

Biodiversity of Bacteria that Dechlorinate Aromatic Chlorides and a New Candidate, *Dehalobacter* sp.

Naoko YOSHIDA^{1,2} and Arata KATAYAMA¹

¹*EcoTopia Science Institute, Nagoya University,
Furo-cho, Chikusa-ku, Nagoya 464-0814, Japan*

²*Laboratory of Microbial Biotechnology, Division of Applied Life Sciences,
Graduate School of Agriculture, Kyoto University,
Oiwake-cho, Kitashirakawa, Sakyo-ku, Kyoto 606-8224, Japan*

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Abstract—Bacteria that dechlorinate aromatic chlorides have been received much attention as a bio-catalyst to cleanup environments polluted with aromatic chlorides. So far, a variety of dechlorinating bacteria have been isolated, which contained members in diverse phylogenetic group such as genera *Desulfitobacterium* and “*Dehalococcoides*”. In this review, we introduced the up-to date knowledge of bacteria that dechlorinate aromatic chlorides and new candidate, *Dehalobacter* spp., as promising bacteria that dechlorinate aromatic chlorides.

Keywords: reductive dehalogenation, aromatic chlorides, *Dehalobacter*

INTRODUCTION

Aromatic chlorides such as chlorinated phenols, benzenes, biphenyls, and dibenzo-*p*-dioxins are compounds of serious environmental concern because of their widespread use and hazardous effects for animals and plants and frequently encountered as persistent pollutants in various environments. Although the aromatic ring of these compounds is generally broken by aerobic bacteria in nature, these compounds are persistent due to their chlorine substitutions. Therefore, biological removal of chlorines from chlorinated compounds, called as “dechlorination”, has been extensively studied over last few decades. So far, four different dechlorination processes for aromatic chlorides have been demonstrated, which are (i) dechlorination by aerobic bacteria, (ii) anaerobic oxidative dechlorination, (iii) dechlorination by phototrophic bacteria, and (iv) dehalorespiration by anaerobic bacteria. The dechlorination of aromatic chlorides by aerobic bacteria has reported for following compounds via variety of enzymes (for review, Janssen *et al.*, 1994); pentachlorophenol is dechlorinated by hydrolase in *Rhodococcus* species (Apajalahti and Salkinoja-Salonen, 1987), dioxygenase in *Sphingomonas* species (Ohstubo *et al.*, 1999), glutathione-*s*-transferase (Orser

et al., 1993a) and monooxygenase (Orser *et al.*, 1993b) in *Flavobacterium* species; 1,2,4-trichlorobenzene is dechlorinated by chloromuconate cycloisomerase and chloromaleylacetate reductase in *Pseudomonas* species (van der Meer *et al.*, 1991). Anaerobic oxidative dechlorination has been reported in the genera *Thauera*, *Pseudomonas*, and *Ochrobactrum*, which mineralize 3-chlorobenzoate under denitrifying conditions (Song *et al.*, 2000). The dechlorination by phototrophic bacteria was observed for 3-chlorobenzoate in the genera *Rhodospirillum* and *Rhodopseudomonas* under anaerobic photosynthetic condition, however the dechlorination occurred for 3-chlorobenzoyl-CoA rather than free 3-chlorobenzoic acid (van der Woude *et al.*, 1994; Eglund *et al.*, 2001). Dehalorespiration reductively dechlorinates aromatic chlorides via respiration by using chlorinated compounds as terminal electron acceptor. This process has been demonstrated for a variety of aromatic chlorides containing chlorinated phenols, benzoates, benzenes, biphenyls, and dibenzo-*p*-dioxins. This review article will focus on dehalorespiration below from respect on the larger spectrum of aromatic chlorides than other three dechlorination processes.

DEHALORESPIRATION BY USING AROMATIC CHLORIDES AS ELECTRON ACCEPTORS

Dehalorespiration was firstly demonstrated in *Desulfomonile tiedjei* of *Deltaproteobacteria* (Deweerd *et al.*, 1990), the isolate increased pH gradient and ATP in the cell as the result of the reductive dechlorination of 3-chlorobenzoate coupled to formate oxidation, suggested that dechlorination supports formation of a proton-motive force which in turn supports ATP synthesis via a proton-driven ATPase (Mohn and Tiedje, 1991). Reductive dechlorination can be indicated as following reaction: $R-Cl + 2[H] \rightarrow R-H + H^+ + Cl^-$. Gibbs free energies for the reaction show the aromatic chlorides are good electron acceptor, yielding -130 to -180 kJ/mol of chlorine removed. Indeed, the redox potentials are in the range from +260 to +480 mV, which are considerably higher than general electron acceptors such as sulfate and carbon dioxide. Although organic acids such as lactate, pyruvate, formate and acetate are used as the electron donors in some species, hydrogen is probably most important electron donors for the dehalorespiration, because of the high affinity of dehalorespiring bacteria for hydrogen. For example, the threshold for the dehalorespiration for tetra- and trichloroethenes are 0.05–0.9 nM. The concentration is considerably higher than acetogenesis, methanogenesis, and sulfate reduction (Luijten *et al.*, 2004), therefore dehalorespiring bacteria out-compete these hydrogenotrophic organisms by using hydrogen of lower concentration which released by the fermentation of organic materials in the environments. A key enzyme of dehalorespiration, reductive dehalogenase (RD), are involved in or anchored to cytoplasmic membrane, and reduces organochlorides as terminal electron acceptor in the respiratory chain. Electrons released in the oxidization of electron donor are transported to RDs via electron carriers such as cytochromes and menaquinones which are different among microbial species. Many RDs are inducible by the exposure with organochloride and repressed by alternative electron acceptors,

indicates that dehalorespiring bacteria also have system to sense organochlorides and regulate their expression of dehalogenases.

BIODIVERSITY OF BACTERIA THAT DEHALORESPIRE WITH AROMATIC HALIDES

Since the isolation of *Desulfomonile tiedjei*, a number of halorespiring bacteria that dechlorinate aromatic chlorides have been isolated and characterized, which belong to three distinct phyla, *Firmicutes* (i.e. *Desulfitobacterium* spp.), *Chloroflexi* (i.e. “*Dehalococcoides*”, o17/DF-1 group), and class *Deltaproteobacteria* (i.e. *Desulfomonile* spp., *Sulfurospirillum* spp.). The spectrum of aromatic chlorides for dehalorespiration is considerably larger than the yet-known other dechlorination metabolisms, and has no correlation with the phylogeny of the bacteria. The dechlorination spectrum, phylogeny, and electron donor of yet-isolated dehalorespiring bacteria for aromatic chlorides are summarized in Table 1.

Anaeromyxobacter

This genus is the group of metal-reducing bacteria containing two isolates, *Anaeromyxobacter dehalogenans* 2CP-1 (Cole *et al.*, 1994; Sanford *et al.*, 2002) and *Anaeromyxobacter* sp. strain FAc12 (Treude *et al.*, 2003). Only strain 2CP-1 is dehalorespiring bacteria, and reduces 2-chlorophenol, 2-bromophenol, and 2,6-chlorophenol by using hydrogen, formate, acetate, succinate, and pyruvate (Cole *et al.*, 1994; Sanford *et al.*, 2002).

Desulfomonile

In genus *Desulfomonile*, two species have been obtained as bacteria dehalorespiring with 3-chlorobenzoate, which are *Desulfomonile tiedjei* (Deweerd *et al.*, 1990) and *Desulfomonile limimaris* (Sun *et al.*, 2001). *D. limimaris* strain DCB-M also dechlorinated chlorines at *meta*-positions from dichloro- and trichlorobenzoate but not for chlorinated phenols, suggesting the dehalogenase in this genus is specific for *meta*-chlorobenzoate.

Desulfovibrio

In this genus, *Desulfovibrio dechloracetivorans* SF3 is the only isolate capable of dehalorespiration (Sun *et al.*, 2000). Strain SF3 dechlorinated chlorines at *ortho*-position of 2-chlorophenol and 2,6-dichlorophenol by coupling with oxidization of acetate.

Sulfurospirillum

Two species have been isolated as dehalorespiring bacteria for tetrachloroethene, *Sulfurospirillum halorespirans* (Luijten *et al.*, 2003) and *Sulfurospirillum multivorans* (Scholz-Muramatsu *et al.*, 1995; Luijten *et al.*, 2003), and only *S. multivorans* has reported to debrominate polychlorinated biphenyl ethers (He *et al.*, 2006).

Table 1. The dehalorespiring isolates and their spectrum of aromatic halides.

Phylum/class	Microorganisms	e-Donors for dehalorespiration	Aromatic organochlorides for dehalorespiration	Reference
<i>Dehaloproteobacteria</i>	<i>Anaeromixobacter dehalogans</i> strain 2CF-1	H ₂ , formate, acetate, succinate, pyruvate	2-MCP, 2-MBP, 2,6-DCP	Cole <i>et al.</i> , 1994; Sanford <i>et al.</i> , 2002
	<i>Desulfomonile tiedjei</i> strain DCB-1		3-CBA	Mohn and Tiedje, 1991
	<i>Desulfomonile liminaris</i> strain DCB-M	H ₂ , formate, pyruvate, lactate, butyrate, benzoate, propionate	3-CBA, 3-BBA, 2,3-DCP, 2,5-DCP, 3,5-DCP, 2,3,5-TCBA	Sun <i>et al.</i> , 2001
	<i>Desulfovibrio dechloroacetivorans</i> strain SF3	Acetate	2-CP, 2,6-DCP	Sun <i>et al.</i> , 2000
<i>Firmicutes</i>	<i>Sulfospirillum multivorans</i> strain DSM 12446	H ₂ , formate, pyruvate, lactate, ethanol, glycerol	PBDEs	Scholz-Muramatsu <i>et al.</i> , 1995; Luijten <i>et al.</i> , 2003; He <i>et al.</i> , 2006
	<i>Desulfotobacterium dehalogans</i> strain JW/U-DC1	H ₂ , formate, pyruvate, lactate	3-Cl-4-OHPA, 2,3-DCP, 2,4-DCP, 2,6-DCP, 2,4,6-TCP, PCP, OH-PCBs, TCMP, TCHQ	Utkin <i>et al.</i> , 1994, 1995; Wiegel <i>et al.</i> , 1999; Milliken <i>et al.</i> , 2004a, b
	<i>Desulfotobacterium chlororespirans</i> strain Co23	H ₂ , formate, pyruvate, lactate, butyrate, crotonate	3-Cl-4-OHPA, 2,3-DCP, 2,4-DCP, 2,6-DCP, 2,4,6-TCP, 2,4,6-TBP, 3-Cl-4-OHBA, TCMP, TCHQ, bromoxynil, ioxynil	Sanford <i>et al.</i> , 1996; Milliken <i>et al.</i> , 2004a, b; Cupples <i>et al.</i> , 2005
	<i>Desulfotobacterium hafniense</i> strain DCB-2	formate, pyruvate, lactate, fumarate, butyrate, succinate, malate, ethanol	PCP, 2,4,5-TCP, 2,4,6-TCP, 2,4-DCP, 3,5-DCP, 3-Cl-4OHPA, TCMP, TCHQ	Madsen and Licht, 1992; Christiansen and Ahning, 1996; Niggemeyer, 2001; Milliken <i>et al.</i> , 2004a, b
	<i>Desulfotobacterium hafniense</i> strain TCP-A	H ₂ , formate, pyruvate, lactate, butyrate, alanine, glutamate	PCP, 2,3,4,5-TeCP, 2,3,5,6-TeCP, 2,3,5-TCP, 2,4,6-TCP, 3,5-DCP, 2,3DCP, 2,4-DCP, 2-CP	Breitenstein <i>et al.</i> , 2001

Phylum/class	Microorganisms	e-Donors for dehalorespiration	Aromatic organochlorides for dehalorespiration	Reference
	<i>Desulfitobacterium hafnense</i> strain PCP-1	formate, pyruvate, lactate, fumarate, butyrate, succinate, malate, ethanol	PCP, 2,3,4,5-TeCP, 2,3,5,6-TeCP, 2,3,4-TCP, 2,3,5-TCP, 2,3,6-TCP, 2,4,5-TCP, 2,4,6-TCP, 3,4,5-TCP, 2,6-DCP, 2,4-DCP, 3,5-DCP, PBDEs	Bouchard <i>et al.</i> , 1996; Robrock <i>et al.</i> , 2008
	<i>Desulfitobacterium metallireducens</i> strain		3-Cl-OHPA	Finneran <i>et al.</i> , 2002
	<i>Desulfitobacterium</i> sp. KBC1	formate, lactate, pyruvate, butyrate	3-Cl-OHPA, 2,4,6-TCP, 2,4-DCP	Tsukagoshi <i>et al.</i> , 2006
	<i>Desulfitobacterium</i> sp. PCE1	formate, lactate, pyruvate, butyrate, succinate, ethanol	3-Cl-OHPA, 2,4,6-TCP, 2,4-DCP, 2-CP, TCMP, TCHQ	Gerritse <i>et al.</i> , 1996; Milliken <i>et al.</i> , 2004a, b
	<i>Dehalobacter restrictus</i> PER-K23	H ₂	PBDEs	Holliger <i>et al.</i> , 1998; Robrock <i>et al.</i> , 2008
<i>Chloroflexi</i>	" <i>Dehalococcoides ethenogenes</i> " strain 195	H ₂	1,2,3,4-TeCDD, 2,3,4,5,6-PeCBP, 1,2,3,4-TeCNP, 1,2,3,4-TeCDF, HCB	Maymo-Gatell <i>et al.</i> , 1997; Fennell <i>et al.</i> , 2004; He <i>et al.</i> , 2006
	" <i>Dehalococcoides</i> " sp. strain CBDB1	H ₂	1,2,3-TCB, HCB, PeCB, 1,2,3,4-TeCDD, 1,2,3-TrCDD, 1,2,3,7,8-PeCDD, Aroclor 1260*	Adrian <i>et al.</i> , 2000; Bunge <i>et al.</i> , 2003; Jayachandran <i>et al.</i> , 2003; Adrian <i>et al.</i> , 2009
	ultramicrobacterium DF-1	H ₂ , formate	2,3,4,5-TeCBP, Aroclor 1260*, HCB	Wu <i>et al.</i> , 2002a, b; May <i>et al.</i> , 2008

MCP, monochlorophenol; MBP, monobromophenol; DCP, dichlorophenol; CBA, chlorobenzene; BBA, bromobenzene; TCBA, trichlorobenzene; PBDEs, polybrominated diphenyl ethers; 3-Cl-4-OHPA, 3-chloro-4-hydroxyphenylacetate; TCP, trichlorophenol; PCP, pentachlorophenol; OH-PCBs, *para*-hydroxylated polychlorinated biphenyls; TCMP, 2,3,5,6-tetrachloro-4-methoxyphenol; TCHQ, tetrachloroquinone; TBP, tribromophenol; 3-Cl-4-OHBA, 3-chloro-4-hydroxybenzoate; bromoxynil, 3,5-dibromo-4-hydroxybenzotriazole; ioxynil, 3,5-ditido-4-hydroxybenzotriazole; TeCP, tetrachlorophenol; TeCDD, tetrachlorodibenzo-*p*-dioxin; PeCBP, pentachlorobiphenyl; TeCNP, tetrachloronaphthalene; TeCDF, tetrachlorodibenzofuran; HCB, hexachlorobenzene; TCB, trichlorobenzene. *Commercial PCB mixtures composed of 60 to 100 different congeners.

Desulfitobacterium

The genus *Desulfitobacterium* includes a large number of dehalorespiring strains, which are able to reduce aliphatic or aromatic chlorides or both. *D. dehalogenans* strain JW/IU-DC1 is the first isolate in this genus (Utkin *et al.*, 1994), and capable of *ortho*-dechlorination for a variety of aromatic chlorides such as 3-chloro-4-hydroxyphenylacetate (3-Cl-4-OHPA), chlorinated phenols (Utkin *et al.*, 1995) and *para*-hydroxylated polychlorinated biphenyls (Wiegel *et al.*, 1999). *D. chlororespirans* strain Co23 has similar *ortho*-dechlorination activity as strain JW/IU-DC1 and respire with chlorinated and brominated phenols, hydroxyphenylacetate, and hydroxybenzoate (Sanford *et al.*, 1996). *D. hafniense* strains DCB-2 and TCP-A have *ortho*- and *meta*-dechlorination activities for chlorinated phenols (Madsen and Licht, 1992; Christiansen and Ahring, 1996; Breitenstein *et al.*, 2001), although strain PCP-1 has *ortho*-, *meta*-, and *para*-dechlorination activities for chlorinated phenols (Bouchard *et al.*, 1996).

Dehalobacter

Dehalobacter sp. is known as obligate dehalorespiring bacteria and includes three strains, and the debromination of polybrominated biphenyl ethers has been demonstrated in a pure culture of *D. restrictus* PER-K23 (He *et al.*, 2006). More detail information about this genus described below.

Dehalococcoides

“*Dehalococcoides ethenogenes*” strain 195 is probably most famous dehalorespiring bacteria because of first isolate as an obligate dehalorespiring bacteria has an advantage in the reduction of tetrachloroethene to ethene, which had never observed in other yet-isolated dehalorespiring bacteria (Maymo-Gatell *et al.*, 1997). Later, “*Dehalococcoides*” sp. CBDB1 and strain 195 reported to reduce considerably larger spectrum of organochlorides than other dehalorespiring bacteria, which are chlorinated ethenes, ethanes, phenols, benzenes, biphenyls, dibenzo-*p*-dioxins, and brominated diphenyl ethers (Adrian *et al.*, 2000, 2009; Bunge *et al.*, 2003; Fennell *et al.*, 2004; He *et al.*, 2006). The genome sequence analysis of the two strains revealed that chromosomes of strain 195 and CBDB1 contain at least 18 and 32 RD-homologues, respectively. These numbers of RD-homologues are much higher than other dehalorespiring bacteria (i.e. two RD-homologues in *Desulfitobacterium hafniense* Y51, nine RD-homologues in *Desulfitobacterium hafniense* DHB-2) and are well agreement with large spectrum of organochlorides in “*Dehalococcoides*” spp. (Kube *et al.*, 2005; Seshadri *et al.*, 2005; Nonaka *et al.*, 2006; *D. hafniense* DCB-2 whole-genome shotgun project, GenBank accession number AAW00000000).

o-17/DF-1 group of *Chloroflexi*

A phylogenetic group of uncultured *Chloroflexi* that dechlorinate polychlorinated biphenyls (PCBs) was designed as *o*-17/DF-1 group (Wu *et al.*, 2002a), and includes an isolate, strain DF-1, which dechlorinate doubly flanked

chlorines from PCBs and chlorinated benzenes (Wu *et al.*, 2002b; May *et al.*, 2008). DF-1 is also obligate dehalorespiring bacteria as “*Dehalococcoides*” and forms a closely related but distinct phylogenetic cluster with “*Dehalococcoides*” spp. based on 16S rRNA gene sequences. Therefore, this group is called as “*Dehalococcoides*”-like group in some cases.

DEHALOBACTER SP. AS NEW CANDIDATE AS BACTERIA THAT DECHLORINATE AROMATIC CHLORIDES

Genus *Dehalobacter* is known obligate halorespiring bacteria and includes three strains: *D. restrictus* strain PER-K23 (Holliger *et al.*, 1998) and *D. restrictus* strain TEA (Wild *et al.*, 1996), which grow on the dechlorination of tetrachloroethene and trichloroethene; and *Dehalobacter* sp. strain TCA1 (Sun *et al.*, 2002), which grows on the dechlorination of 1,1,2-trichloroethane and 1,1-dichloroethane. *D. restrictus* strain PER-K23 has also been reported to debrominate PBDEs as do *Sulfurospirillum* sp., *Desulfitobacterium* sp. and “*Dehalococcoides*” sp. (He *et al.*, 2006; Robrock *et al.*, 2008), although the capacities of *Dehalobacter* spp. to dechlorinate aromatic chlorides have not been reported so far. Recently, we demonstrated the reductive dechlorination of an aromatic chloride, 4,5,6,7-tetrachlorophthalide (fthalide), in a soil-free enrichment culture (designated KFL culture) that containing *Dehalobacter* species in phylum *Firmicutes* (Yoshida *et al.*, 2009a). In the KFL culture, *Dehalobacter* species increased in the population corresponding to the dechlorination of fthalide and members of other yet-isolated dehalorespiring bacteria were not detected. Indeed, other reports also detected *Dehalobacter* species in several microbial communities that dechlorinate aromatic chlorides such as 1,2,3-trichlorobenzene dechlorinating consortium (von Wintzingerode *et al.*, 1999) and a sediment culture containing “*Dehalococcoides*” sp. that dechlorinated 2,3,4,5-tetrachlorobiphenyl (2,3,4,5-TeCB) (Yan *et al.*, 2006). Indeed, we additionally demonstrated the KFL culture dechlorinated PCBs and 1,2,3-trichlorodibenzo-*p*-dioxin (Yoshida *et al.*, 2009b). In the dechlorination assay for PCBs, KFL culture dechlorinated *para*-, *meta*- and *ortho*-substituted chlorines including only doubly flanked but singly flanked chlorine of PCBs, which have never observed in yet-isolated PCB dechlorinators. Surprisingly, the KFL culture also dechlorinated hexachlorobenzene, 2,4,6-trichlorophenol in addition to PCBs and PCDD, although tetrachloroethene, trichloroethene, and beta-hexachlorinated cyclohexane (beta-HCH) were not dechlorinated at all (unpublished data). These findings suggest that *Dehalobacter* sp. is probably a new candidate to dechlorinate aromatic chlorides of large spectrum as do “*Dehalococcoides*” spp. The potential dechlorination spectrum of *Dehalobacter* sp. based on previously published papers and our unpublished data was summarized in Table 2.

FUTURE PERSPECTIVE

Dehalorespiring bacteria have been extensively investigated as beneficial bio-catalysts for application in bioremediation technology for last few decades.

Table 2. The potential dechlorination spectrum of *Dehalobacter* spp.

Isolate/consortium	Dehalogenation activities	Reference
<i>Dehalobacter restrictus</i> strain PER-K23	PCE → cis-DCE PBDE	Holliger <i>et al.</i> , 1998 Robrock <i>et al.</i> , 2008
<i>D. restrictus</i> strain TEA	PCE → cis-DCE	Wild <i>et al.</i> , 1996
<i>Dehalobacter</i> sp. strain TCA1	1,1,2-TCA → VC	Sun <i>et al.</i> , 2002
sediment microcosm containing <i>Dehalobacter</i> sp.	2,3,4,5-TeCB → 2-MCB	Yan <i>et al.</i> , 2006
co-culture of <i>Dehalobacter</i> sp. and <i>Sedimentibacter</i> sp.	β-HCH → MCB, benzene	van Doesburg <i>et al.</i> , 2005
enrichment culture containing <i>Dehalobacter</i> sp.	1,1,1-TCA → 1,1-DCA and MCA	Grostorm <i>et al.</i> , 2009
co-culture of <i>Dehalobacter</i> sp. and <i>Acetobacterium</i> sp.	1,2-DCA → ethene	Grostorm and Edwards, 2009
enrichment culture containing <i>Dehalobacter</i> sp.	ftalide → 4-MCPH	Yoshida <i>et al.</i> , 2009a
	2,3,4,5-TeCB → 2-MCB, 4-MCB	Yoshida <i>et al.</i> , 2009b
	2,3,4-TricB → 2-MCB, 4-MCB	Yoshida <i>et al.</i> , 2009b
	1,2,3-TricDD → 2,3-DiCDD	Yoshida <i>et al.</i> , 2009b
	HCB → DiCBs	Yoshida <i>et al.</i> (unpublished data)
	2,4,6-TCP → 4-MCP	Yoshida <i>et al.</i> (unpublished data)

PCE, tetrachloroethene; cis-DCE, cis-dichloroethene; PBDE, polybrominated diphenyl ethers; TCA, trichloroethane; VC, vinyl chloride; TeCB, trichlorobenzene; TeCB, tetrachlorobiphenyl; beta-HCH, beta-hexachlorocyclohexane; DCA, dichloroethane; MCA, monochloroethane; ftalide, 4,5,6,7-tetrachlorophthalide; MCPH, monochlorophthalide; TeCB, tetrachlorobiphenyl; MCB, monochlorobiphenyl; TriCB, trichlorobiphenyl; TricDD, trichlorodibenzo-*p*-dioxin; DiCDD, dichlorodibenzo-*p*-dioxin; HCB, hexachlorobenzene; TCP, trichlorophenol; MCP, monochlorophenol.

Interestingly, these works revealed the presences of many obligate dehalorespiring bacteria spanning different phyla (i.e. *Dehalobacter* sp. of *Firmicutes*, and “*Dehalococcoides*” spp. and ultramicrobacterium DF-1 of *Chloroflexi*) and frequent detection of their relative phylotypes in environments without historical anthropogenic exposure of organic halides. The molecular biology revealed highly developed mechanisms of dehalorespiration in the dehalorespiring bacteria. These findings indicate that the dehalogenation metabolism including dehalorespiration is likely ancient evolutionary process corresponding to ecological niche abundant with natural organohalides rather than recent evolutionary events triggered by anthropogenic pollution of organohalides. In fact, 3,000 organohalides are biologically or abiotically synthesized in the environments in addition to 15,000 anthropogenic organohalides, which are potential substrate for dehalogenating bacteria for long before human introduction of organohalides (Hileman, 1993; Gribble *et al.*, 2000; Öberg, 2002). Several reports have supported this speculation so far; microbial debromination of bromophenols that produced by a marine sponge as metabolites (Ahn *et al.*, 2003); dechlorination of chlorinated hydroquinone, a metabolites synthesized by fungi, via bacterial dehalorespiration (Milliken *et al.*, 2004a, b); widespread distribution of reductive dehalogenase and the debromination activities for bromophenols in marine subsurface sediments (Futagami *et al.*, 2009). We also recently enriched several dehalogenating bacteria including *Dehalobacter* species with natural organohalides (data not shown). These observations now opened up a field of great interest, natural global halogen cycle and the fundamental role of microorganisms that dehalogenate organohalides.

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REFERENCES

- Adrian, L., U. Szewzyk, J. Wecke and H. Gorisch (2000): Bacterial dehalorespiration with chlorinated benzenes. *Nature*, **408**, 580–583.
- Adrian, L., V. Dudkova, K. Demnerova and D. L. Bedard (2009): “*Dehalococcoides*” sp. strain CBDB1 extensively dechlorinates the commercial polychlorinated biphenyl mixture Aroclor 1260. *Appl. Environ. Microbiol.*, **75**, 4516–4524.
- Ahn, Y. B., S. K. Rhee, D. E. Fennell, L. J. Kerkhof, U. Hentschel and M. M. Haggblom (2003): Reductive dehalogenation of brominated phenolic compounds by microorganisms associated with the marine sponge *Aplysina aerophoba*. *Appl. Environ. Microbiol.*, **69**, 4159–4166.
- Apajalahti, J. H. and M. S. Salkinoja-Salonen (1987): Dechlorination and *para*-hydroxylation of polychlorinated phenols by *Rhodococcus chlorophenicus*. *J. Bacteriol.*, **169**, 675–681.
- Bouchard, B., R. Beaudet, R. Villemur, G. McSween, F. Lepine and J. G. Bisaillon (1996): Isolation and characterization of *Desulfitobacterium frappieri* sp. nov., an anaerobic bacterium which reductively dechlorinates pentachlorophenol to 3-chlorophenol. *Int. J. Syst. Bacteriol.*, **46**, 1010–1015.
- Breitenstein, A., A. Saano, M. Salkinoja-Salonen, J. R. Andreesen and U. Lechner (2001): Analysis of a 2,4,6-trichlorophenol-dehalogenating enrichment culture and isolation of the dehalogenating

- member *Desulfitobacterium frappieri* strain TCP-A. *Arch. Microbiol.*, **175**, 133–142.
- Bunge, M., L. Adrian, A. Kraus, M. Opel, W. G. Lorenz *et al.* (2003): Reductive dehalogenation of chlorinated dioxins by an anaerobic bacterium. *Nature*, **421**, 357–360.
- Christiansen, N. and B. K. Ahring (1996): *Desulfitobacterium hafniense* sp. nov., an anaerobic, reductively dechlorinating bacterium. *Int. J. Syst. Bacteriol.*, **46**, 442–448.
- Cole, J. R., A. L. Cascarelli, W. W. Mohn and J. M. Tiedje (1994): Isolation and characterization of a novel bacterium growing via reductive dehalogenation of 2-chlorophenol. *Appl. Environ. Microbiol.*, **60**, 3536–3542.
- Cupples, A. M., R. A. Sanford and G. K. Sims (2005): Dehalogenation of the herbicides bromoxynil (3,5-dibromo-4-hydroxybenzotrile) and ioxynil (3,5-diiodo-4-hydroxybenzotrile) by *Desulfitobacterium chlororespirans*. *Appl. Environ. Microbiol.*, **71**, 3741–3746.
- Deweerd, K. A., L. Mandelco, R. S. Tanner, C. R. Woese and J. M. Suflita (1990): *Desulfomonile tiedjei* gen. nov. and sp. nov., a novel anaerobic, dehalogenating, sulfate-reducing bacterium. *Arch. Microbiol.*, **154**, 23–30.
- Egland, P. G., J. Gibson and C. S. Harwood (2001): Reductive, coenzyme A-mediated pathway for 3-chlorobenzoate degradation in the phototrophic bacterium *Rhodospseudomonas palustris*. *Appl. Environ. Microbiol.*, **67**, 1396–1399.
- Fennell, D. E., I. Nijenhuis, S. F. Wilson, S. H. Zinder and M. M. Haggblom (2004): *Dehalococcoides ethenogenes* strain 195 reductively dechlorinates diverse chlorinated aromatic pollutants. *Environ. Sci. Technol.*, **38**, 2075–2081.
- Finneran, K. T., H. M. Forbush, C. V. VanPraagh and D. R. Lovley (2002): *Desulfitobacterium metallireducens* sp. nov., an anaerobic bacterium that couples growth to the reduction of metals and humic acids as well as chlorinated compounds. *Int. J. Syst. Evol. Microbiol.*, **52**, 1929–1935.
- Futagami, T., Y. Morono, T. Terada, A. H. Kaksonen and F. Inagaki (2009): Dehalogenation activities and distribution of reductive dehalogenase homologous genes in marine subsurface sediments. *Appl. Environ. Microbiol.*, **75**, 6905–6909.
- Gerritse, J., V. Renard, T. M. P. Gomes, P. A. Lawson, M. D. Collins and J. C. Gottschal (1996): *Desulfitobacterium* sp. strain PCE1, an anaerobic bacterium that can grow by reductive dechlorination of tetrachloroethene or *ortho*-chlorinated phenols. *Arch. Microbiol.*, **165**, 132–140.
- Gribble, G. W. (2000): The natural production of organobromine compounds. *Environ. Sci. Pollut. Res.* **7**, 37–47.
- Grosten, A. and E. A. Edwards (2009): Characterization of a *Dehalobacter* coculture that dechlorinates 1,2-dichloroethane to ethene and identification of the putative reductive dehalogenase gene. *Appl. Environ. Microbiol.*, **75**, 2684–2693.
- Grosten, A., W. W. M. Chan and E. A. Edwards (2009): 1,1,1-Trichloroethane and 1,1-dichloroethane reductive dechlorination kinetics and co-contaminant effects in a *Dehalobacter*-containing mixed culture. *Environ. Sci. Technol.*, **43**, 6799–6807.
- He, J. Z., K. R. Robrock and L. Alvarez-Cohen (2006): Microbial reductive debromination of polybrominated diphenyl ethers (PBDEs). *Environ. Sci. Technol.*, **40**, 4429–4434.
- Hileman, B. (1993): Concerns broaden over chlorine and chlorinated hydrocarbons. *Chem. Eng. News*, **71**, 11–20.
- Holliger, C., D. Hahn, H. Harmsen, W. Ludwig, W. Schumacher *et al.* (1998): *Dehalobacter restrictus* gen. nov. and sp. nov., a strictly anaerobic bacterium that reductively dechlorinates tetra- and trichloroethene in an anaerobic respiration. *Arch. Microbiol.*, **169**, 313–321.
- Janssen, D. B., F. Pries and J. R. Vanderploeg (1994): Genetics and biochemistry of dehalogenating enzymes. *Ann. Rev. Microbiol.*, **48**, 163–191.
- Jayachandran, G., H. Gorisch and L. Adrian (2003): Dehalorespiration with hexachlorobenzene and pentachlorobenzene by *Dehalococcoides* sp. strain CBDB1. *Arch. Microbiol.*, **180**, 411–416.
- Kube, M., A. Beck, S. H. Zinder, H. Kuhl, R. Reinhardt and L. Adrian (2005): Genome sequence of the chlorinated compound respiring bacterium *Dehalococcoides* species strain CBDB1. *Nature Biotechnology*, **23**, 1269–1273.
- Luijten, M., J. de Weert, H. Smidt, H. T. S. Boschker, W. M. de Vos *et al.* (2003): Description of *Sulfurospirillum halorespirans* sp. nov., an anaerobic, tetrachloroethene-respiring bacterium,

- and transfer of *Dehalospirillum multivorans* to the genus *Sulfurospirillum* as *Sulfurospirillum multivorans* comb. nov. *Int. J. Syst. Evol. Microbiol.*, **53**, 787–793.
- Luijten, M., W. Roelofs, A. A. M. Langenhoff, G. Schraa and A. J. M. Stams (2004): Hydrogen threshold concentrations in pure cultures of halo-respiring bacteria and at a site polluted with chlorinated ethenes. *Environ. Microbiol.*, **6**, 646–650.
- Madsen, T. and D. Licht (1992): Isolation and characterization of an anaerobic chlorophenol-transforming bacterium. *Appl. Environ. Microbiol.*, **58**, 2874–2878.
- May, H. D., G. S. Miller, B. V. Kjellerup and K. R. Sowers (2008): Dehalorespiration with polychlorinated biphenyls by an anaerobic ultramicrobacterium. *Appl. Environ. Microbiol.*, **74**, 2089–2094.
- Maymo-Gatell, X., Y. T. Chien, J. M. Gossett and S. H. Zinder (1997): Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene. *Science*, **276**, 1568–1571.
- Milliken, C. E., G. P. Meier, J. E. M. Watts, K. R. Sowers and H. D. May (2004a): Microbial anaerobic demethylation and dechlorination of chlorinated hydroquinone metabolites synthesized by basidiomycete fungi. *Appl. Environ. Microbiol.*, **70**, 385–392.
- Milliken, C. E., G. P. Meier, K. R. Sowers and H. D. May (2004b): Chlorophenol production by anaerobic microorganisms: Transformation of a biogenic chlorinated hydroquinone metabolite. *Appl. Environ. Microbiol.*, **70**, 2494–2496.
- Mohn, W. W. and J. M. Tiedje (1991): Evidence for chemiosmotic coupling of reductive dechlorination and ATP synthesis in *Desulfomonile tiedjei*. *Arch. Microbiol.*, **157**, 1–6.
- Niggemyer, A., S. Spring, E. Stackebrandt and R. F. Rosenzweig (2001): Isolation and characterization of a novel As(V)-reducing bacterium: Implications for arsenic mobilization and the genus *Desulfitobacterium*. *Appl. Environ. Microbiol.*, **67**, 5568–5580.
- Nonaka, H., G. Keresztes, Y. Shinoda, Y. Ikenaga, M. Abe *et al.* (2006): Complete genome sequence of the dehalorespiring bacterium *Desulfitobacterium hafniense* Y51 and comparison with *Dehalococcoides ethenogenes* 195. *J. Bacteriol.*, **188**, 2262–2274.
- Öberg, G. (2002): The natural chlorine cycle-fitting the scattered pieces. *Appl. Microbiol. Biotechnol.*, **58**, 565–581.
- Ohtsubo, Y., K. Miyauchi, K. Kanda, T. Hatta, H. Kiyohara *et al.* (1999): PcpA, which is involved in the degradation of pentachlorophenol in *Sphingomonas chlorophenolica* ATCC39723, is a novel type of ring-cleavage dioxygenase. *FEBS Lett.*, **459**, 395–398.
- Orser, C. S., J. Dutton, C. Lange, P. Jablonski, L. Y. Xun and M. Hargis (1993a): Characterization of a *Flavobacterium* glutathione-S-transferase gene involved in reductive dechlorination. *J. Bacteriol.*, **175**, 2640–2644.
- Orser, C. S., C. C. Lange, L. Y. Xun, T. C. Zahrt and B. J. Schneider (1993b): Cloning, sequence-analysis, and expression of the *Flavobacterium* pentachlorophenol-4-monoxygenase gene in *Escherichia coli*. *J. Bacteriol.*, **175**, 411–416.
- Robrock, K. R., P. Korytar and L. Alvarez-Cohen (2008): Pathways for the anaerobic microbial debromination of polybrominated diphenyl ethers. *Environ. Sci. Technol.*, **42**, 2845–2852.
- Sanford, R. A., J. R. Cole, F. E. Löffler and J. N. Tiedje (1996): Characterization of *Desulfitobacterium chlororespirans* sp. nov., which grows by coupling the oxidation of lactate to the reductive dechlorination of 3-chloro-4-hydroxybenzoate. *Appl. Environ. Microbiol.*, **62**, 3800–3808.
- Sanford, R. A., J. R. Cole and J. M. Tiedje (2002): Characterization and description of *Anaeromyxobacter dehalogenans* gen. nov., sp. nov., an aryl-halo-respiring facultative anaerobic myxobacterium. *Appl. Environ. Microbiol.*, **68**, 893–900.
- Scholz-Muramatsu, H., A. Neumann, M. Messmer, E. Moore and G. Diekert (1995): Isolation and characterization of *Dehalospirillum multivorans* gen. nov., sp. nov., a tetrachloroethene-utilizing, strictly anaerobic bacterium. *Arch. Microbiol.*, **163**, 48–56.
- Seshadri, R., L. Adrian, D. E. Fouts, J. A. Eisen, A. M. Phillippy *et al.* (2005): Genome sequence of the PCE-dechlorinating bacterium *Dehalococcoides ethenogenes*. *Science*, **307**, 105–108.
- Song, B. K., N. J. Palleroni and M. M. Haggblom (2000): Isolation and characterization of diverse halobenzoate-degrading denitrifying bacteria from soils and sediments. *Appl. Environ. Microbiol.*, **66**, 3446–3453.
- Sun, B. L., J. R. Cole, R. A. Sanford and J. M. Tiedje (2000): Isolation and characterization of

- Desulfovibrio dechloracetivorans* sp. nov., a marine dechlorinating bacterium growing by coupling the oxidation of acetate to the reductive dechlorination of 2-chlorophenol. *Appl. Environ. Microbiol.*, **66**, 2408–2413.
- Sun, B. L., J. R. Cole and J. M. Tiedje (2001): *Desulfomonile limimaris* sp. nov., an anaerobic dehalogenating bacterium from marine sediments. *Int. J. Syst. Evol. Microbiol.*, **51**, 365–371.
- Sun, B. L., B. M. Griffin, H. L. Ayala-del-Rio, S. A. Hashsham and J. M. Tiedje (2002): Microbial dehalorespiration with 1,1,1-trichloroethane. *Science*, **298**, 1023–1025.
- Treude, N., D. Rosencrantz, W. Liesack and S. Schnell (2003): Strain FAc12, a dissimilatory iron-reducing member of the *Anaeromyxobacter* subgroup of *Myxococcales*. *Fems Microbiol. Ecol.*, **44**, 261–269.
- Tsakagoshi, N., S. Ezaki, T. Uenaka, N. Suzuki and R. Kurane (2006): Isolation and transcriptional analysis of novel tetrachloroethene reductive dehalogenase gene from *Desulfitobacterium* sp. strain KBC1. *Appl. Microbiol. Biotechnol.*, **69**, 543–553.
- Utkin, I., C. Woese and J. Wiegel (1994): Isolation and characterization of *Desulfitobacterium dehalogenans* gen. nov., sp. nov., an anaerobic bacterium which reductively dechlorinates chlorophenolic compounds. *Int. J. Syst. Bacteriol.*, **44**, 612–619.
- Utkin, I., D. D. Dalton and J. Wiegel (1995): Specificity of reductive dehalogenation of substituted *ortho*-chlorophenols by *Desulfitobacterium dehalogenans* JW/IU-DC1. *Appl. Environ. Microbiol.*, **61**, 346–351.
- van der Meer, J. R., R. I. L. Eggen, A. J. B. Zehnder and W. M. Devos (1991): Sequence-analysis of the *Pseudomonas* sp. strain-P51 Tcb gene-cluster, which encodes metabolism of chlorinated catechols—Evidence for specialization of catechol 1,2-dioxygenases for chlorinated substrates. *J. Bacteriol.*, **173**, 2425–2434.
- van der Woude, B. J., M. Deboer, N. M. J. Vanderput, F. M. Vandergeld, R. A. Prins and J. C. Gottschal (1994): Anaerobic degradation of halogenated benzoic-acids by photoheterotrophic bacteria. *Fems Microbiol. Lett.*, **119**, 199–207.
- van Doesburg, W., M. H. A. van Eekert, P. J. M. Middeldorp, M. Balk, G. Schraa and A. J. M. Stams (2005): Reductive dechlorination of beta-hexachlorocyclohexane (beta-HCH) by a *Dehalobacter* species in coculture with a *Sedimentibacter* sp. *Fems Microbiol. Ecol.*, **54**, 87–95.
- von Wintzingerode, F., B. Selent, W. Hegemann and U. B. Gobel (1999): Phylogenetic analysis of an anaerobic, trichlorobenzene transforming microbial consortium. *Appl. Environ. Microbiol.*, **65**, 283–286.
- Wiegel, J., X. M. Zhang and Q. Z. Wu (1999): Anaerobic dehalogenation of hydroxylated polychlorinated biphenyls by *Desulfitobacterium dehalogenans*. *Appl. Environ. Microbiol.*, **65**, 2217–2221.
- Wild, A., R. Hermann and T. Leisinger (1996): Isolation of an anaerobic bacterium which reductively dechlorinates tetrachloroethene and trichloroethene. *Biodegradation*, **7**, 507–511.
- Wu, Q. Z., J. E. M. Watts, K. R. Sowers and H. D. May (2002a): Identification of a bacterium that specifically catalyzes the reductive dechlorination of polychlorinated biphenyls with doubly flanked chlorines. *Appl. Environ. Microbiol.*, **68**, 807–812.
- Wu, Q. Z., C. E. Milliken, G. P. Meier, J. E. M. Watts, K. R. Sowers and H. D. May (2002b): Dechlorination of chlorobenzenes by a culture containing bacterium DF-1, a PCB dechlorinating microorganism. *Environ. Sci. Technol.*, **36**, 3290–3294.
- Yan, T., T. M. LaPara and P. J. Novak (2006): The effect of varying levels of sodium bicarbonate on polychlorinated biphenyl dechlorination in Hudson River sediment cultures. *Environ. Microbiol.*, **8**, 1288–1298.
- Yoshida, N., L. Ye, D. Baba and A. Katayama (2009a): A novel *Dehalobacter* species is involved in extensive 4,5,6,7-tetrachlorophthalide dechlorination. *Appl. Environ. Microbiol.*, **75**, 2400–2405.
- Yoshida, N., L. Ye, D. Baba and A. Katayama (2009b): Reductive dechlorination of polychlorinated biphenyls and dibenzo-*p*-dioxins in an enrichment culture containing *Dehalobacter* species. *Microb. Environ.*, **24**, 343–346.