – diversity, genomics and ecophysiology of ‘Dehalococcoides’ spp. in contaminated environments

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Summary
The fate and persistence of chlorinated organics in the environment have been a concern for the past 50 years. Industrialization and extensive agricultural activities have led to the accumulation of these pollutants in the environment, while their adverse impact on various ecosystems and human health also became evident. This review provides an update on the current knowledge of specialized anaerobic bacteria, namely ‘Dehalococcoides’ spp., which are dedicated to the transformation of various chlorinated organic compounds via reductive dechlorination. Advances in microbiology and molecular techniques shed light into the diversity and functioning of Dehalococcoides spp. in several different locations. Recent genome sequencing projects revealed a large number of genes that are potentially involved in reductive dechlorination. Molecular approaches towards analysis of diversity and expression especially of reductive dehalogenase-encoding genes are providing a growing body of knowledge on biodegradative pathways active in defined pure and mixed cultures as well as directly in the environment. Moreover, several successful field cases of bioremediation strengthen the notion of dedicated degraders such as Dehalococcoides spp. as key players in the restoration of contaminated environments.

Chlorine-containing chemicals like hexachlorobenzene (HCB), tetra- and trichloroethenes (PCE and TCE), dichlorodiphenyltrichloroethane (DDT), dioxins, polychlorinated biphenyls (PCBs), chlorophenols (CPs) and chlorofluorocarbons (CFCs) are persistent pollutants in our environment. Recognition of the ability of microorganisms to degrade these hazardous compounds opened up a new vista for the microbially mediated remediation of polluted environments. In addition, it also triggered the scientific community to undertake continued efforts towards the discovery, isolation and characterization of new microbial species. Among these, ‘Dehalococcoides’ spp. represent dedicated degraders, which are specialized in the anaerobic transformation of chlorinated organic contaminants that may otherwise persist in the environment for decades. In 1997, Maymó-Gatell and co-authors isolated the first anaerobic bacterium, ‘Dehalococcoides ethenogenes’ strain 195 (Maymo-Gatell et al., 1997), that can transform toxic PCE completely to non-toxic ethene via the process of reductive dechlorination. Since then, Dehalococcoides spp. were found to be dechlorinating a variety of hazardous chlorinated pollutants like CPs, polychlorinated dibenzo-p-dioxins (Fennell et al., 2004), PCB congeners (Bedard et al., 2007), chloroethanes (Grostern and Edwards, 2006; Duhamel and Edwards, 2007) and chlorinated benzenes (Adrian et al., 2000; Fennell et al., 2004). Dehalococcoides is a taxon of many irregularities. Even though the genomes of several representatives of this genus are among the smallest found in free-living bacteria (Kube et al., 2005; Seshadri et al., 2005), they also contain the highest number of putative reductive dehalogenase (rdh) genes that code for the key enzymes mediating reductive dechlorination, within all known dechlorinating genera. Regardless of their general specialization to reductive dechlorination, every strain isolated so far has its own choice of favourite chlorinated compound(s). The unusual dependence of Dehalococcoides spp. on chlorinated organic compounds for their
growth made them interesting research subjects to study their application in bioremediation. Yet our knowledge about presence, activity and capabilities of members of this genus in the environment is rather limited, including their response to changes in environmental conditions. This review provides a summary of the present knowledge on the role of Dehalococcoides spp. in degradation of chlorinated organic contaminants and the traits of this interesting group of microorganisms.

Pollution of chlorinated compounds and their bioremediation

Chlorine-containing organics (Table 1) are often believed to originate exclusively from industrial pollution. However, many living organisms (e.g. marine sponges or terrestrial antagonistic microorganisms as a part of their defense mechanisms) produce them naturally whereas chlorinated compounds are also released as a result of, for example, eruptions of volcanoes, forest fires and geothermal processes (Griebler et al., 2004; Bengtson et al., 2009). Nevertheless, it is their extensive industrial (e.g. solvent, metal degreasing, rubber production) and agricultural (e.g. pesticide component) application over the past 50 years that resulted in their deposition in various environments, especially in soils, groundwater aquifers and sediments (Bailey, 2001; Meijer et al., 2003; Barber et al., 2005; Hageman et al., 2006; Weber et al., 2008). Due to their physicochemical properties (Table 1), exposure to these compounds can have carcinogenic and lethal effects on biota. Therefore, the production and application of most of these compounds is no longer allowed in 90 countries since the Stockholm convention in 2001 (Decision No. 2455/2001/EC, 2001; UNEP, 2005). Finding the suitable clean-up techniques for contaminated environments, however, remains challenging. Remediation of soils and groundwater can be achieved via physicochemical methods such as thermal cleaning, chemical oxidation or adsorption of pollutants on activated carbon (Lai et al., 2007), whereas there are no in situ remediation technologies for sediments other than complete removal of the contaminated sediment (Wenning et al., 2006). Moreover, the high ecological disturbance that these physicochemical treatment methods can cause in the environment makes them unsuitable solutions in the long term (Wenning et al., 2006). Other than harsh physicochemical treatments, a far more preferable option is bioremediation. During bioremediation chlorinated contaminants are largely transformed by microorganisms although degradation by higher organisms is also reported. Phytoremediation, where plants are employed to assimilate, degrade, metabolize or detoxify chlorinated compounds, is an effective bioremediation method (Susarla et al., 2002). For example, poplar trees were shown to assimilate and degrade TCE to 2,2,2-trichloroethanol, trichloroacetic acid and dichloroacetic acid (Newman et al., 1997). Recently, it has also been shown that the presence of these trees may stimulate the transformation of PCE in the subsurface (James et al., 2009). In this study, in the test location populated with hybrid poplar trees PCE pollution was reduced by over 99%, in comparison with 2% removal in an unplanted control. Moreover, several plant species, especially varieties of Cucurbita pepo ssp. pepo (squash), were shown to extract milligrams of PCBs from soil in approximately 8 weeks time (Zeeb et al., 2006).

Lately the generation of transgenic plants to improve the phytoremediation of these pollutants resulted in several promising demonstrations of TCE, 1,2-dichloroethane (DCA) and chlorophenol removal in several laboratory scale tests (Wang et al., 2004; Dowling and Doty, 2009; James and Strand, 2009). In many ecosystems, fungi are among the major decomposers. Most fungi are robust organisms and are generally tolerant to high levels of pollution (Singh, 2006). Fungal lignocellulolytic enzymes have been related to the degradation of various pollutants when used in combination with mediators and reactive radicals. Being the most commonly studied example, white-rot fungi are able to detoxify a wide range of pollutants including chlorinated organics, with lignin and manganese peroxidases (Tortella et al., 2005; Field and Sierra-Alvarez, 2008).

The bacterial degradation of chlorinated pollutants can be a result of fortuitous co-metabolic conversion, or it may contribute to the energy metabolism of the degrading organism. During the latter metabolic processes, chlorinated compounds are used either as carbon source or as electron acceptor (coupled to the oxidation of an electron donor), depending on the oxidation state of the compound. Although many chlorinated compounds may be transformed under aerobic conditions, the majority of polychlorinated compounds, such as those discussed in this review, are recalcitrant to aerobic degradation. Due to the electronegative nature of the chlorine atom, oxidation of the carbon backbone in the chlorinated compound becomes thermodynamically unfavourable (Wohlfarth and Diekert, 1997), especially in polychlorinated compounds. As a result they serve as energetically favourable electron acceptors in microbial metabolism in anoxic environments such as sediments, subsurface soils and groundwater aquifers. Consequently, anaerobic bacteria, which can use these compounds as electron acceptors in a process termed organohalide respiration, are good candidates for bioremediation (van Eekert and Schraa, 2001). Within the organohalide-respiring bacteria, Dehalococcoides spp. and related isolates within the Chloroflexi represent a special case in the anaerobic detoxification of halogenated organic contaminants. It has been shown that several other bacteria belonging to the δ- and
Table 1. Sources, biological impacts and physicochemical properties of chlorinated organic compounds that have been reported to be degraded by ‘Dehalococcoides’ spp.

|                  | HCB\(^{ab}\) | PCE/TCE\(^c\) | PCB\(^{ad}\) | Dioxins\(^a\) | CPs\(^e\) |
|------------------|--------------|---------------|--------------|--------------|---------|
| Natural sources  | Volcanic activity, minerals  | Volcanic activity, barley  | Volcanic activity  | Forest fires  | Metabolites of microbes, sponges  |
| Anthropogenic sources | Pesticide synthesis, waste incineration, dye production | Solvent (dry cleaning, metal cleansing), grain fumigation | Insulating fluid, microscope oil, stabilizing additive | Coal fired utilities, waste incineration, metal smelting, diesel truck, bleaching | Pesticides, bleaching wood pulp |
| Abiotic degradation Effects | Photolysis | None | Ultrasound | Photolysis | Photolysis |
| Molecular weight | 285 | 165/131 | Various | Various (from 84–322) | Various (from 128–266) |
| Water solubility (mg l\(^{-1}\)) | 0.005 | 150/1280 | 0.0027–0.42 × 10\(^{-3}\) | Insoluble | 10–905 |
| Vapour pressure (kPa)\(^f\) | 0.1 × 10\(^{-3}\) | 1.9/7.8 | 1.1 × 10\(^{-3}\)–1.3 × 10\(^{-7}\) | Insoluble | 1–12.7 × 10\(^{-3}\) |

\(^{a}\) Gribble (2003).  
\(^{b}\) Hexachlorobenzene [Agency for Toxic Substances and Disease Registry (ATSDR), 2002].  
\(^{c}\) PCE: Tetrachloroethene and TCE: Trichloroethene (US EPA, 1985).  
\(^{d}\) Polychlorinated biphenyls. There are theoretically 209 different PCB congeners, although only about 130 of these were found in commercial PCB mixtures (UNEP Chemicals, 1999).  
\(^{e}\) Chlorophenols [Agency for Toxic Substances and Disease Registry (ATSDR), 1999].  
\(^{f}\) Only PCE is illustrated.  
\(^{g}\) At 20°C.  
NA, not available.
"Dehalococcoides ethenogenes" sp MB
"Dehalococcoides" sp VS
"Dehalococcoides" sp FL2
"Dehalococcoides" sp CDBB1
"Dehalococcoides" sp DCB5
"Dehalococcoides" sp GT
"Dehalococcoides" sp BAY1

Lahn Cluster
Tidal Flat Cluster
"Dehalogenimonas lykanthroporepellens" BL-DC-9

"Dehalobium chlorococercia" DF-1

Desulfitobacterium hafniense GBFH
Desulfitobacterium hafniense TCP-A
Desulfitobacterium hafniense Y51
Desulfitobacterium hafniense TCE-1
Desulfitobacterium hafniense DCB-2
Desulfitobacterium hafniense PCP-1
Desulfitobacterium hafniense PCE-S
Desulfitobacterium hafniense JH1
Desulfitobacterium hafniense G2
Desulfitobacterium chlororespirans
Desulfitobacterium dehalogenans
Desulfitobacterium sp PCE-1
Desulfitobacterium sp Viet-1
Desulfitobacterium sp KBC1
Desulfitobacterium dichlororespirans
Desulfitobacterium metaliredoxens
Desulfitobacterium sp CR1
Dehalobacter sp WL
Dehalobacter sp 1,1-DCA1
Dehalobacter sp E1
Dehalobacter restrictus TEA
Dehalobacter restrictus PER-K23
Dehalobacter sp TCA1
Dehalobacter sp MS

An aeromycobacter dehalogenans
An aeromycobacter sp K
An aeromycobacter sp Fw109-5

Desulfuromonas michiganensis
Desulfuromonas chloroethenica
Geobacter lovleyi
Geobacter thiogenes
Desulfomonile liminaria
Desulfomonile tiedjei

Desulfovibrio sp PCP1
Desulfovibrio sp TBP-1
Desulfovibrio dechloracetovorans

Sulfurospirillum halorespirans
Sulfurospirillum multivorans
Sulfurospirillum sp JPD-1

Chloroflexi
Firmicutes
Delta-Proteobacteria
Epsilon-Proteobacteria

The phylogenetic tree of dechlorinating bacteria based on bacterial 16S rRNA sequences. Alignment and phylogenetic analysis were performed with the ARB software using the most recent release of the ARB-SILVA project (SILVA 96) (Ludwig et al., 2004; Pruesse et al., 2007), and the tree was constructed using the neighbour joining method. The reference bar indicates the branch length that represents 10% sequence divergence. Boldface lettering indicates completed or ongoing genome sequencing.

ε-Proteobacteria (Anaeromycobacter, Desulfuromonas, Desulfomonile, Desulfovibrio, Geobacter, Sulfurospirillum) or to the low-GC Gram-positive bacteria (Desulfitobacterium, Dehalobacter) are also able to degrade chlorinated organic contaminants through organohalide respiration (Fig. 1) (Smidt and de Vos, 2004). However, with the exception of Dehalobacter spp., none of these species are as specialized as Dehalococcoides, and they are reported to grow as well, for example, by metal reduction, denitrification or fermentation. © 2009 The Authors
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The little bacteria that can: the genus Dehalococcioides

Dehalococcioides is a genus of strictly anaerobic Gram-negative bacteria that to the best of our knowledge are restricted to gaining energy from the reduction of chlorinated compounds by organohalide respiration. Cultured Dehalococcioides spp. isolates have an irregular, spherical shape (approximately 0.5 μm) often referred to as coccoid. These mesophilic (25–40°C) bacteria prefer neutral pH environments. Their growth on alternative electron acceptors such as oxygen, nitrate or sulfate has never been reported (Kube et al., 1998). Gibbs free energy (ΔG°) generated with reductive dechlorination of chlorinated compounds could range from −130 to −180 kJ mol⁻¹ per chlorine removed. The redox potential thus generated is comparable to the redox potential of denitrification and higher than that generated by sulfate reduction. As a result it was suggested that reductively dechlorinating bacteria could out-compete sulfate reducers and methanogens for reducing equivalents when the formation of reducing equivalents is rate limiting (Dolfing, 2003). Dehalococcioides spp. are also capable of degrading chlorinated aliphatic compounds, i.e. 1,2-DCA, via so-called dihaloelimination. In this process two neighbouring chlorine atoms are concurrently replaced via the formation of a double bond between the two carbon atoms. Dihaloelimination requires less H₂ for the removal of chlorine atoms than reductive hydrogenolysis, thus its energy balance is more favourable under H₂-limited conditions (Smidt and de Vos, 2004).

As the ecologists’ quest prevails to delve Becking and Beijerinck’s long running argument: ‘Everything is everywhere, but the environment selects’ (Beijerinck, 1913; Becking, 1934), the application of biomolecular tools, including the PCR amplification and sequencing of 16S ribosomal RNA (rRNA) genes from environmental samples, enables to study the full extent of microbial diversity and describe the biogeographical patterns exhibited by microorganisms at large spatial scales (Fierer and Jackson, 2006; Martiny et al., 2006). With the growing interest in Dehalococcioides’ presence and functioning in the environment, several studies were conducted using Dehalococcioides-specific 16S rRNA gene-based approaches in uncontaminated and chlorinated ethene contaminated sediments, soils and groundwater aquifers (Löffler et al., 2000; Hendrickson et al., 2002; Kittelmann and Friedrich, 2008). Currently, more than 100 16S rRNA gene sequences of cultured and uncultured Dehalococcioides spp. have been deposited to the database of the National Center for Biotechnology Information (NCBI). The 16S rRNA gene in Dehalococcioides spp. is highly conserved throughout the entire genus (Fig. 1); however, various studies showed that this group is functionally very diverse (Maymo-Gatell et al., 1997; Adrian et al., 2000; Hendrickson et al., 2002; He et al., 2003; Duhamel et al., 2004; Krajmalnik-Brown et al., 2004). Eight Dehalococcioides strains have been isolated, mainly for their ability to degrade chlorinated ethenes (Table 2). Functional differences in these isolates can be observed in the chlorinated compound transformed and in the transformation end-products. For example, the first isolate of the genus Dehalococcioides ethenogenes strain 195 can completely dechlorinate PCE to ethene, although degradation of vinyl chloride (VC) to ethene is co-metabolic (Maymo-Gatell et al., 1997). Dehalococcioides ethenogenes strain 195 can also dechlorinate HCB to 1,3-DCB (dichlorobenzene), 1,4-DCB, 1,2-DCB and 1,3,5-TCB (trichlorobenzene). In contrast to D. ethenogenes strain 195, Dehalococcioides sp. CBDB1 dechlorinates HCB to 1,3-DCB, 1,4-DCB and 1,3,5-TCB, and recently also transformation of PCE and TCE to trans-DCE was observed (Adrian et al., 2007b). Dehalococcioides spp. are difficult to maintain in pure culture (Maymo-Gatell et al., 1997; Adrian et al., 2000; He et al., 2003); they are more easily maintained in a microbial community, on which they depend for H₂ supply, as long as ideal growth conditions are provided (Duhamel et al., 2004; Holmes et al., 2006). Examples include chlorinated ethene transforming Cornell (Maymo-Gatell et al., 1997), Victoria (Hendrickson et al., 2002), Pinellas (Harkness et al., 1999), KB-1 (Duhamel et al., 2002) and ANAS cultures (Richardson et al., 2002). In addition to Dehalococcioides spp., two other distantly related isolates within the Chloroflexi have recently been obtained (Fig. 2). The marine ‘Dehalobium chlorococcæ’ DF-1 is able to dechlorinate a variety of PCBs (May et al., 2008). Most recently, ‘Dehalogenimonas lykanthroporepellens’ BL-DC-9 has been isolated from contaminated groundwater. This microorganism dechlorinates polychlorinated alkanes (Yan et al., 2009). Like Dehalococcioides spp., both isolates are strictly hydrogenotrophic.

Several enrichment studies showed the presence of Dehalococcioides spp. in different locations and environ-
ments in the Northern Hemisphere (mainly concentrated in North America, Europe and Japan). Dehalococcoides-containing enrichment cultures originating from river sediments have been shown to dechlorinate PCB and dioxin congeners, PCE, TCE and a number of chlorinated benzenes (Ballerstedt et al., 2004; Yoshida et al., 2005; Bedard et al., 2007; Bunge et al., 2007; Futamata et al., 2007). Besides sediment enrichments, dechlorination by Dehalococcoides was also reported in groundwater aquifers (Bowman et al., 2006; Bürgmann et al., 2008; Imfeld et al., 2008; Lee et al., 2008; Himmelheber et al., 2009) and a denitrifying membrane-biofilm reactor (Chung et al., 2003). Few studies have demonstrated that the bioaugmentation with reductively dehalogenating cultures can result in complete dechlorination of PCE and TCE to ethene (Ellis et al., 2000; Major et al., 2002; Lendvay et al., 2003; Schuetz et al., 2008). The maximum reported growth rates of Dehalococcoides spp. in pure and enrichment cultures are in the range of 0.2–0.4 day⁻¹ under laboratory conditions (Maymo-Gatell et al., 1997; Cupples et al., 2003; Adrian et al., 2007b; Duhamel and Edwards, 2007). Additionally, quantitative analyses of the Dehalococcoides spp. 16S rRNA gene at chlorinated ethene bioremediation sites (soil and groundwater) revealed abundances of 10⁵–10⁷ copies per gram material (Lendvay et al., 2003; Sleep et al., 2006). Recently, a groundwater bioremediation simulation study showed that growth rates obtained in laboratory conditions could also be replicated in large-scale experiments, which resulted in up to 10⁵ 16S rRNA gene copies l⁻¹ (Vainberg et al., 2009). Hence, these pilot- as well as field-scale bioremediation tests with Dehalococcoides-containing cultures offer promising results for the further use of these microorganisms.

In spite of all the information obtained in physiological studies very little is known about the diversity, distribution and functioning of Dehalococcoides in different environments although they were detected at several contaminated locations. Hendrickson and co-authors have demonstrated the presence of Dehalococcoides spp. in soil and groundwater samples from 24 sites scattered throughout North America and Europe (Hendrickson et al., 2002). Up to 200 μM PCE could be dechlorinated, and complete dechlorination to ethene could be correlated to the presence of Dehalococcoides spp. in the sampling locations. Recently, we conducted a large-scale survey focusing on presence, activity and dechlorination potential of Dehalococcoides spp. in river sediments and floodplain soils from different polluted locations in Europe (Fig. 3) (Taş, 2009). Almost all of the tested sediment and soil samples showed the capacity to dechlorinate HCB and/or chlorinated ethenes irrespective of the in situ contaminant levels. Nevertheless, the HCB transformation rates observed in the laboratory-scale microcosms and the number of 16S rRNA gene copies of Dehalococcoides spp. in the environmental samples did not show a strong correlation. In these river systems, Dehalococcoides spp. relative abundance was furthermore shown to change significantly along temporal and spatial gradients, but was also found to be influenced by other environmental factors such as water temperature (Taş et al., 2009).

As non-fermentative microorganisms Dehalococcoides spp. and their organohalide-respiring relatives Dehalobium chloroeroearia DF-1 and Dehalogenomas lykanthrorepellens DC-9 depend on the H₂ supply from other microorganisms for their growth (Smidt and de Vos, 2004; May et al., 2008; Yan et al., 2009). Recently, it has also been suggested that the activity of Dehalococcoides spp.
in situ conditions is linked to the performance of fermentative communities (Röling et al., 2007). Therefore, it is crucial to have insight in factors affecting nutrient fluxes and microbial communities involved in carbon, nitrogen and sulfur (C, N, S) cycling in the river basins to be able to understand the survival and functioning of *Dehalococcoides* spp. in different geographical locations. Because there are considerable differences between dechlorination capabilities of the known *Dehalococcoides* strains despite 16S rRNA identities of >99%, their sole presence based on the detection of the 16S rRNA gene in an environment does not guarantee successful in situ dechlorination of a specific pollutant. Consequently, molecular tools that target metabolic activities of the entire microbial communities in the environment are needed to have a canonical assessment of the conditions.

**Discoveries from *Dehalococcoides* spp. genomes**

Our knowledge gap concerning the properties of *Dehalococcoides* spp. is closing rapidly with the developments in high-throughput sequencing technologies. Full-genome sequence analyses revealed that *D. ethenogenes* strain 195 (GenBank Accession No. NC_002936) and strain CBDB1 (NC_007356) genomes are approximately 1.47 and 1.39 million base pairs (Mbp) respectively. Both genomes comprise single circular chromosomes with 1591 predicted protein coding sequences (CDs) in strain 195 (Seshadri et al., 2005) and 1458 CDs in strain CBDB1 (Table 3). Up to 1217 of the CDs from strain CBDB1 have orthologous genes in *D. ethenogenes* strain 195 (83.5%) (Kube et al., 2005). Strain BAV1 (NC_009455) has a genome of 1.34 Mbp with 1385 CDs based on information provided in the Integrated Microbial Genomes (IMG) database, release March 2009 (Markowitz et al., 2008). All of these genomes are among the smallest for free-living bacteria. Different *Dehalococcoides* spp. genomes share many common properties. For example, one copy of each rRNA gene is present in all *Dehalococcoides* genomes (Kube et al., 2005; Seshadri et al., 2005). In strains 195, CBDB1 and BAV1 the 16S rRNA gene is spatially separated from 5S and 23S rRNA genes. Comparative analysis of available *Dehalococcoides* genomes showed that 70% of all genes in these genomes have a high sequence and contextual conservation (McMurdie et al., 2008). Interestingly, *D. ethenogenes* strain 195 possesses a nitrogenase-encoding operon, which is missing in strain CBDB1. Even though this finding suggests that *D. ethenogenes* strain 195 can fix nitrogen, diazotrophic growth of the *Dehalococcoides* strains has not yet been reported.

Different *Dehalococcoides* strains contain different numbers of *rdh* genes that encode protein, which have been proven or predicted to catalyse the dechlorination reaction. When compared with the genomes of other dechlorinating bacteria, *Dehalococcoides* have
Table 3. Comparison of whole-genome sequence statistics for reductively dechlorinating bacteria as presented in the Integrated Microbial Genomes (IMG/M) database, March 2009 (Markowitz et al., 2008).

| Genome name                  | Phylum/genus          | Bases (Mbp) | GC (%) | Genes | CDs | RNA | 16S | Orthologues | Paralogues |
|------------------------------|-----------------------|-------------|--------|-------|-----|-----|-----|-------------|------------|
| 2CP-C Anaeromyxobacter dehalogenans | Proteobacteria         | 5.01        | 0.75   | 4419  | 4361| 58  | 2   | 4290        | 2468       |
| Anaeromyxobacter SZ           | Firmicutes            | 3.87        | 0.55   | 3514  | 3476| 38  | 1   | 3287        | 1858       |
| Geobacter Desulfitobacterium hafniense DCB-2 | Firmicutes          | 5.28        | 0.48   | 4801  | 4712| 89  | 5   | 4597        | 2921       |
| Desulfitobacterium Y51        | Firmicutes            | 5.73        | 0.47   | 5137  | 5060| 77  | 6   | 4765        | 3200       |
| Dehalococcoides sp. strain 195 | Chloroflexi           | 1.47        | 0.49   | 1641  | 1591| 51  | 51  | 1426        | 628        |
| Dehalococcoides sp. strain VS  | Chloroflexi           | 2.39        | 0.55   | 2160  | 2096| 64  | 1   | 2003        | 892        |

Genomes of strains 195, CBDB1 and BAV1 have 17, 32 and 10 rdh genes, respectively, whereas only seven rdh genes were identified in the genome of Desulfotobacterium hafniense DCB-2, four rdh genes in D. hafniense Y51 and two rdh genes in Geobacter lovleyi SZ and Anaeromyxobacter dehalogenans (Thomas et al., 2008). The draft genome of strain VS contains the highest number of rdh genes (36 full-length genes) ever found in a single bacterial genome (McMurdie et al., 2008). Similarly, 14 and 19 rdh genes were detected via PCR amplification in Dehalococcoides sp. strains FL2 and DCMB5 respectively (Holscher et al., 2004; Bunge et al., 2008). Twelve rdh genes from strain CBDB1 have orthologues in D. ethenogenes strain 195 genome with 86.4–95.4% sequence identity. In D. ethenogenes strain 195 and strain CBDB1 genomes almost all of the rdh genes (except DET0079, TCE reductive dehalogenase tceA in D. ethenogenes strain 195 and cbdbA1583 in strain CBDB1) were found to be located in close proximity to genes for transcription regulators, and were predicted to be transcribed in the direction of DNA synthesis, which suggests tight regulation of rdh activity (Kube et al., 2005; Seshadri et al., 2005). However, the function of only a small number of these genes is known. Only two rdh genes from strain 195, DET0079 and DET0318, have been characterized as TCE (tceA) and PCE (pceA) reductive dehalogenases respectively (Fung et al., 2007). Another tceA gene was identified in Dehalococcoides sp. strain FL2 (GenBank Accession No. AY165309) (He et al., 2005). The cbdbA84 gene from strain CBDB1 was recently designated as a chlorobenzene reductive dehalogenase (cbra), which is involved in dechlorination of 1,2,3,4-TeCB and 1,2,3-TCB (Adrian et al., 2005). Additionally, two VC reductase genes were identified from strain BAV1 (bvcrA, Dehalobacterium sp. BAV1_0847) (Krajmalnik-Brown et al., 2004) and strain VS (vcra, GenBank Accession No. AY322364) (Muller et al., 2004). Since metabolic function cannot be inferred from Dehalococcoides phylogeny, detection methods based on process-specific biomarkers are necessary to describe the bioremediation capacity and activity of Dehalococcoides in the environment. Therefore, genes like rdh and the corresponding gene products that are specific to functions of interest can serve as useful biomarkers in monitoring of different Dehalococcoides activities.

In the past years microarrays were shown to be useful tools for such monitoring activities and characterization of microbial communities (Zhou, 2003; Wang et al., 2009). Furthermore, functional gene arrays (FGAs), which target functional genes such as nitrogenases, cellulases etc., allow fast and comprehensive analysis of metabolic potential and activity of microbial communities in the environment by targeting a large number of genes or their transcripts in one single experiment (Wu et al., 2001;
Taroncher-Oldenburg et al., 2003; Steward et al., 2004; Zhou et al., 2008). Up to date the most extensive FGA platform is the GeoChip (He et al., 2007), which targets approximately 10,000 catabolic genes involved in major biogeochemical cycles, including those of carbon, nitrogen and sulfur, as well as organic pollutant degradation. Analysis of HCB-contaminated sediments in the Ebro river basin (Spain) using the GeoChip amended with probes targeting 153 rhd genes showed that rhd gene diversity changed significantly between different sampling locations (Taş et al., 2009). More specifically, sediment samples taken at a site with high HCB pollution (Lacorte et al., 2006) were dominated by rhd genes of Dehalococcoides spp. strain CBDB1 and D. ethenogenes strain 195. In contrast samples, which were characterized by more diffuse pollution with a broader range of contaminants, a wide spectrum of rhd genes was detected including those from various other organohalide-respiring microorganisms (Fig. 4). However, it should be noted that microarrays can only detect known sequences, which can cause an underestimation of functional gene diversity and abundance in environments for which limited sequence information is available. Application of FGAs in combination with newly developed techniques such as high-throughput non-gel-based proteomics (Maron et al., 2007) and sequencing of the metatranscriptome offers a remarkable promise. Recent studies on D. ethenogenes strain 195 and Dehalococcoides spp. strain CBDB1 transcriptomes suggested continuous transcription of rhd genes such as tceA (Johnson et al., 2008) and cbrA (Wagner et al., 2009) during different growth phases. As a result gene transcripts of such genes can be studied using transcriptomic techniques with FGAs, in combination with proteomics methods (Morris et al., 2006; Morris et al., 2007) to identify the proteins with significant functional impact.

Future perspectives: reductive dechlorination, systems microbiology and microbial networks

The broad aim of systems microbiology is to define and understand the relationships between the individual components that build a cellular organism, a community and an ecological niche (Vieites et al., 2009). As a result, in the past, the focus of systems microbiology was on microbial isolates or enrichments (McHardy and Rigoutsos, 2007). To date, the majority of the research conducted in the field of reductive dechlorination has been predominantly focused on the identification of genes and proteins directly responsible for the dechlorination process (Cuppes et al., 2003; Muller et al., 2004; Holmes et al., 2006; Adrian et al., 2007a; McMurdie et al., 2007; West et al., 2008; Wagner et al., 2009). These experimental studies, so far,
allowed the analysis and characterization of several key genes. However, it is becoming evident that to understand microbial functions or functioning of microbial communities one must study the entire system (Vietes et al., 2009). The body of research summarized in this review also supports this idea and suggests that with molecular assays targeting ribosomal and process-specific functional genes such as those encoding reductive dehalogenases, it will remain difficult to understand the full extent of the process, since the dechlorination process comprises an integral part of a complex web of metabolic and regulatory interactions (Rahm et al., 2006; West et al., 2008; Wagner et al., 2009). The application of novel, more comprehensive methods like whole genome shotgun (WGS) sequencing of environmental DNA and mRNA (functional metagenomics) (Tringe et al., 2005; Kalyuzhnaya et al., 2008), the establishment of large-scale databases which contain metagenomic data from different environments (Seshadri et al., 2007; Pignatelli et al., 2009; Vogel et al., 2009) as well as the development of new computational resources for comparative (meta)genomic analyses (Peterson et al., 2001; Alm et al., 2005; Markowitz et al., 2006) enable us to develop and analyse data sets (and microbial networks) which so far are believed to be the closest to the actual environmental situations. Thus, today, it can be proposed to leave reductionist approaches that are limited to only one or a few selected biomarkers, and to study reductive dechlorination and the function of Dehalococcoides spp. in larger communities and in the environments in which they belong. As the functional properties of such communities are elucidated, we will be able to assess the true role and importance of Dehalococcoides spp. in the environment.

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