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

Naoko YOSHIDA¹,² and Arata KATAYAMA¹

¹EcoTopia Science Institute, Nagoya University, Furo-cho, Chikusa-ku, Nagoya 464-0814, Japan
²Laboratory of Microbial Biotechnology, Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Oiwake-cho, Kitashirakawa, Sakyo-ku, Kyoto 606-8224, Japan

(Received 18 January 2010; accepted 27 January 2010)

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-chlorobenzyol-CoA rather than free 3-chlorobenzoic acid (van der Woude et al., 1994; Egland 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 trichlotoethenes 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 careers 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. “*Dehalococccoides*”, 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 liminmaris* (Sun *et al.*, 2001). *D. liminmaris* 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 oxidation 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.

<table>
<thead>
<tr>
<th>Phylum/Class</th>
<th>Microorganisms</th>
<th>e-Donors for dehalorespiration</th>
<th>Aromatic organochlorides for dehalorespiration</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Deltaproteobacteria</strong></td>
<td>Anaeromixobacter dehalogans strain 2CP-1</td>
<td>$H_2$, acetate, succinate, pyruvate</td>
<td>2-MCP, 2-MBP, 2,6-DCP</td>
<td>ortho</td>
</tr>
<tr>
<td></td>
<td>Desulfomonile tiedjei strain DCB-1</td>
<td></td>
<td>3-CBA</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Desulfomonile liminarius strain DCB-M</td>
<td>$H_2$, pyruvate, lactate, butyrate, propionate</td>
<td>3-CBA, 3-BBA, 2,3-DCP, 2,5-DCP, 3,5-DCP, 2,3,5-TCP</td>
<td>meta</td>
</tr>
<tr>
<td><strong>Firmicutes</strong></td>
<td>Desulfotobacterium dehalogans strain JW/IU-DC1</td>
<td>$H_2$, pyruvate, lactate</td>
<td>3-Cl-4-OHPA, 2,3-DCP, 2,4-DCP, 2,6-DCP, PCP, OH-PCBs, TCMP, TCHQ</td>
<td>ortho</td>
</tr>
<tr>
<td></td>
<td>Desulfotobacterium chlororespirants strain Co23</td>
<td>$H_2$, formate, pyruvate, lactate, butyrate, crotonate</td>
<td>3-Cl-4-OHPA, 2,3-DCP, 2,4-DCP, 2,6-DCP, 2,4,6-TCP, 3-Cl-4-OHBA, TCMP, TCHQ, bromoxylin, ioxyni</td>
<td>ortho</td>
</tr>
<tr>
<td></td>
<td>Desulfotobacterium hafniense strain DCB-2</td>
<td>formate, pyruvate, lactate, fumarate, butyrate, succinate, maleate, ethanol</td>
<td>PCP, 2,4,5-TCP, 2,4,6-TCP, 3-Cl-4-OHPA, TCMP, TCHQ</td>
<td>ortho, meta</td>
</tr>
<tr>
<td></td>
<td>Desulfotobacterium hafniense strain TCP-A</td>
<td>$H_2$, pyruvate, lactate, butyrate, alanine, glutamate</td>
<td>PCP, 2,3,4,5-TeCP, 2,3,5,6-TeCP, 2,3,5,6-TeCP, 2,4,6-TCP, 3,5-DCP, TCHQ</td>
<td>ortho, meta</td>
</tr>
<tr>
<td>Phylum/Class</td>
<td>Microorganisms</td>
<td>e-Donors for dehalorespiration</td>
<td>Aromatic organochlorides for dehalorespiration</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------</td>
<td>----------------</td>
<td>-------------------------------</td>
<td>---------------------------------------------</td>
<td>-----------</td>
</tr>
<tr>
<td><strong>Desulfotobacterium hafniense</strong> strain PCP-1</td>
<td>formate, pyruvate, lactate, fumarate, butyrate, succinate, malate, ethanol</td>
<td>PCP, 2,3,4,5-TeCP, 2,3,5,6-TeCP, 2,3,4,6-TCP, 2,4,5-TCP, 2,4,6-TCP, 3,4,5-TCP, 2,6-DCP, 2,4-DCP, 3,5-DCP, PBDEs</td>
<td>ortho, meta, para</td>
<td>Bouchard et al., 1996; Robrock et al., 2008</td>
</tr>
<tr>
<td><strong>Desulfotobacterium metallireducens</strong> strain</td>
<td>formate, pyruvate, butyrate</td>
<td>3-Cl-OHPA</td>
<td>ortho</td>
<td>Finneran et al., 2002</td>
</tr>
<tr>
<td><strong>Desulfotobacterium sp. KB1</strong></td>
<td>formate, lactate, pyruvate, butyrate</td>
<td>3-Cl-OHPA, 2,4,6-TCP, 2,4-DCP</td>
<td>ortho</td>
<td>Tsukagoshi et al., 2006</td>
</tr>
<tr>
<td><strong>Desulfotobacterium sp. PCE1</strong></td>
<td>formate, lactate, pyruvate, butyrate, succinate, ethanol</td>
<td>3-Cl-OHPA, 2,4,6-TCP, 2,4-DCP, 2-CP, TCMP, TCHQ</td>
<td>ortho</td>
<td>Gerrise et al., 1996; Milliken et al., 2004a, b</td>
</tr>
<tr>
<td><strong>Dehalobacter restrictus</strong> strain PER-K23</td>
<td>formate, lactate, pyruvate, butyrate, succinate, ethanol</td>
<td>3-Cl-OHPA, 2,4,6-TCP, 2,4-DCP, 2-CP, TCMP, TCHQ</td>
<td>ortho</td>
<td>Helliger et al., 1998; Robrock et al., 2008</td>
</tr>
<tr>
<td><strong>Chloroflexi</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Dehalococcoides ethenogenes</strong> strain 195</td>
<td>H₂</td>
<td>1,2,3,4-TeCDD, 2,3,4,5-TeCPB, 1,2,3,4-TeCP, 1,2,3,4-TeCDF, HCB</td>
<td>meta, para (PeCBP), per, lateral (PCDDs)</td>
<td>Maymo-Gatell et al., 1997; Fennell et al., 2004; He et al., 2006</td>
</tr>
<tr>
<td><strong>Dehalococcoides” sp. strain CBDB1</strong></td>
<td>H₂</td>
<td>1,2,3-TCB, HCB, PeCB, 1,2,3,4-TeCDD, 1,2,3,4-TeCDF, HCB</td>
<td>meta, para (Aroclor 1260), per, lateral (PCDDs)</td>
<td>Adian et al., 2000; Bunge et al., 2003; Jayachandran et al., 2003; Adian et al., 2009</td>
</tr>
<tr>
<td><strong>ultramicrobacterium DF-1</strong></td>
<td>formate</td>
<td>2,3,4,5-TeCBP, Aroclor 1260, HCB</td>
<td>double-flanked chlorines</td>
<td>Wu et al., 2002a, b; May et al., 2008</td>
</tr>
</tbody>
</table>

MCP, monochlorophenol; MBP, monobromophenol; DCP, dichlorophenol; CBA, chlorobenzene; BBA, bromobenzene; TCBA, trichlorobenzene; PBDEs, polychlorinated dibenzo-p-dioxins; 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, tetrachlorohydroquinone; TBP, tribromophenol; 3-Cl-4-OHBA, 3-chloro-4-hydroxybenzoate; bromoxynil, 3,5-dibromo-4-hydroxybenzonitrile; ioxynil, 3,5-diiodo-4-hydroxybenzonitrile; TeCP, tetrachlorophenol; TeCDD, tetrachlorodibenzo-p-dioxin; TeCBP, pentachlorobenzyphenyl; TeCNP, tetrachloronaphthalene; TeCDF, tetrachlorodibenzo furan; 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 respires 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 AAAW00000000).

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
Biodiversity of Dechlorinating Bacteria

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-trichlorobenzen 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-trichlororobenzo-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>
<thead>
<tr>
<th>Isolation/consortium</th>
<th>Dehalogenation activities</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehalobacter restrictus strain PER-K23</td>
<td>PCE $\rightarrow$ cis-DCE</td>
<td>Holliger et al., 1998</td>
</tr>
<tr>
<td>Dehalobacter restrictus strain TEA</td>
<td>PCE $\rightarrow$ cis-DCE</td>
<td>Robbeck et al., 2008</td>
</tr>
<tr>
<td>Dehalobacter restrictus strain TCA1</td>
<td>1,1,2-TCA $\rightarrow$ VC</td>
<td>Wild et al., 1996</td>
</tr>
<tr>
<td>Dehalobacter sp.</td>
<td>1,2-DCA $\rightarrow$ 1,1-DCA and MCA</td>
<td>Sun et al., 2002</td>
</tr>
<tr>
<td>co-culture of Dehalobacter sp. and Sedimentibacter sp.</td>
<td>β-HCH $\rightarrow$ MCB, benzene</td>
<td>van Doebrug et al., 2005</td>
</tr>
<tr>
<td>co-culture of Dehalobacter sp. and Acetobacterium sp.</td>
<td>1,2-DCA $\rightarrow$ ethene</td>
<td>Groenewald and Edwards, 2009</td>
</tr>
<tr>
<td>enrichment culture containing Dehalobacter sp. and Acetobacterium sp.</td>
<td>2,3,4,5-TeCB $\rightarrow$ 2-MCB, 4-MCB</td>
<td>Yan et al., 2006</td>
</tr>
<tr>
<td></td>
<td>2-MCB, 4-MCB</td>
<td>Groenewald and Edwards, 2009</td>
</tr>
<tr>
<td></td>
<td>2,3,4-TriCB $\rightarrow$ 2-MCB, 4-MCB</td>
<td>Yoshida et al., 2006a</td>
</tr>
<tr>
<td></td>
<td>2-MCB, 4-MCB</td>
<td>Yoshida et al., 2006b</td>
</tr>
<tr>
<td></td>
<td>1,2-DCA $\rightarrow$ 1,1-DCBs and 2,3-DCCD</td>
<td>Grostern and Edwards, 2009</td>
</tr>
<tr>
<td></td>
<td>1,2-DCA $\rightarrow$ 1,1-DCBs</td>
<td>Yoshida et al., 2006b</td>
</tr>
<tr>
<td></td>
<td>2,3,4,5-TeCB $\rightarrow$ 2-MCB, 4-MCB</td>
<td>Grostern and Edwards, 2009</td>
</tr>
<tr>
<td></td>
<td>2,4,6-TCP $\rightarrow$ 4-MCP</td>
<td>Grostern and Edwards, 2009</td>
</tr>
<tr>
<td></td>
<td>2-MCB, 4-MCB</td>
<td>Yoshida et al., 2006a</td>
</tr>
<tr>
<td></td>
<td>2,3,4,5-TeCB $\rightarrow$ 2-MCB, 4-MCB</td>
<td>Grostern and Edwards, 2009</td>
</tr>
<tr>
<td></td>
<td>2,4,6-TCP $\rightarrow$ 4-MCP</td>
<td>Grostern and Edwards, 2009</td>
</tr>
<tr>
<td></td>
<td>2-MCB, 4-MCB</td>
<td>Yoshida et al., 2006b</td>
</tr>
</tbody>
</table>

Table 2. The potential dechlorination spectrum of Dehalobacter spp.
Interestingly, these works revealed the presence 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 an 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 metabolite synthesized by fungi, via bacterial dehalorespiration (Mülliken *et al*., 2004a, b); widespread distribution of reductive dehalogenase and the dechlorination 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.

**Acknowledgments**—This work was partly supported by the Environmental Technology Development Fund of the Ministry of the Environment; a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (B2:17310045); the New Energy and Industrial Technology Development Organization.

**REFERENCES**


member Desulfitobacterium frappieri strain TCP-A. Arch. Microbiol., 175, 133–142.
Luijten, M., J. de Weert, H. Smidt, H. T. S. Boschker, W. M. de Vos et al. (2003): Description of Sulfurospirillum halorespians sp. nov., an anaerobic, tetrachloroethene-respiring bacterium,


Sun, B. L., J. R. Cole, R. A. Sanford and J. M. Tiedje (2000): Isolation and characterization of


N. Yoshida (e-mail: ysd75@kais.kyoto-u.ac.jp)