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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.

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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

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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 defence 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 unsustainable 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

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Table 1. Sources, biological impacts and physicochemical properties of chlorinated organic compounds that have been reported to be degraded by '*Dehalococcoides*' spp.

	HCB[a,b]	PCE/TCE[c]	PCBs[a,d]	Dioxins[a]	CPs[e]
	[f,g]				
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	Photolysis	None	Ultrasound	Photolysis	Photolysis
Effects	Immune system and liver damage, cancer	Liver and kidney damage, neurotoxicity, possibly cancer	Skin rashes, dizziness, liver damage, reproductive damage, possibly cancer	Cancer, hepatotoxicity birth defects, endocrine disruption	Cancer, birth defects
Molecular weight	285	165/131	Various	Various (from 84–322)	Various (from 128–266)
Water solubility (mg l^{-1})	0.005	150/1280	$0.0027–0.42 \times 10^{-3}$	Insoluble	10–905
Vapour pressure (kPa)[g]	0.1×10^{-3}	1.9/7.8	$1.1 \times 10^{-3}–1.3 \times 10^{-7}$ (Criado *et al.*, 2002)	NA	$1–12.7 \times 10^{-3}$

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.

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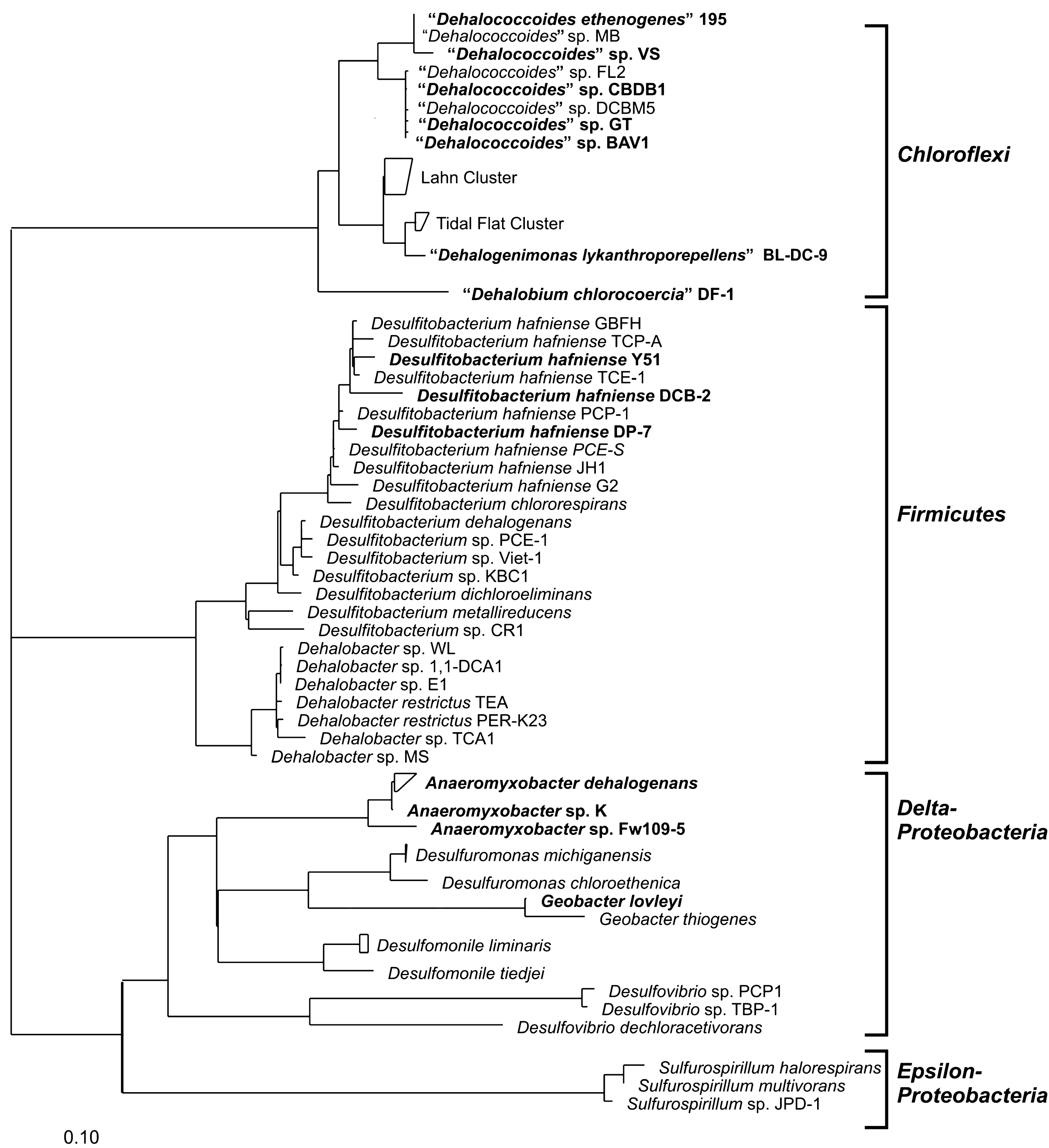

Fig. 1. Phylogenetic tree of dechlorinating bacteria based on bacterial 16S rRNA sequences. Alignment and phylogenetic analysis were performed with the ARB software using the most recent release of the ARB-SILVA project (SILVA 96) (Ludwig *et al.*, 2004; Pruesse *et al.*, 2007), and the tree was constructed 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* (*Anaeromyxobacter*, *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.

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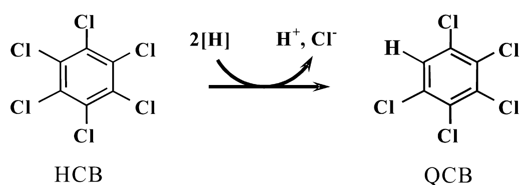

Fig. 2. Reductive dechlorination of hexachlorobenzene (HCB) to pentachlorobenzene (QCB).

The little bacteria that can: the genus *Dehalococcoides*

Dehalococcoides 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 *Dehalococcoides* 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.*, 2005; Seshadri *et al.*, 2005). Reductive dechlorination by *Dehalococcoides* spp. occurs via the replacement of a chlorine atom in the chlorinated compound by hydrogen (reductive hydrogenolysis) and results in a net input of one proton and two electrons (Fig. 2) (Holliger *et al.*, 1998). Gibbs free energy ($\Delta G^{0\prime}$) 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). *Dehalococcoides* 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 H_2 for the removal of chlorine atoms than reductive hydrogenolysis, thus its energy balance is more favourable under H_2-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 *Dehalococcoides*' presence and functioning in the environment, several studies were conducted using *Dehalococcoides*-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 *Dehalococcoides* spp. have been deposited to the database of the National Center for Biotechnology Information (NCBI). The 16S rRNA gene in *Dehalococcoides* 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 *Dehalococcoides* 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 *Dehalococcoides 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). *Dehalococcoides 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, *Dehalococcoides* 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). *Dehalococcoides* 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 H_2 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 *Dehalococcoides* spp., two other distantly related isolates within the *Chloroflexi* have recently been obtained (Fig. 2). The marine '*Dehalobium chlorocoercia*' 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 *Dehalococcoides* spp., both isolates are strictly hydrogenotrophic.

Several enrichment studies showed the presence of *Dehalococcoides* spp. in different locations and environ-

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Table 2. Isolated strains of '*Dehalococcoides*' spp. and the chlorinated substrates they transform.

	Chlorinated compound reduced	End-products	References
'*Dehalococcoides ethenogenes*' strain 195	PCE and TCE	Ethene	Maymo-Gatell *et al.* (1997)
	HCB	1,3-DCB, 1,4-DCB, 1,2-DCB and 1,3,5-TCB	Fennell *et al.* (2004)
	2,3-DCP and 2,3,4-TCP	3-MCP	Adrian *et al.* (2007b)
	1,2 DCA	Ethene	Maymo-Gatell *et al.* (1999)
Dehalococcoides sp. BAV1	VC	Ethene	He *et al.* (2003)
Dehalococcoides sp. CBDB1	HCB	1,3-DCB, 1,4-DCB and 1,3,5-TCB	Adrian *et al.* (2000)
	PCE and TCE	*Trans*-1,2-dichloroethene	Adrian *et al.* (2007b)
	2,3-DCP and 2,3,4-TCP	3-MCP	Adrian *et al.* (2007b)
	Polychlorinated dioxins	Dichloro-dioxins	Bunge *et al.* (2003)
	Polychlorinated biphenyls (Aroclor1260)	Various	Adrian *et al.* (2009)
Dehalococcoides sp. VS	VC	Ethene	Cupples *et al.* (2003)
Dehalococcoides sp. FL2	TCE	*Cis*-1,2-dichloroethene and *trans*-1,2-dichloroethene	He *et al.* (2005)
Dehalococcoides sp. GT	TCE	Ethene	Sung *et al.* (2006)
Dehalococcoides sp. DCMB5	1,2,4-Trichlorodibenzo-*p*-dioxin 1,2,3-TCB	2-Monochlorodibenzo-*p*-dioxin 1,3-DCB	Bunge *et al.* (2008)
Dehalococcoides sp. Strain MB	PCE and TCE	*Trans*-1, 2-dichloroethene	Cheng and He (2009)

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.*, 2008). 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; Scheutz *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 10^2–10^7 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 10^{12} 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 chlorocoercia* DF-1 and *Dehalogenimonas lykanthroporepellens* DC-9 depend on the H_2 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.

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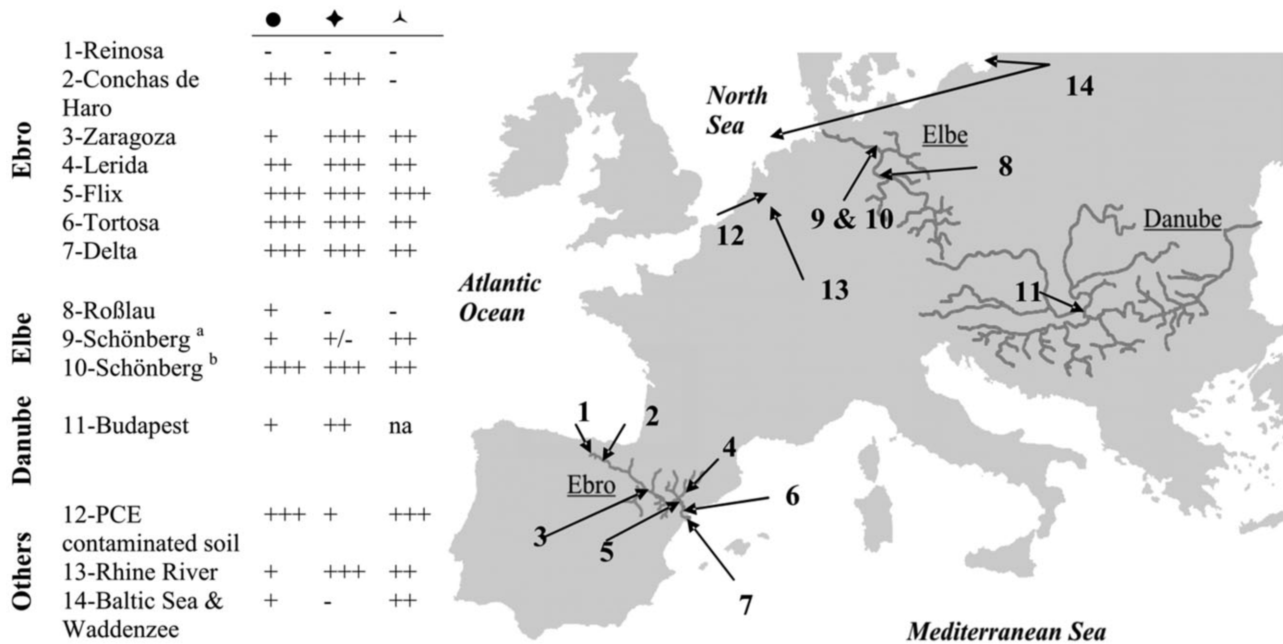

Fig. 3. Summary of results from the locations studied by Taş (2009) with cultivation-dependent and -independent molecular methods. ●: ‘*Dehalococcoides*’ spp. detection with 16S rRNA and/or 16S rRNA gene-targeted methods; ✦: HCB transformation; ⅄: chlorinated ethene transformation; (–) no detection or no transformation; (+/++/+++) low to high rRNA copies or long to short lag phases in HCB and chlorinated ethene transformation; na: not available; ([a]) soil and ([b]) river sediment sample from Schönberg, Germany. Map was redrawn from OpenStreetMap (http://www.openstreetmap.org).

in *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, diazotropic 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 the

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Table 3. Comparison of whole-genome sequence statistics for reductively dechlorinating bacteria as presented in 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	*rdh* genes
Anaeromyxobacter dehalogenans 2CP-C	*Proteobacteria* *Anaeromyxobacter*	5.01	0.75	4419	4361	58	2	4290	2468	2
Geobacter lovleyi SZ	*Proteobacteria* *Geobacter*	3.87	0.55	3514	3476	38	1	3287	1858	2
Desulfitobacterium hafniense DCB-2	*Firmicutes* *Desulfitobacterium*	5.28	0.48	4801	4712	89	5	4597	2921	7
Desulfitobacterium hafniense Y51	*Firmicutes* *Desulfitobacterium*	5.73	0.47	5137	5060	77	6	4765	3200	4
'*Dehalococcoides ethenogenes*' strain 195	*Chloroflexi* '*Dehalococcoides*'	1.47	0.49	1641	1591	51	1	1426	628	17
'*Dehalococcoides*' sp. BAV1	*Chloroflexi* '*Dehalococcoides*'	1.34	0.47	1436	1385	51	1	1327	488	10
'*Dehalococcoides*' sp. CBDB1	*Chloroflexi* '*Dehalococcoides*'	1.39	0.47	1516	1458	58	1	1378	541	32
'*Dehalococcoides*' sp. VS	*Chloroflexi* '*Dehalococcoides*'	2.39	0.55	2160	2096	64	1	2003	892	36

Genes: total gene count; CDs: coding sequences; RNA: number of rRNA, tRNA and other RNA genes; 16S: number of 16S rRNA gene copies; Orthologues: number of genes in orthologues; Paralogues: number of genes in paralogues; *rdh* genes: confirmed and predicted reductive dehalogenase-encoding genes.

highest number of *rdh* genes in their genomes (Table 3). 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 *Desulfitobacterium 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.*, 2007a). Additionally, two VC reductase genes were identified from strain BAV1 (*bvcrA*, DehaBAV1_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*s 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;

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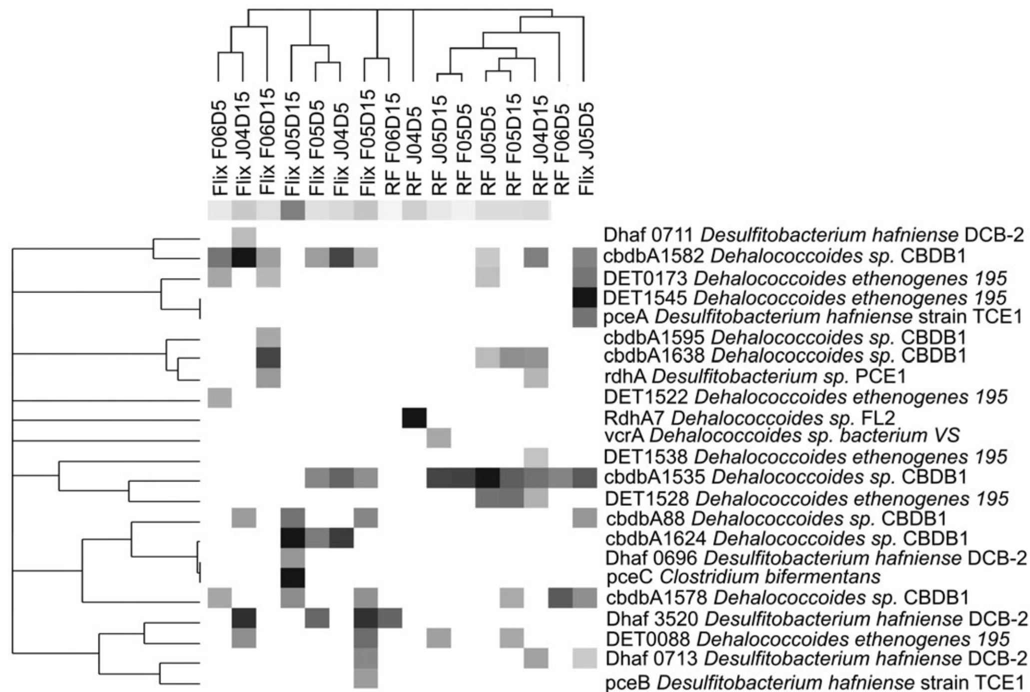

Fig. 4. Hierarchical cluster analysis of *rdh* gene profiles based on GeoChip functional gene array hybridization signals for samples from Flix and Rice Fields (RF, river delta) in the Ebro River. HCB is reported to be the dominant chlorinated contaminant in Ebro's basin where location Flix bares the highest HCB pollution (Lacorte *et al.*, 2006). White represents no hybridization above background level and grey represents positive hybridization. The grey-scale intensity indicates differences in hybridization signal intensity, with black representing the strongest signals. Samples are represented according to sampling month, year and sampling depth (i.e. F06D5: February 2006 depth 0–5 cm; J04D15: June 2004 depth 10–15 cm). For accession numbers of *rdh* gene targets, see Taş and colleagues (2009).

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 *rdh* genes showed that *rdh* 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 *rdh* 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 *rdh* 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 *rdh* 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 (Cupples *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,

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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 (Vieites *et al.*, 2009). The body of research summarized in this review also supports this idea and suggests that with biomolecular 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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