Chapter 9
The Genus *Desulfitobacterium*

Taiki Futagami and Kensuke Furukawa

Abstract To date, 22 *Desulfitobacterium* strains have been isolated. From them a total of six distinct species have been proposed: *D. hafniense*, *D. dehalogenans*, *D. chlororespirans*, *D. metallireducens*, *D. dichloroeliminans*, and *D. aromaticivorans*. The isolated strains are strictly anaerobic, mesophilic, and grow in the neutral pH range. The cells are slightly curved rods ranging from 2 to 7 μm in length and 0.3 to 1 μm in width. Most of the *Desulfitobacterium* strains have been isolated as organohalide-respiring bacteria (OHRB) and show versatile dehalogenation of both chlorinated aliphatic and aromatic compounds such as chloroethenes and chlorophenols. The *Desulfitobacterium* strains are phylogenetically classified into the phylum *Firmicutes* (Gram-positive bacteria). The closest related OHRB genus of *Desulfitobacterium* is *Dehalobacter*, the members of which are strict OHRB within the phylum *Firmicutes* (see Chap. 8). In contrast, the *Desulfitobacterium* strains isolated to date are not strict OHRB. In addition to the ability to respire with organohalides, most isolates can grow fermentatively on pyruvate and can utilize a variety of electron acceptors, including sulfite, thiosulfate, fumarate, Fe(III), and Mn(IV). Complete genome information is available for four *Desulfitobacterium* strains and draft information is available for five strains. The complete genomes range from 3.62 to 5.73 Mbp, with GC content ranging from 44.2 to 47.5 % and the number of predicted coding sequences ranging from 3340 to 5060. Consistent with their physiological diversity, the *Desulfitobacterium* genome has been shown to encode a variety of respiratory reductases, including reductive dehalogenases.

T. Futagami (✉)
Education and Research Center for Fermentation Studies, Faculty of Agriculture, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan
e-mail: futagami@chem.agri.kagoshima-u.ac.jp

K. Furukawa
Faculty of Food Science and Nutrition, Department of Food and Bioscience, Beppu University, Kitaishigaki 82, Beppu, Oita 874-8501, Japan
e-mail: kfurukaw@nm.beppu-u.ac.jp

L. Adrian and F.E. Löffler (eds.), *Organohalide-Respiring Bacteria*,
DOI 10.1007/978-3-662-49875-0_9

Abbreviations Used in Text

AQDS	Anthraquinone-2,6-disulfonate
3-Cl-4-OHPA	3-chloro-4-hydroxyphenylacetic acid
DCA	Dichloroethane
DCP	Dichlorophenol
DMSO	Dimethyl sulfoxide
OHRB	Organohalide-respiring bacteria
PCE	Tetrachloroethene
PCP	Pentachlorophenol
RDase	Reductive dehalogenase
TCE	Trichloroethene
TCP	Trichlorophenol

Abbreviations Used in Tables

AQDS	Anthraquinone-2,6-disulfonate
BP	Bromophenol
bromoxynil	3,5-dibromo-4-hydroxybenzonitrile
CD	Carbon dichloride
CF	Chloroform
3-Cl-4-OHPA	3-chloro-4-hydroxyphenylacetic acid
CP	Chlorophenol
CT	Carbon tetrachloride
Cysteate	Alanine-3-sulfonate
DBP	Dibromophenol
DCA	Dichloroethane
DCHQ	Dichlorohydroquinone
DCP	Dichlorophenol
HCB	Hexachlorobenzene
Ioxynil	3,5-diiodo-4-hydroxybenzonitrile
Isethionate	2-hydroxyethanesulfonate
PCE	Tetrachloroethene
PCP	Pentachlorophenol
TCA	Trichloroethane
TCE	Trichloroethene
TCHQ	2,3,5,6-tetrachlorohydroquinone
TCMP	2,3,5,6-tetrachloro-4-methoxyphenol
TCP	Trichlorophenol
TeCP	Tetrachlorophenol
VC	Vinyl chloride

9.1 Discovery

The *Desulfitobacterium* strains have been isolated primarily as organohalide-respiring bacteria (OHRB) from organohalide-contaminated environments. The first *Desulfitobacterium* isolate was strain DCB-2 (for [aryl-] dechlorinating bacterium no. 2, currently known as *Desulfitobacterium hafniense* DCB-2) (Madsen and Licht 1992). Strain DCB-2 was isolated as a chlorinated phenol-degrading bacterium from a stable trichlorophenol (TCP)-dechlorinating consortium enriched from municipal digestor sludge in Copenhagen, Denmark. At that time, strain DCB-2 was reported to dehalogenate chlorophenols at the *ortho* and *meta* positions. This activity differed from that reported for *Desulfomonile tiedjei* DCB-1, which was the first isolate of the OHRB, dechlorinating only from the *meta* position (Shelton and Tiedje 1984; DeWeerd et al. 1990). Strain DCB-2 was later designated *D. hafniense* DCB-2 (Christiansen and Ahring 1996a). The species name *hafniense* reflects that Copenhagen was the place of isolation.

The nomenclature of the genus *Desulfitobacterium* was defined upon isolation of the second strain, *D. dehalogenans* JW/IU-DC1, in Athens, Georgia, USA (Utkin et al. 1994). The strain JW/IU-DC1 can dechlorinate at the *ortho* position of both chlorophenols and chlorophenylacetates (Utkin et al. 1994, 1995) and was the first *Desulfitobacterium* strain for which energy conservation through reductive dechlorination of 3-chloro-4-hydroxyphenylacetic acid (3-Cl-4-OHPA) was shown (Mackiewicz and Wiegel 1998). The genus name *Desulfitobacterium* describes a rod-shaped bacterium that reduces sulfite. However, several *Desulfitobacterium* isolates that do not reduce sulfite have been reported, including *D. metallireducens* 853-15A and *Desulfitobacterium* sp. PR (Finneran et al. 2002; Ding et al. 2014). A total of 22 *Desulfitobacterium* strains exhibiting a wide dehalogenation spectrum have been isolated and characterized to date.

9.2 Isolation and Habitat

Desulfitobacterium strains are distributed worldwide, having been isolated from Denmark, the United States, Vietnam, The Netherlands, Germany, Canada, Japan, Finland, and Poland (Table 9.1). Most *Desulfitobacterium* strains have been isolated during the course of studies of reductive dechlorination processes. Therefore, the target organochlorines in each of these studies were generally used as the electron acceptors in enrichment cultures. A total of 15 *Desulfitobacterium* strains (*D. hafniense* strains DCB-2, TCE1, TCP-A, PCE-S, PCP-1, Y51; *D. dehalogenans* JW/IU-DC1; *D. chlororespirans* Co23; *D. dichloroeliminans* DCA1; and *Desulfitobacterium*. sp. strains Viet1, KBC1, PCE1, B31e3, JH1, PR) have been isolated from enrichment cultures containing the organochlorines tetrachloroethene (PCE), trichloroethene (TCE), 2,4,6-TCP, 2,3-dichlorophenol (2,3-DCP), pentachlorophenol (PCP), 3-Cl-4-OHPA, 1,2-dichloroethane (1,2-DCA), or 1,1,1-trichloroethane (Suyama et al. 2001; Gerritse et al. 1999; Breitenstein et al. 2001;

Table 9.1 *Desulfitobacterium* strains in order of the year they were first reported

Organism	Stock center no.	Source of isolation	Geographic origin	Reference
D. hafniense DCB-2^T	DSM 10664	Municipal digestor sludge	Copenhagen, Denmark	Madsen and Licht (1992)
D. dehalogenans JW/IU-DC1^T	DSM 9161, ATCC 51507	A freshwater sediment collected from a pond of a wooded area	The Sandy Creek Nature Park, Athens, Georgia, USA	Utkin et al. (1994)
Desulfitobacterium sp. PCE1	DSM 10344	Soil contaminated with chloroethene	The Netherlands	Gerritse et al. (1995, 1996)
D. chlororespirans Co23^T	ATCC 700175, DSM 11544	Compost soil	Lansing, Michigan, USA	Sanford et al. (1996)
D. hafniense PCP-1	ATCC 700357, DSM 12420	A mixture of anaerobic sewage sludge and soil samples that had been contaminated with PCP	Quebec, Canada	Bouchard et al. (1996)
D. hafniense PCE-S	DSM 14645	Soil contaminated with chloroethenes	Eppelheim, Germany	Miller et al. (1997), Goris et al. (2015)
Desulfitobacterium sp. Viet1	NA	Parfume River sediment	Huế, Vietnam	Löffler et al. (1997)
D. dehalogenans XZ-1	ATCC 700041	A freshwater	Athens, Georgia, USA	Wiegel et al. (1999), ATCC website
D. hafniense TCE1	DSM 12704[a]	Soil obtained from a chloroethene-polluted location	Breda, The Netherlands	Gerritse et al. (1999)
D. hafniense TCP-A	DSM 13557	Sediment of the river Saale	Germany	Breitenstein et al. (2001)
D. hafniense DP7	DSM 13498	Fresh fecal sample of a healthy 28-year-old female person	The Netherlands	van de Pas et al. (2001b)
D. hafniense Y51	NBRC 109954	Soil contaminated with PCE	Fukuoka, Japan	Suyama et al. (2001)
D. hafniense GBFH	NA	Arsenic-contaminated sediments	Coeur d'Alene River delta, Lake Coeur d'Alene, Idaho, USA	Niggemyer et al. (2001)
D. metallireducens 853-15A^T	ATCC BAA-636, DSM 15288	Uranium-contaminated anaerobic aquifer sediment	Floodplain of the San Juan River, Shiprock, NM, USA	Finneran et al. (2002)

(continued)

Table 9.1 (continued)

Organism	Stock center no.	Source of isolation	Geographic origin	Reference
D. hafniense G2	DSM-16228	Subsurface smectite bedding of the Twiggs Clay formation of late Eocene age	Georgia, USA	Shelobolina et al. (2003)
D. dichloroeliminans DCA1^T	BCCM/LMG P-21439	Soil matrix of an anoxic water-saturated layer (1 m in depth) that had been exclusively polluted with 50 mg/kg 1,2-DCA for 30 years	NR	De Wildeman et al. (2003)
Desulfitobacterium sp. RPf35Ei	NA	Methanogenic granular sludge and sediments	Outokumpu's Pyhäsalmi mine, Finland	Kaksonen et al. (2004)
Desulfitobacterium sp. KBC1	NA	Soil sample from crop field	Ibaraki, Japan	Tsukagoshi et al. (2006)
Desulfitobacterium sp. B31e3	NA	Unsaturated subsurface soils contaminated with chloroethene	NR	Yoshida et al. (2007)
D. hafniense JH1	NA	Ditch sludge (mixed with sewage) contaminated with PCE and halogenated aliphatic compounds	Gifu, Japan	Chang et al. (2000), Fletcher et al. (2008)
D. aromaticivorans UKTL^T	DSM 19510, JCM 15765	Soil of a former coal gasification site	Gliwice, Poland	Kunapuli et al. (2010)
Desulfitobacterium sp. PR	NA	An anaerobic mixed culture enriched from a bioreactor maintained to perform dechlorination of chloroethenes and chloroethanes	NR	Ding et al. (2014)

[a] Currently not available. Type strains are indicated by a superscript capital T. *NA* not available; *NR* not reported; *PCP* pentachlorophenol; *PCE* tetrachloroethene, *DCA* dichloroethane

Miller et al. 1997; Bouchard et al. 1996; Madsen and Licht 1992; Sanford et al. 1996; Löffler et al. 1997; Tsukagoshi et al. 2006; Utkin et al. 1994; Gerritse et al. 1996; De Wildeman et al. 2003; Yoshida et al. 2007; Fletcher et al. 2008; Ding et al. 2014). In contrast, six *Desulfitobacterium* strains (*D. hafniense* strains G2, DP7, and GBFH; *Desulfitobacterium* sp. RPf35Ei; *D. metallireducens* 853-15A; and *D. aromaticivorans* UKTL) were isolated in the absence of organohalides (Shelobolina et al. 2003; van de Pas et al. 2001b; Niggemyer et al. 2001; Kaksonen et al. 2004; Finneran et al. 2002; Kunapuli et al. 2010).

Desulfitobacterium strains have been isolated from a variety of anaerobic environments, including digestor sludge, sewage sludge, ditch sludge mixed with sewage, methanogenic granular sludge, freshwater sediment, river sediment, arsenic-contaminated sediments, uranium mill tailing site floodplain, active mines, compost soil, soil samples from crop fields, soil from a former coal gasification site, and human feces (Table 9.1). Studies indicate that *Desulfitobacterium* spp. are widely distributed in anaerobic terrestrial environments, but not in marine environments. Molecular ecological studies have confirmed the ubiquitous distribution of *Desulfitobacterium* spp. For example, in one study, *Desulfitobacterium* spp. were detected in 31 of 48 soil samples from Canada (mainly from Quebec), which included 24 samples from contaminated industrial sites (Lanthier et al. 2001). In addition, *Desulfitobacterium* spp. as well as other OHRB have been detected with a high frequency in samples from chloroethenes-contaminated sites (Davis et al. 2002; Yang et al. 2005; Yoshida et al. 2007; Dowideit et al. 2010; Rouzeau-Szynalski et al. 2011; Kranzioch et al. 2013). Thus, the *Desulfitobacterium* spp. are one of the most abundant OHRB in the natural environment.

Methods involving serial dilution or single colony isolation on agar plates or in agar shake cultures have been used to isolate *Desulfitobacterium* strains. In addition, flow cytometric sorting was utilized in the isolation of *Desulfitobacterium* sp. KBC1 (Tsukagoshi et al. 2006). Anaerobic media containing target organohalides in combination with an electron donor such as pyruvate works well when attempting to isolate organohalide-respiring *Desulfitobacterium* strains. Yeast extract can be used as a source of vitamins and trace elements when culturing *Desulfitobacterium* strains. Antimicrobial agents may also be useful for *Desulfitobacterium* enrichment, as evidenced by the isolation of *D. hafniense* DP7 using the antibacterial agent aztreonam (50 μg/L), which is a β-lactam compound that inhibits the growth of aerobic Gram-negative bacteria (van de Pas et al. 2001b). Sequence analyses identified a vancomycin resistance gene cluster in the genome of *D. hafniense* Y51 (Kalan et al. 2009; Kruse et al. 2014a). Thus, exploiting vancomycin resistance might also be useful for the enrichment and isolation of *Desulfitobacterium* strains. Pasteurization is also useful for the isolation of spore-forming *Desulfitobacterium* strains (see Sect. 9.3).

The *Desulfitobacterium* strains can be preserved as frozen or freeze-dried stocks (Spring and Rosenzweig 2006). For long-term storage, anaerobic 50 % glycerol stocks are kept at −80 °C or in liquid nitrogen. Glycerol stocks remain viable for at least 5 years when stored at −80 °C (authors' unpublished data). The procedures used for manipulating the stocks under anaerobic conditions are

important for the long-term preservation of *Desulfitobacterium* strains. Although freeze-drying is also suitable for long-term storage, several *Desulfitobacterium* strains are sensitive to the freeze-drying process and display low survival rates after lyophilization. For these strains, preparation of cell suspensions in freshly prepared medium supplemented with 5 % dimethyl sulfoxide (DMSO) as a cryoprotectant and subsequent storage in liquid nitrogen is recommended (Spring and Rosenzweig 2006).

9.3 Morphology, Physiology, and Growth Characteristics

Morphologically, the *Desulfitobacterium* strains appear as slightly curved rods (Fig. 9.1). The cells range from 2 to 7 μm in length and 0.3 to 1 μm in width (Table 9.2). Variation in cell length (exceeding 10 μm) has been reported for *D. dichloroeliminans* DCA1 (De Wildeman et al. 2003). In addition, a coupled cell structure was reported for *Desulfitobacterium* sp. PR (Ding et al. 2014), which may form filaments composed of up to 10 cells. Most *Desulfitobacterium* isolates are motile and have from 1 to 6 flagella. Nonmotile cells without flagella have been reported for *D. hafniense* PCP-1 (Bouchard et al. 1996). The motility characteristics of *D. hafniense* JH1 and *Desulfitobacterium* sp. PR have not been described, but these cells also do not have flagella (Fletcher et al. 2008; Ding et al. 2014).

The *Desulfitobacterium* strains are mesophilic and grow at neutral pH. The optimum growth temperature and pH are in the ranges of 25–40 °C and 6.5–7.8, respectively (Table 9.2). Ten *Desulfitobacterium* strains are capable of forming terminal endospores. The capability of forming spores is likely associated with ensuring survival during heat stress, as evidenced by the observation that *D. hafniense* strains DCB-2, PCP-1, and TCP-A survived pasteurization during the isolation process (Madsen and Licht 1992; Bouchard et al. 1996; Breitenstein et al. 2001); during the isolation of *D. hafniense* DCB-2, the dechlorinating enrichment consortium was incubated at 80 °C for 60 min (Madsen and Licht 1992). Other research has confirmed that *D. chlororespirans* Co23 and *D. hafniense* GBFH can survive heat treatment (Sanford et al. 1996; Niggemyer et al. 2001). On the other hand, the non-spore forming strains *Desulfitobacterium* sp. PCE1 and *D. metallireducens* 853-15A do not survive heat treatment (Gerritse et al. 1996; Finneran et al. 2002); incubation at 85 °C for 20 min irreversibly inhibits the growth of *Desulfitobacterium* sp. PCE1 (Gerritse et al. 1996).

Although the known *Desulfitobacterium* strains are strict anaerobes, they are slightly oxygen tolerant. For example, *D. dehalogenans* JW/IU-DC1 was shown to dechlorinate 3-Cl-4-OHPA under aerobic condition with 2 % air, indicating its tolerance for oxygen (Utkin et al. 1994). It has also been reported that *D. hafniense* strains DCB-2 and GBFH, *D. dichloroeliminans* DCA1, and *Desulfitobacterium* sp. strain PR could survive aerobic conditions for 24 h (Madsen and Licht 1992; Niggemyer et al. 2001; De Wildeman et al. 2003; Ding et al. 2014).

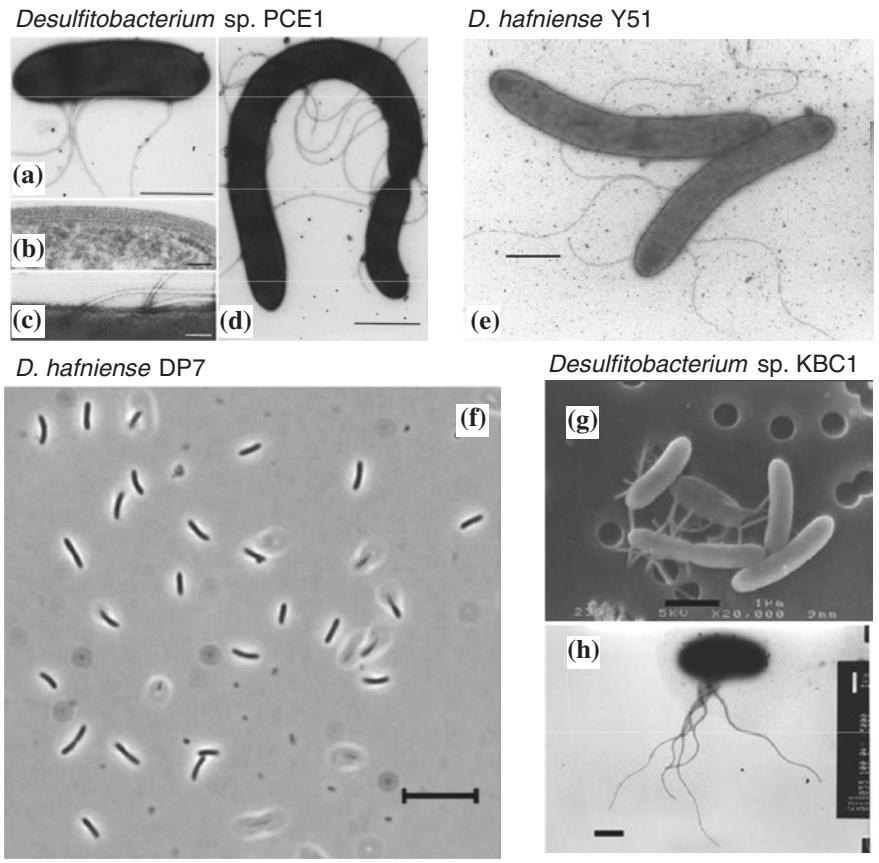

Fig. 9.1 Morphology of *Desulfitobacterium* strains. **a**–**d** Electron micrographs of negatively stained exponential-phase cells of *Desulfitobacterium* sp. PCE1. **a** Cell with four laterally attached flagella. **b** Ultrathin section revealing the thick Gram-positive cell wall. **c** S-layer surrounding the cell wall and **d** dividing long, curved cell of *Desulfitobacterium* sp. PCE1. Bars indicate 1 μm in (**a**) and (**d**) and 0.1 μm in (**b**) and (**c**). **e** Electron micrograph of negatively stained exponential-phase cells of *D. hafniense* Y51. Bar, 1 μm. **f** Phase contrast light micrograph of *D. hafniense* DP7. Bar, 10 μm. (**g**–**h**). **g** Scanning electron micrograph and **h** transmission electron micrograph of exponential-phase cells of *Desulfitobacterium* sp. KBC1. Bar, 1 μm. Photos were taken from Gerritse et al. (1996), van de Pas et al. (2001b), Furukawa et al. (2005), and Tsukagoshi et al. (2006) with permission

Characterization of the cellular fatty acid composition is frequently used in microbial taxonomy. The fatty acid composition has been reported for *D. dehalogenans* JW/IU-DC1, *D. hafniense* strains DCB-2 and PCP-1, *D. chlororespirans* Co23, *D. metallireducens* 853-15A, and *D. aromaticivorans* UKTL. Spring and Rosenzweig (2006) reported that the major fatty acids in *D. dehalogenans* JW/IU-DC1, *D. hafniense* strains DCB-2 and PCP-1, *D. chlororespirans* Co23, and *D. metallireducens* 853-15A are 14:0 (4.0–22.8 %), 16:1 *cis*9 (6.4–13.0 %),

Table 9.2 Physiological features of *Desulfitobacterium* strains

Organism	Cell size (long × wide μm)	Motility	Flagella	Sporulation	GC content (%)[a]	Optimum temperature	Optimum pH	Reference
D. hafniense DCB-2	3.3–6 × 0.6–0.7	+	+	+	47.5	37	ND	Madsen and Licht (1992), Christiansen and Ahring (1996a), Kim et al. (2012)
D. dehalogenans JW/IU-DC1	2.5–4 × 0.7	+	+	–	45	38	7.5	Utkin et al. (1994), van de Pas et al. (2001b), Kruse et al. (2014b)
Desulfitobacterium sp. PCE1	2–7 × 0.6–0.8	+	+	–	45	34–37	7.2	Gerritse et al. (1996)
D. chlororespirans Co23	3–5 × 0.5–1	+	ND	+	48.8	37	6.8–7.5	Sanford et al. (1996), van de Pas et al. (2001b)
D. hafniense PCP-1	2–4.5 × 0.7	–	–	+	47.5	38	7.5	Bouchard et al. (1996)
D. hafniense PCE-S	6 × 0.6	+	ND	+	47.3	37	ND	Miller et al. (1997), Goris et al. (2015)
Desulfitobacterium sp. Viet1	ND	ND	ND	ND	ND	ND	7.5	Löffler et al. (1997)
D. dehalogenans XZ-1	ND	ND	ND	ND	ND	ND	ND	Wiegel et al. (1999)
D. hafniense TCE1	2–4 × 0.6–0.8	+	+	–	47.5	35	7.2	Gerritse et al. (1997, 1999), van de Pas et al. (2001b)
D. hafniense TCP-A	2.5–5 × 0.6	+	ND	+	47.3	ND	ND	Breitenstein et al. (2001)
D. hafniense DP7	4–6 × 0.6	ND	+	–	47.6	34–40	7.2–7.4	van de Pas et al. (2001b)
D. hafniense Y51	5–7 × 0.8–1	+	+	–	47.4	37	6.5–7.5	Suyama et al. (2001), Nonaka et al. (2006)
D. hafniense GBFH	2–4 × 0.3–0.5	+	+	+	ND	37–38	7.5	Niggemyer et al. (2001)

(continued)

Table 9.2 (continued)

Organism	Cell size (long × wide μm)	Motility	Flagella	Sporulation	GC content (%)[a]	Optimum temperature	Optimum pH	Reference
D. metallireducens 853-15A	2–5 × 0.5	–	ND	–	41.8	30	7	Finneran et al. (2002)
D. hafniense G2	2–4 × 0.6–0.8	+	+	+	ND	ND	ND	Shelobolina et al. (2003)
D. dichloroeliminans DCA1	2–5 × 0.5–0.7	+	ND	ND	44.2	25–30	7.2–7.8	De Wildeman et al. (2003)
Desulfitobacterium sp. RPf35Ei	2.5–4 × 0.3–0.5	ND	ND	+	ND	35	ND	Kaksonen et al. (2004)
Desulfitobacterium sp. KBC1	2–3	+	+	ND	46	34	7.5	Tsukagoshi et al. (2006)
Desulfitobacterium sp. B31e3	2–3 × 0.4–0.5	+	ND	+		ND	7.0	Yoshida et al. (2007)
D. hafniense JH1	ND	ND	ND	–	ND	ND	ND	Chang et al. (2000), Fletcher et al. (2008)
D. aromaticivorans UKTL	2.3–4 × 0.5	+	ND	+	47.4	30	6.6–7.0	Kunapuli et al. (2010)
Desulfitobacterium sp. PR	2–5 × 0.3–0.35	–	ND	–	43.5	27–33	7.1-7.5	Ding et al. (2014)

[a]GC content of strains DCA1, PCP-1, PCE-S, TCP-A, DP7, 853-15A, and PCE1 were from their genome information. *ND* not determined

16:0 (4.4–25.6 %), and 18:1 *cis*11 (0.5–13.6 %). The fatty acid profiles of *D. hafniense* strains DCB-2 and PCP-1 are similar. In contrast, the fatty acid compositions of *D. dehalogenans* JW/IU-DC1 and *D. metallireducens* 853-15A differ. The characteristic fatty acids were identified as 16:0, 14:0, and 16:1 *cis*9 in *D. dehalogenans* JW/IU-DC1; 18:1 *cis*11 dimethylacetal, 18:1 *cis*1, and 16:1 *cis*9 in *D. hafniense* strains DCB-2 and PCP-1; 16:0, 16:1 *cis*9, and 14:0 in *D. chlororespirans* Co23; and 14:0, 16:1 *cis*9 dimethylacetal, and an unidentified fatty acid with an equivalent chain length of 13.52 in *D. metallireducens* 853-15A (Spring and Rosenzweig 2006). In addition, Kunapuli et al. (2010) investigated the fatty acid composition of *D. aromaticivorans* UKTL, with *D. chlororespirans* Co23 serving as a control, and found that strain UKTL contains 15:0 iso, but not 18:1ω7*c* fatty acids, in contrast to strain Co23. These data have to be used with a caution because fatty acid composition can change with factors such as medium composition and growth phase.

The cytochrome and quinone contents have been reported for several *Desulfitobacterium* strains. The *D. hafniense* strains DCB-2 and TCE1, *D. metallireducens* 853-15A, and *Desulfitobacterium* sp. PCE1 were shown to contain cytochrome c (Christiansen and Ahring 1996a; Gerritse et al. 1996, 1999; Finneran et al. 2002). Menaquinone-7 was identified as the primary menaquinone of *D. hafniense* TCP-A and *D. aromaticivorans* UKTL (Breitenstein et al. 2001; Kunapuli et al. 2010).

The *Desulfitobacterium* strains can also grow fermentatively; fermentative growth on pyruvate has been reported for most *Desulfitobacterium* isolates except for *D. metallireducens* 853-15A (Finneran et al. 2002) (Table 9.3). The fermentative growth yield of *D. dehalogenans* JW/IU-DC1 on pyruvate is approximately 14 g of dry cell weight per mole of pyruvate (van de Pas et al. 2001a). Tryptophan and serine also reportedly support the fermentative growth of *D. hafniense* DCB-2 and *D. hafniense* TCE1, respectively (Christiansen and Ahring 1996a; Gerritse et al. 1999).

Formate, lactate, and pyruvate generally serve as electron donors for *Desulfitobacterium* strains. In addition, most *Desulfitobacterium* strains exhibit *O*-demethylation activity. The *O*-demethylation was reported to be involved in the intermediary metabolism for methoxylated organochlorines such as tetrachloroguaiacol, tetrachloroveratrole, pentachloroanisole, and 3,5-dichloro-4-methoxyphenol in *D. hafniense* strains PCP-1 and DCB-2, *D. chlororespirans* Co23, and *D. dehalogenans* JW/IU-DC1 (Dennie et al. 1998; Milliken et al. 2004b). Then, the utilization of phenyl methyl ethers, vanillate and syringate, as electron donors via *O*-demethylation was reported for *D. hafniense* strains DCB-2, PCE-S, DP7, G2, PCP-1, TCP-A, and Y51, *D. chlororespirans* Co23, and *D. dehalogenans* JW/IU- 1 (Neumann et al. 2004; Mingo et al. 2014). Enzymes involved in *O*-demethylation have been biochemically characterized in *D. hafniense* strains DCB-2 and PCE-S (Kreher et al. 2008; Studenik et al. 2012). The methyl group from phenyl methyl ethers is transferred to tetrahydrofolate and considered to be further used as an electron donor via acetyl-CoA formation. Because phenyl methyl ethers are lignin decomposition products, *Desulfitobacterium* spp. are

Table 9.3 Primary metabolic features of *Desulfitobacterium* isolates

Organism	Electron donors							Electron acceptors														Reference
	Fermentatoin of pyruvate	Formate	Hydrogen	Lactate	Pyruvate	Vanillate	Syringate	Fumarate	Sulfate	Sulfur	Sulfite	Thiosulfate	AQDS	Isethionate	Cysteate	Nitrate	Fe(III)	As(V)	Se(VI)	Mn(IV)	U(VI)	
D. hafniense DCB-2	+	+	−	+	+	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	Madsen and Licht (1992), Christiansen and Ahring (1996a), Lie et al. (1999), Niggemyer et al. (2001), Neumann et al. (2004), Milliken and May (2007), Kim et al. (2012), Mingo et al. (2014)
D. dehalogenans JW/IU-DC1	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	−	+	+	+	Utkin et al. (1994), Lovley et al. (1998), Lie et al. (1999), Niggemyer et al. (2001), Cervantes et al. (2002), Luijten et al. (2004), Fletcher et al. (2010), Mingo et al. (2014)
Desulfitobacterium sp. PCE1	+	+	−	+	+	ND	ND	+	−	ND	+	+	+	+	+	+	−	−	+	−	+	Gerritse et al. 1996), Lie et al. (1999), Gerritse et al. (1999), Cervantes et al. (2002), Luijten et al. (2004), Fletcher et al. (2010)

(continued)

Table 9.3 (continued)

Organism	Fermentatoin of pyruvate	Electron donors						Electron acceptors														Reference
		Formate	Hydrogen	Lactate	Pyruvate	Vanillate	Syringate	Fumarate	Sulfate	Sulfur	Sulfite	Thiosulfate	AQDS	Isethionate	Cysteate	Nitrate	Fe(III)	As(V)	Se(VI)	Mn(IV)	U(VI)	
D. chlororespirans Co23	+	+	+	+	+	+	+	−	−	+	+	+	+	−	−	−	+	−	+	+	+	Sanford et al. (1996), Lie et al. (1999), Niggemyer et al. (2001), Luijten et al. (2004), Fletcher et al. (2010), Mingo et al. (2014)
D. hafniense PCP-1	+	+	−	+	+	+	+	+	−	+	+	+	ND	ND	ND	+	+	+	+	+	ND	Bouchard et al. (1996), Niggemyer et al. (2001), Mingo et al. (2014)
D. hafniense PCE-S	+	+	ND	ND	+	+	+	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Miller et al. (1997), Neumann et al. (2004), Ye et al. (2010), Mingo et al. (2014)
Desulfitobacterium sp. Viet1	+	ND	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	−	−	ND	ND	ND	ND	ND	+	Löffler et al. (1997), Lie et al. (1999), Fletcher et al. (2010)
D. dehalogenans XZ-1	ND	ND	ND	ND	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Wiegel et al. (1999)
D. hafniense TCE1	+	+	+	+	+	ND	ND	+	−	ND	+	+	ND	ND	ND	+	+	+	+	+	ND	Gerritse et al. (1999), Luijten et al. (2004)
D. hafniense TCP-A	+	+	+	+	+	+	+	+	−	ND	+	+	ND	ND	ND	+	ND	ND	ND	ND	ND	Breitenstein et al. (2001), Mingo et al. (2014)
D. hafniense DP7	+	+	+	+	+	+	+	+	−	ND	+	+	+	ND	ND	+	+	−		+	ND	van de Pas et al. (2001b), Luijten et al. (2004), Mingo et al. (2014)

(continued)

Table 9.3 (continued)

Organism	Fermentatoin of pyruvate	Electron donors						Electron acceptors														Reference
		Formate	Hydrogen	Lactate	Pyruvate	Vanillate	Syringate	Fumarate	Sulfate	Sulfur	Sulfite	Thiosulfate	AQDS	Isethionate	Cysteate	Nitrate	Fe(III)	As(V)	Se(VI)	Mn(IV)	U(VI)	
D. hafniense Y51	ND	+	ND	+	+	+	+	+	+	ND	+	ND	ND	ND	ND	+	ND	ND	ND	ND	ND	Suyama et al. (2001), Peng et al. (2012), Mingo et al. (2014)
D. hafniense GBFH	+	+	−	+	+	ND	ND	+	−	+	+	+	ND	ND	ND	−	+	+	+	+	ND	Niggemyer et al. (2001)
D. metallireducens 853-15A	−	+	−	+	+	−	−	−	−	+	−	+	+	ND	ND	−	+	ND	−	+	ND	Finneran et al. (2002), Mingo et al. (2014)
D. hafniense G2	+	+	+	+	+	+	+	+	−	−	+	+	+	ND	ND	+	+	ND	ND	−	+	Shelobolina et al. (2003), Mingo et al. (2014)
D. dichloroeliminans DCA1	ND	+	+	+	ND	ND	ND	−	−	ND	+	+	ND	ND	ND	+	ND	ND	ND	ND	ND	De Wildeman et al. (2003)
Desulfitobacterium sp. RPf35Ei	ND	ND	ND	+	ND	ND	ND	ND	−	ND	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Kaksonen et al. (2004)
Desulfitobacterium sp. KBC1	+	+	−	+	+	ND	ND	+	−	ND	+	+	ND	ND	ND	−	ND	ND	ND	ND	ND	Tsukagoshi et al. (2006)
Desulfitobacterium sp. B31e3	ND	ND	+	+	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Yoshida et al. (2007)
D. hafniense JH1	+	+	+	ND	+	ND	ND	ND	−	ND	+	ND	ND	ND	ND	+	+	ND	ND	ND	+	Fletcher et al. (2008, 2010)
D. aromaticivorans UKTL	+	+	−	−	+	ND	ND	−	−	−	+	+	ND	ND	ND	−	+	ND	ND	−	ND	Kunapuli et al. (2010)
Desulfitobacterium sp. PR	+	+	+	−	+	ND	ND	ND	−	ND	−	−	ND	ND	ND	−	ND	ND	ND	ND	ND	Ding et al. (2014)

AQDS anthraquinone-2,6-disulfonate; *Cysteate* alanine-3-sulfonate; *Isethionate* 2-hydroxyethanesulfonate; *ND* not determined

thought to be involved in biological lignin transformation. *D. hafniense* strains DCB-2 and PCE-S grown on fumarate and vanillate or syringate yield approximately 15 g of dry cell weight per mole of methyl moiety converted (Neumann et al. 2004).

Compounds capable of supporting the growth of *Desulfitobacterium* strains as electron acceptor include sulfite, thiosulfate, fumarate, nitrate, anthraquinone-2,6-disulfonate (AQDS, a humic acid analog), isethionate (2-hydroxyethanesulfonate), and cysteate (adenine-3-sulfonate). *Desulfitobacterium* strains generally cannot use sulfate and nitrite as electron acceptors with the exception that *D. hafniense* Y51 reduces sulfate (Suyama et al. 2001). Inorganic metals such as Fe(III), Mn(IV), Se(VI), As(V), and U(VI) can also be used as electron acceptors by *Desulfitobacterium* spp., and strains GBFH, G2, 853-15A, and UKTL have been isolated as metal reducers without demonstrable organohalide respiration. *D. hafniense* GBFH was isolated as an As(V)-reducing bacterium from arsenic-contaminated sediments (Niggemyer et al. 2001). *D. hafniense* G2 can utilize U(VI), Fe(III) and AQDS as electron acceptors (Shelobolina et al. 2003). *D. metallireducens* 853-15A can utilize AQDS, chelated Fe(III) (not crystalline Fe[III] oxide), humic acids, Mn(IV), colloidal sulfur, Se(IV), and Cr(VI) as electron acceptors (Finneran et al. 2002). *D. aromaticivorans* UKTL was isolated as an iron-reducing bacterium capable of anaerobic degradation of monoaromatic hydrocarbons, including toluene, phenol, and *p*-cresol (Kunapuli et al. 2010). The *Desulfitobacterium* strains initially isolated as OHRB also have the potential to reduce a variety of electron acceptors including metals (Table 9.3). Thus, the *Desulfitobacterium* spp. play important roles in the natural cycles of a variety of compounds other than organohalides.

9.4 Phylogeny

The genus *Desulfitobacterium* belongs to the phylum *Firmicutes*, class *Clostridia*, order *Clostridiales*, and family *Peptococcaceae* (Lupa and Wiegel 2009). Members of the family *Peptococcaceae* are anaerobes, and the *Desulfitobacterium* spp. which are strictly anaerobic. However, in contradiction to the name *Peptococcaceae*, the known *Desulfitobacterium* strains are not cocci, but curved rods throughout all growth phases, similar to some other *Peptococcaceae* genera (e.g., *Dehalobacter*; see Chap. 8) (Fig. 9.1). This apparent contradiction derives from the fact that the family *Peptococcaceae* was composed of three genera of cocci (*Peptococcus*, *Peptostreptococcus*, and *Ruminococcus*) when it was proposed (Rogosa 1971). As the phylum *Firmicutes* is composed of Gram-positive bacteria, the *Desulfitobacterium* spp. are recognized as Gram positive. However, Gram stain analyses determined that five isolates, including *D. hafniense* strains DCB-2, PCP-1, and Y51, *D. chlororespirans* Co23, and *Desulfitobacterium* sp. B31e3, are actually staining Gram negative (Bouchard et al. 1996; Christiansen and Ahring 1996a; Sanford et al. 1996; Suyama et al. 2001) (Table 9.2). In the

case of *D. hafniense* PCP-1, the cells stain Gram negative, but observation of ultrathin cross section of a strain PCP-1 by electron microscopic analysis indicated that this strain is Gram positive. The thick Gram-positive cell wall structure is also evident in ultrathin sections of *Desulfitobacterium* sp. PCE1 (Fig. 9.1b). Thus, it appears that staining is insufficient for determining whether *Desulfitobacterium* strains are Gram positive or Gram negative. On the other hand, the cell envelope

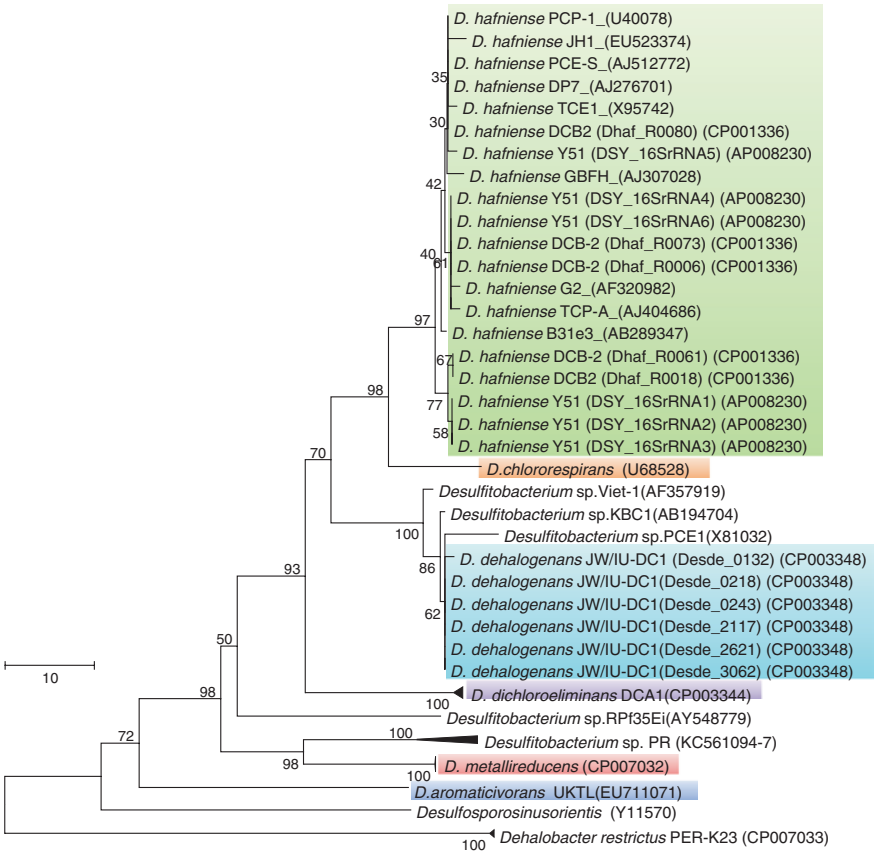

Fig. 9.2 Phylogenetic relationships of *Desulfitobacterium* isolates based on 16S rRNA gene sequences. Sequence accession numbers are indicated in parentheses. Branch supporting values (%) were evaluated with 1000 bootstrap replications. The tree was constructed using the neighbor-joining method based on an alignment of almost-complete 16S rRNA gene sequences. The 100- to 200-bp insertion sequence harboring the 5′ end (Villemur et al. 2007) was excluded using the complete gap deletion option in MEGA version 6 software (Tamura et al. 2013). *Desulfosporosinus orientis* and *Dehalobacter restrictus* PER-K23 were used as outgroups. Multiple copies of 16S rRNA gene sequences of *D. hafniense* strains Y51 and DCB-2, *D. dehalogenans* JW/IU-DC-1, *D. dichloroeliminans* DCA1, *D. metallireducens* 853-15A, and *Dehalobacter restrictus* PER-K23 were identified based on genome information. In those cases, locus tags are indicated in the parentheses. Different species are highlighted in different colors. The scale bar represents 10 % estimated sequence divergence

architecture can give a clearer definition than the staining properties (Sutcliffe 2010). From this point of view, the *Desulfitobacterium* spp. should be recognized as monoderm that have an envelope with one membrane.

The phylogenetic relationships of *Desulfitobacterium* isolates based on 16S rRNA gene sequences are shown in Fig. 9.2. The *Desulfitobacterium* isolates are classified into six species: *D. hafniense*, *D. dehalogenans*, *D. dichloroeliminans*, *D. chlororespirans*, *D. metallireducens*, and *D. aromaticivorans*. Currently, no species name has been assigned to six strains: Viet1, PCE1, RPf35Ei, KBC1, B31e3, and PR. The *D. hafniense* strains PCP-1, DP7, TCP-A, G2, and TCE1 were previously classified as *D. frappieri*, but the species name *frappieri* is no longer used based on detailed 16S rRNA gene analyses. As a result, *D. frappieri* has been reclassified as *D. hafniense*. This confusion can be attributed to the size of the 16S rRNA gene in *D. hafniense* PCP-1 (formerly *D. frappieri* PCP-1) (Bouchard et al. 1996, reviewed in Villemur et al. 2006). The 16S rRNA gene of strain PCP-1 is 1655-bp long, whereas that of *D. hafniense* DCB-2 is 1530-bp long. It is now known that the *Desulfitobacterium* genome encodes multiple 16S rRNA genes and shows intra-genomic heterogeneity (Villemur et al. 2007). Denaturing gradient gel electrophoresis (DGGE) analysis indicated that 16S rRNA gene copy numbers in *Desulfitobacterium* strains vary from 2 to 7. Heterogeneity with respect to 16S rRNA gene copies is caused by 100- to 200-bp insertions in the 5′ region, the region that differs between strains PCP-1 and DCB-2. The 16S rRNA gene of strain PCP-1 has a 128-nt insertion that is absent in the 16S rRNA gene of strain DCB-2 (Bouchard et al. 1996). The insertions are predicted to form an energetically stable loop when they are transcribed. In addition, reverse transcriptase-PCR analyses have demonstrated that most of the observed insertions in the 16S rRNA gene of *Desulfitobacterium* strains are excised from the mature 16S rRNA transcripts. Although such insertion sequences are rarely observed in bacterial 16S rRNA genes, they are also found in several other bacterial genera, such as *Desulfotomaculum* and *Anaerospora* (Patel et al. 1992; Woo et al. 2005).

9.5 Reductive Dehalogenation Characteristics

The reductive dehalogenation activities of *Desulfitobacterium* isolates are summarized in Table 9.4. The reductive dehalogenation spectrum of the strains does not correlate with their phylogenetic relationships and seems to depend heavily upon the enzyme reductive dehalogenase (RDase), which is a terminal reductase in the organohalide respiratory pathway expressed in each *Desulfitobacterium* isolate (Hug et al. 2013).

The OHRB exhibits two types of reductive dehalogenation: hydrogenolysis and dihaloelimination (Mohn and Tiedje 1992; Holliger et al. 1998; Smidt and de Vos, 2004). In hydrogenolysis, the halogen substituents of alkyl or aryl halides are replaced with hydrogen atoms. In contrast, in dihaloelimination, two halogen substituents of alkyl halides are removed from adjacent carbon atoms. Both types of reductive dehalogenation have been observed in *Desulfitobacterium* strains.

Table 9.4 Dehalogenation activity of *Desulfitobacterium* strains

Organism	Dehalogenated	Not dehalogenated	Reference
D. hafniense DCB-2	PCE (weak activity), 3-Cl-4-OHPA, PCP, 2,3,4,5-TeCP, 2,4,5- and 2,4,6-TCPs, 2,3-, 2,4-, and 3,5-DCPs, TCMP, TCHQ	TCE, 3,4,5-TCP, 3,4-DCP, CPs	Madsen and Licht (1992), Christiansen and Ahring (1996a), Gerritse et al. (1999), Milliken et al. (2004b), Mac Nelly et al. (2014)
D. dehalogenans JW/IU-DC1	PCE (weak activity), 3-Cl-4-OHPA, PCP, TeCPs, 2,3,4-, 2,3,6-, and 2,4,6-TCPs, 2,3-, 2,4-, and 2,6-DCPs, TCMP, TCHQ, 2,6-dichloro-4-R-phenol (R: -H, -Cl, -F, -NO_2, -COOH, -$COOCH_3$), 2-chloro-4-R-phenol (R: -Cl, -F, -Br, -NO_2, -COOH, -$COOCH_3$, -CH_2COOH), 3,3″5,5″ tetrachloro-4,4″dihydroxybiphenyl congeners, 3,4″5-trichloro-4- hydroxy-biphenyl, 3,5-dichloro-4-hydroxybi-phenyl, 2,6-DBP, 2-BP, 2-bromo-4-CP, 2-bromo-4-methylphenol	TCE, 2,3,5-, 2,4,5-, and 3,4,5-TCPs, 3,4-, 3,5-, and 2,5-DCPs, CPs, 3-fluoro-4-hydroxyphenylacetate	Utkin et al. (1994, 1995), Wiegel et al. (1999), Gerritse et al. (1999), Milliken et al. (2004b)
Desulfitobacterium sp. PCE1	PCE, TCE (weak activity), 3-Cl-4-OHPA, 2,3,5,6-TeCP, 2,4,6-TCP, 2,4-DCP, 2-CP,TCMP, TCHQ, 2,3,5-trichlorohydroquinone	DCE, CF, CD, PCP, 2,3,5-TCP, HCB, 2,5- and 3,4-dichlorobenzoates, 2,3,6-trichlorobenzoate, trichloroac-etate, hexachloroethane, 4-chlorophe-nylacetate, 2,5-DCHQ, 2,6-DCHQ, 2-chloro-1,4-hydroquinone	Gerritse et al. (1996), Milliken et al. (2004a, b)
D. chlororespirans Co23	3-Cl-4-OHPA, 2,4,6-TCP, 2,3,- and 2,6-DCPs, TCMP, TCHQ, 3-chloro-4-hydroxy-benzoate, 3,5-dibromo-4-hydroxybenzoate, 2,4,6-tribromophenol, bromoxynil, ioxynil	PCE, PCP, 2,3,5-TCP, 2,4- and 2,5-DCPs, CPs, 3-chlorobenzo-ate, 3-chloro-L-tyrosine, 3-chloro-anisaldehyde, 2-BP, 2-iodophenol, 2-fluorophenol	Sanford et al. (1996), Milliken et al. (2004b), Cupples et al. (2005)

(continued)

Table 9.4 (continued)

Organism	Dehalogenated	Not dehalogenated	Reference
D. hafniense PCP-1	PCE, PCP, 2,3,4,5- and 2,3,5,6-TeCPs, 2,3,4-, 2,3,5-, 2,3,6-, 2,4,5-, 2,4,6-, and 3,4,5-TCPs, 2,4-, 2,6-, and 3,5-DCPs, TCMP, TCHQ, 3,5-dichloro-4-hydroxybiphenyl; polychlorinated catechol, guaiacol, veratrole, anisole, aniline, nitrobenzene and pyridine; 2,4,6-tribromophenol, 2,4-DBP	TCE, 3-Cl-4-OHPA, 2,3-, 2,5-, and 3,4-DCPs. CPs, 2,2″5″trichloro-4- hydroxybiphenyl, 3,5-dichloro-2-hydroxybiphenyl, 2″5″dichloro-2-hydroxybiphenyl, 2,3,5- and 2,4,6- trichlorobenzoates, 2,3,5- and 2,4,6-trifluorophenols	Bouchard et al. (1996), Dennie et al. (1998), Milliken et al. (2004b)
D. hafniense PCE-S	PCE, TCE, 2,3-dichloropropene, tribromoethene, *cis*- and *trans*- dibromoethenes, 2,3-dibromopropene	DCEs, 3-Cl-4-OHPA, PCP, 2,4,6-TCP	Miller et al. (1997), Ye et al. (2010)
Desulfitobacterium sp. Viet1	PCE, 2,4,6-TCP, 2,4-DCP	TCE, 2,3,5- and 2,4,5-TCPs, 2,5-, 2,6-, and 3,4-DCPs	Löffler et al. (1997), Tront et al. (2006)
D. dehalogenans XZ-1	3,3″5,5″tetrachloro-4,4″dihydroxybiphenyl congeners, 3,4″5-trichloro-4-hydroxybiphenyl, 3,5-dichloro-4-hydroxybiphenyl	ND	Wiegel et al. (1999)
D. hafniense TCE1	PCE, TCE, CT (weak activity), CF (weak activity)	*cis*- and *trans*-DCEs, CD, 3-Cl-4-OHPA, 2,4,6-TCP, 1,2-dichloropropane, TCMP, TCHQ	Gerritse et al. (1999), Milliken et al. (2004b)
D. hafniense TCP-A	PCE (weak activity), PCP, 2,3,4,5- and 2,3,5,6-TeCPs, 2,3,5- and 2,4,6-TCPs, 2,3-, and 2,4-, 3,5-DCPs, 2-CP (weak activity)	3-CP, 4-CP	Breitenstein et al. (2001)
D. hafniense DP7	ND	PCE, 3-Cl-4-OHPA, PCP, 2,3-, 2,4-, 2,5-, and 2,6-DCPs, CPs, TCMP, TCHQ	van de Pas et al. (2001b), Milliken et al. (2004b)
D. hafniense Y51	PCE, TCE, 1,1,1,2,2,3,3-heptachloropropane, hex- and pentachloroethanes, 1,1,1,2- and 1,1,2,2-tetrachloroethane	DCEs, hexachloro-1,3-butadiene, 1,1,1- and 1,1,2-TCAs, 1,1,2,3-tetrachloropropane, *o*-, *m*-, and *p*-chlorobenzoates, CF, CT, CD	Suyama et al. (2001), Futagami et al. (2006b)

(continued)

Table 9.4 (continued)

Organism	Dehalogenated	Not dehalogenated	Reference
D. hafniense GBFH	ND	3-Cl-4-OHPA	Niggemyer et al. (2001)
D. metallireducens 853-15A	PCE, TCE, 3-Cl-4-OHPA	ND	Finneran et al. (2002)
D. hafniense G2	PCE, TCE	ND	Shelobolina et al. (2003)
D. dichloroeliminans DCA1	1,2-DCA, 1,1,2-TCA, *meso* and DL stereoisomers of 2,3- dichlorobutane, 1,2-dichlorobutane, 1,2- dichloropropane	VC, hexa-, penta-, and tetrachloroethanes, chlorinated methanes, monochloroalkanes, nonvicinal dichloroalkanes	De Wildeman et al. (2003)
Desulfitobacterium sp. RPf35Ei	ND	ND	Kaksonen et al. (2004)
Desulfitobacterium sp. KBC1	PCE, 2,4,6-TCP, 2,4-DCP	TCE, CPs	Tsukagoshi et al. (2006)
Desulfitobacterium sp. B31e3	PCE, TCE	*cis*-DCE, PCP, 2,4,6-TCP, 2,4-DCP, CPs	Yoshida et al. (2007)
D. hafniense JH1	PCE, TCE	*cis*- and *trans*-DCEs, VC, 1,1,2-TCA 1,1- and 1,2-DCAs, CT, CF, CD, 1,2,3-trichloropropane, 1,2-dichloropropane, 2-chlorotoluene, HCB, 3-chloro-4-hydroxybenzoate	Fletcher et al. (2008)
D. aromaticivorans UKTL	ND	ND	Kunapuli et al. (2010)
Desulfitobacterium sp. PR	PCE, 1,1,1- and 1,1,2-TCA, CF	TCE, PCP, 2,4,6-TCP	Ding et al. (2014), Zhao et al. (2015)

PCE tetrachloroethene; *3-Cl-4-OHPA* 3-chloro-4-hydroxyphenylacetic acid; *PCP* pentachlorophenol; *TeCP* tetrachlorophenol; *TCP* trichlorophenol; *DCP* dichlorophenol; *TCMP* 2,3,5,6-tetrachloro-4-methoxyphenol; *TCHQ* 2,3,5,6-tetrachlorohydroquinone; *TCE* trichloroethene; *CP* chlorophenol; *DBP* dibromophenol; *BP* bromophenol; *CF* chloroform; *CD* carbon dichloride; *HCB* hexachlorobenzene; *DCHQ* dichlorohydroquinone; *bromoxynil* 3,5-dibromo-4-hydroxybenzonitrile; *ioxynil* 3,5-diiodo-4-hydroxybenzonitrile; *TCA* trichloroethane; *CT* carbon tetrachloride; *DCA* dichloroethane; *VC* vinyl chloride; *ND* not determined

Most *Desulfitobacterium* strains can dechlorinate chloroaliphatic and/or chloroaromatic compounds. In addition, several *Desulfitobacterium* strains were found to dehalogenate organobromine and organoiodine compounds. However, to date, no dehalogenation activity has been reported for four *Desulfitobacterium* strains: *D. hafniense* DP7 and GBFH, *Desulfitobacterium* sp. RPf35Ei, and *D. aromaticivorans* UKTL. Of these strains, *D. hafniense* DP7 was characterized as a non-dechlorinator. Strain DP7 was isolated from human feces (nonpolluted environment) and does not dechlorinate monochlorophenols, 2,3-, 2,4-, 2,5-, and 2,6-DCPs, PCP, PCE, 3-Cl-4-OHPA, 2,3,5,6-tetrachloro-4-methoxyphenol, or tetrachlorohydroquinone (van de Pas et al. 2001b; Milliken et al. 2004a).

9.5.1 Dehalogenation of Chloroaliphatic Compounds

As it is one of the most common environmental pollutants, bacterial dechlorination of chloroethenes has been widely studied. The *Desulfitobacterium* spp. identified to date do not dechlorinate DCE or vinyl chloride and can be classified into one of two categories: PCE to *cis*-DCE dechlorinators or PCE to TCE dechlorinators (Table 9.4). The former category includes *D. hafniense* strains TCE1, PCE-S, Y51, G2, JH1, *D. metallireducens* 853-15A, *Desulfitobacterium* sp. B31e3, *D. hafniense* DCB-2, and *D. dehalogenans* JW/IU-DC1, whereas the latter includes *D. hafniense* PCP-1 and *Desulfitobacterium* sp. strains Viet1, PCE1, and KBC1. In the case of strains DCB-2 and JW/IU-DC1, they dechlorinate PCE to TCE slowly via cometabolic processes, which do not provide energy for growth, when the cells are cultured in the presence of 3-Cl-4-OHPA as an inducer (Gerritse et al. 1999). In addition, *D. hafniense* TCP-A and *Desulfitobacterium* sp. PR also dechlorinate PCE to TCE slowly, but the reaction does not require inducers (Breitenstein et al. 2001; Ding et al. 2014).

The unique reaction utilized by *Desulfitobacterium* strains for the dechlorination of chloroaliphatic compounds is as follow. *D. hafniense* Y51 dechlorinates hexachloroethane, pentachloroethane, 1,1,1,2-tetrachloroethane, and 1,1,2,2-tetrachloroethane (Suyama et al. 2001). *D. dichloroeliminans* DCA1 can dechlorinate 1,1,2-trichloroethane, 1,2-DCA, *meso* and DL stereoisomers of 2,3-dichlorobutane, 1,2-dichlorobutane, and 1,2-dichloropropane (De Wildeman et al. 2003). Strain DCA1 dechlorinate these compounds via the dichloroelimination system. *Desulfitobacterium* sp. PR reductively dechlorinates both 1,1,1-trichloroethane and chloroform (Ding et al. 2014). Strain PR can dechlorinate 1,1,1-trichloroethane completely to monochloroethane and dechlorinates chloroform (trichloromethane) to predominantly carbon dichloride (dichloromethane) and trace amounts of monochloromethane. Efficient degradation of chloroform has been reported only in strain PR among the *Desulfitobacterium* strains described to date.

The PCE/TCE RDase PceA has thus far been purified and characterized in *D. hafniense* strains PCE-S, Y51, and TCE1 (Miller et al. 1998; Suyama et al. 2002; Maillard et al. 2003). The PceA-encoding gene is found in the conserved

pceA-pceB-pceC-pceT gene cluster in *D. hafniense* strains TCE1 and Y51. PceB is predicted to be a membrane anchor protein for PceA. The *pceT* gene encodes a trigger factor protein that binds to the twin arginine translocation (Tat) signal sequence of PceA (Morita et al. 2009; Maillard et al. 2011). The degradation gene cluster is sometimes located on a mobile element, and in fact, the *pce* gene cluster forms a catabolic transposon in *D. hafniense* strains TCE1 and Y51 (Maillard et al. 2005; Futagami et al. 2006a, b; Duret et al. 2012).

The activity of the PCE RDase PrdA of *Desulfitobacterium* sp. KBC1 is controlled at the transcriptional level (Tsukagoshi et al. 2006). Transcription of the *prdA* gene in strain KBC1 is induced by PCE. In contrast, the cultivation conditions reportedly affect PceA activity in *D. hafniense* Y51, without transcriptional regulation. Because the *pceA* gene is located on the transposable element in strain Y51, the activity of PceA decreases when cells are grown in the absence of an organochlorine substrate due to loss of the *pce* gene cluster through transposition (Reinhold et al. 2012). Vitamin B_{12} (a corrinoid) also reportedly affects the stability of the *pce* genes (Reinhold et al. 2012). Corrinoids play a significant role in regulating RDase catalytic activity as cofactors. Exogenous vitamin B_{12} hampers the transposition of the *pce* gene cluster, although the exogenous vitamin B_{12} does not appear to be incorporated into the PceA precursor. In addition, when strain Y51 is grown in the absence of an organochlorine substrate, the intracellular corrinoid level decreases and the PceA precursor forms catalytically inactive and corrinoid-free aggregates.

9.5.2 Dehalogenation of Chloroaromatic Compounds

The dechlorination of chlorophenols and 3-Cl-4-OHPA has also been well studied. Dechlorination of these chloroaromatic compounds has been reported in *D. hafniense* strains DCB-2 and TCP-A, *D. dehalogenans* strains JW/IU-DC1 and XZ-1, *Desulfitobacterium* sp. strains Viet1, PCE1, and KBC1, *D. chlororespirans* Co23, and *D. metallireducens* 853-15A (Table 9.4). The spectrum of haloaromatic compound dechlorination has been characterized most thoroughly in *D. hafniense* PCP-1 and *D. dehalogenans* JW/IU-DC1 (reviewed in Villemur 2013), e.g., *D. hafniense* PCP-1 can dechlorinate the *ortho*, *meta*, and *para* positions of chlorophenols, in this strain, PCP is converted to 3-chlorophenol via 2,3,4,5-tetrachlorophenol, 3,4,5-TCP, and 3,5-DCP (Bouchard et al. 1996). In strain PCP-1, *ortho*-dechlorination of PCP to 3,4,5-TCP proceeds rapidly, and after sufficient accumulation, 3,4,5-DCP is *meta*- and *para*-dechlorinated to 3,5-DCP and 3-chlorophenol. Thus, two different RDases are involved in PCP dechlorination in strain PCP-1. *Ortho*-dechlorination activity is induced by PCP, 2,4,6-TCP, 2,3,4-TCP, 2,3,5-TCP, 2,6-DCP, and 2,4-DCP, whereas *meta*- and *para*-dechlorination activities are induced by 3,4,5-TCP and 3,5-DCP (reviewed in Villemur 2013).

In general, RDases preferentially target highly halogenated compounds as substrates for reductive attack over compounds exhibiting lower degrees of halogenation. For example, to date, no DCE- or vinyl chloride-respiring *Desulfitobacterium* strains have been isolated, as mentioned above (see Sect. 9.5.1). In the case of chlorophenols, nine strains have been tested for dechlorination of monochlorophenols, but only *Desulfitobacterium* sp. PCE1 was found to dechlorinate 2-chlorophenol efficiently (Gerritse et al. 1996). Dechlorination of 2,3,5-trichlorohydroquinone has been reported only in strain PCE1.

With respect to chloroaromatic compounds other than chlorophenols and 3-Cl-4-OHPA, *D. hafniense* PCP-1 dechlorinates organochlorides containing a phenyl or pyridine ring substituted by hydroxyl, nitro, methoxy, or amino groups (Bouchard et al. 1996; Dennie et al. 1998). Polychlorinated nitrobenzene is dechlorinated after reduction of the nitro group to an amino group. In addition, *D. dehalogenans* strains JW/IU-DC1 and XZ-1 and *D. hafniense* strain PCP-1 were reported to dechlorinate chlorinated hydroxybiphenyls such as 3,5-dichloro-4-hydroxybiphenyl (Dennie et al. 1998; Wiegel et al. 1999).

The enzyme chlorophenol-RDase (CprA) has been purified and characterized in *D. hafniense* strains PCP-1 and DCB-2, *D. dehalogenans* JW/IU-DC1, *Desulfitobacterium* sp. PCE1, and *D. chlororespirans* Co23 (Christiansen et al. 1998; van de Pas et al. 1999, 2001a; Krasotkina et al. 2001; Löffler et al. 1996; Boyer et al. 2003; Thibodeau et al. 2004; Bisaillon et al. 2010). These enzymes exhibit features similar to the RDases PceA, such as the presence of a Tat signal sequence, two Fe–S clusters, and a corrinoid cofactor. However, the chlorophenol-RDase CrdA purified from *D. hafniense* PCP-1 exhibits different features (Boyer et al. 2003). CrdA *ortho*-dechlorinates PCP and 2,4,6-TCP. Like other RDases, CrdA contains a corrinoid cofactor, but this enzyme also has a LysM domain that may be involved in binding to peptidoglycan (Bateman et al. 2000, reviewed in Villemur 2013). The *crdA* gene has been found in *D. hafniense* strains DCB-2, DP7, TCP-1, Y51 and TCE1, *Desulfitobacterium* sp. PCE1, *D. dehalogenans* JW/IU-DC1, and *D. chlororespirans* Co23 (Gauthier et al. 2006; Nonaka et al. 2006). Transcription of *crdA* in *D. hafniense* Y51 was shown to be upregulated 225-fold by TCE (Peng et al. 2012), although strain Y51 has not been tested for chlorophenol dechlorination activity.

The larger components of the RDase gene cluster have been identified in the upstream and downstream region of the *cprA* gene in *D. dehalogenans* JW/IU-DC1. This gene cluster consists of *cprA*, *cprB*, *cprC*, *cprD*, *cprT*, *cprK*, *cprZ*, and *cprE* (Smidt et al. 2000). In contrast to the *pce* gene cluster, the *cpr* cluster encodes the transcriptional regulator CprK, a member of the CRP-FNR (cAMP-binding protein/fumarate nitrate reduction regulatory protein) family. Transcription of the *cprA* gene is strictly regulated by CprK (Smidt et al. 2000; Gábor et al. 2008). CprK is the most thoroughly studied transcriptional regulator in the OHRB (see Chap. 15).

9.5.3 Dehalogenation of Organobromine and Organoiodine Compounds

Little information is available regarding the reductive dehalogenation of organobromine and organoiodine compounds as compared with organochlorine compounds (Table 9.4). *D. dehalogenans* JW/IU-DC1 can debrominate 2-bromo-4-chlorophenol, 2-bromo-4-methylphenol, 2,6-dibromophenol, and 2-bromophenol (Utkin et al. 1995). *D. hafniense* PCP-1 can debrominate 2,4,6-tribromophenol into 4-bromophenol in the absence of inducers (Dennie et al. 1998). *D. chlororespirans* Co23 can debrominate 2,4,6-tribromophenol, bromoxynil (3,5-dibromo-4-hydroxybenzonitrile), ioxynil (3,5-diiodo-4-hydroxybenzonitrile), and the bromoxynil metabolite 3,5-dibromo-4-hydroxybenzoate (Sanford et al. 1996; Cupples et al. 2005). 2,4,6-tribromophenol is debrominated into 4-bromophenol by strain Co23 (Sanford et al. 1996). Bromoxynil and ioxynil are herbicides used for the postemergence control of dicotyledonous weeds in cereal crops. Bromoxynil is stoichiometrically converted into 4-cyanophenol and the metabolite 3,5-dibromo-4-hydroxybenzoate, which is then converted to 4-hydroxybenzoate. On the other hand, ioxynil is stoichiometrically converted into 4-cyanophenol in the presence of 3-chloro-4-hydroxybenzoate as an inducer. Strain Co23 can utilize bromoxynil and 3,5-dibromo-4-hydroxybenzoate as growth-supporting electron acceptors (Cupples et al. 2005). *D. hafniense* PCE-S can debrominate and grow on *cis*- and *trans*-dibromoethenes (Ye et al. 2010). Strain PCE-S converts *cis*- and *trans*-dibromoethenes to vinyl bromide and ethene. The PceA of strain PCE-S was initially purified as a PCE/TCE RDase (Miller et al. 1998), but the enzyme was later found to also catalyze the debromination of tribromoethene, *cis*- and *trans*-dibromoethenes (Ye et al. 2010).

Defluorination has not been observed by *Desulfitobacterium* spp. or any other OHRB. *Desulfitobacterium dehalogenans* JW/IU-DC1 does not defluorinate 3-fluoro-4-hydroxyphenylacetate (Utkin et al. 1995). *Desulfitobacterium hafniense* PCP-1 does not defluorinate 2,4,6-trifluorophenol or 2,3,5-trifluorophenol (Dennie et al. 1998), and *D. chlororespirans* Co23 does not grow in medium containing 2-fluorophenol as an electron acceptor (Sanford et al. 1996).

9.6 Bioremediation

The isolation of and ecological studies pertaining to *Desulfitobacterium* spp. has demonstrated that these organisms play a significant role in the natural attenuation of organohalide contaminants such as chloroethenes (see Sect. 9.2). For the bioremediation of chloroethenes, however, it is noteworthy that the genus *Dehalococcoides* is known as a key player. This is due to their ability to respire with DCE and vinyl chloride, a potential that has thus far been found solely in the genus *Dehalococcoides* (see Chap. 6).

Desulfitobacterium strains have been examined in both laboratory- and field-scale bioremediation studies. For example, the applicability of bioaugmentation with *D. hafniense* strains PCP-1 and Y51, *D. dehalogenans* JW/IU-DC1, or *D. dichloroeliminans* DCA1 for the degradation of PCP, PCE, 3-Cl-4-OHPA, and 1,2-DCA, respectively, has been demonstrated (El Fantroussi et al. 1997; Beaudet et al. 1998; Lanthier et al. 2000; Lee et al. 2001; De Wildeman et al. 2004). In addition, bioaugmentation using strain DCA1 for the removal of 1,2-DCA at an industrial site in Belgium was also demonstrated (Maes et al. 2006). The success of bioremediation efforts, however, depends largely on environmental factors, such as soil composition (reviewed in Villemur et al. 2006). For example, Lanthier et al. (2000) reported the dechlorination of PCP in a PCP-amended soil rich in organic matter, but no dechlorination was observed in sandy soil amended with PCP, highlighting the impact of factors such as organic matter and toxicity associated with other pollutants on the viability of OHRB. Geochemical properties such as redox conditions and pH also significantly impact the success of in situ bioremediation efforts.

Desulfitobacterium strains have also been used in investigations aimed at increasing bioremediation efficiency. The effect of PCE dense nonaqueous phase liquid on dechlorination by *Desulfitobacterium* strains Viet1 and PCE1 was examined via mathematical modeling (Amos et al. 2007; Huang and Becker 2011). Strain Viet1 was used to investigate the environmental fate of 2,4-DCP sequestered by the aquatic plant *Lemna minor* (Tront et al. 2006). In the environment, aquatic plants are known to take up and sequester various organohalines, such as chlorophenols (Newman and Reynolds 2004).

Desulfitobacterium strains have been utilized in bioreactors for treating organohaline-contaminated wastes. For example, *D. hafniense* strains DCB-2 and PCP-1 were inoculated into an up-flow anaerobic-sludge bed reactor used for PCP degradation (Christiansen and Ahring 1996b). This PCP degrading bioreactor using strain PCP-1 is one of the most thoroughly studied systems of its type (e.g., the localization of strain PCP-1 in the reactor granules and biofilm was demonstrated by fluorescence in situ hybridization) (Tartakovsky et al. 1999; Lanthier et al. 2000, 2002, 2005). Degradation of 2,4,6-TCP in anaerobic granular sludge bioaugmented with *D. hafniense* strains PCP-1 and TCP-A and *D. chlororespirans* Co23 has also been reported (Puyol et al. 2011). The *Desulfitobacterium* spp. were also found to be enriched in a deiodination bioreactor for 5-amino-2,4,6-triiodoisophthalic acid (a core structure of X-ray contrast media) (Lecouturier et al. 2003). These studies have demonstrated that *Desulfitobacterium* spp. are key components in dechlorination bioreactors.

Desulfitobacterium spp. can be employed not only for organohalide detoxification but also for the removal of metals. The solubility of a metal significantly affects its mobility in the environment. Therefore, altering a metal contaminant's solubility through bioreduction is one approach for removing it. For example, several reports have described metal bioremediation strategies employing *Desulfitobacterium* strains to reduce soluble U(VI) to insoluble U(IV) (Fletcher et al. 2010; Boyanov et al. 2011).

Tools such as real-time PCR targeting the 16S rRNA and RDase genes of OHRB, and stable-isotope fractionation analysis are important for monitoring bioremediation efforts and evaluating their success. These tools were also developed for *Desulfitobacterium* spp. as one of important class of OHRB (see Chaps. 25 and 26).

9.7 Genomic Features

9.7.1 Basic Genome Facts

Complete genome sequences have been published for *D. hafniense* strains Y51 and DCB-2 and *D. dehalogenans* JW/IU-DC1 (Nonaka et al. 2006; Kim et al. 2012; Kruse et al. 2014b). In addition, the complete genome sequence for *D. dichloroeliminans* DCA1 and draft genome sequences for *D. hafniense* strains PCE-S (Goris et al. 2015), PCP-1, TCP-A, and DP7, *D. metallireducens* 853-15A, and *Desulfitobacterium* sp. PCE1 are available in the National Center for Biotechnology Information (NCBI) database.

The complete genomes of *Desulfitobacterium* strains vary widely in size, ranging from 3.62 to 5.73 Mbp, with similar GC content, ranging from 44.2 to 47.5 % (Table 9.5). No plasmids have been identified by genomic analyses. The number of coding sequences (CDSs) identified ranges from 3340 to 5060, which is consistent with the differences in genome size. As described in Sect. 9.4, multiple copies of rRNA operons (in the range from 2 to 7) are found in *Desulfitobacterium* genomes.

The number of RDase genes differs in each strain (ranging from 2 to 7), which explains the differences in their dehalogenation spectra. Analyses of the *D. hafniense* strains Y51 and DCB-2 genomes revealed the existence of versatile terminal reductases, such as DMSO reductase superfamily proteins, sulfite

Table 9.5 *Desulfitobacterium* complete genome information

Organism	*D. hafniense* Y51	*D. hafniense* DCB-2	*D. dehalogenans* JW/IU-DC1	*D. dichloroeliminans* DCA1
Size (Mb)	5.73	5.28	4.32	3.62
GC%	47.4	47.5	45	44.2
CDSs	5060	4883	4011	3340
rRNA operon	6	5	6	6
tRNA	59	74	75	73
Rdase genes	2	7	6	1
RefSeq accession number	NC_007907.1	NC_011830.1	NC_018017.1	NC_019903.1
INSDC accession number	AP008230.1	CP001336.1	CP003348.1	CP003344.1
Reference	Nonaka et al. (2006)	Kim et al. (2012)	Kruse et al. (2014b)	Unpublished

reductase, nitrate reductase, and fumarate reductase, in addition to RDases (Nonaka et al. 2006; Kim et al. 2012). This finding is consistent with the growth phenotype of the *Desulfitobacterium* strains, with the exception that the nitrate reductase gene cluster (Nap system) was not identified in the genome of strain DCB-2, even though it can grow using nitrate as an electron acceptor.

As the *Desulfitobacterium* strains are slightly oxygen tolerant, the genomes of *D. hafniense* strains Y51 and DCB-2 encode putative catalases, superoxide dismutases, and rubrerythrin-rubredoxin enzymes that may be involved in imparting oxygen tolerance (Nonaka et al. 2006; Kim et al. 2012). In addition, consistent with its ability to form spores, genes involved in spore formation are present in strain DCB-2 (Kim et al. 2012).

9.7.2 Functional Genes and Metabolic Pathways

Genomic information has enabled the prediction of metabolic pathways in *Desulfitobacterium* strains. Central metabolic pathways for *D. hafniense* strains Y51 and DCB-2 have been proposed (Nonaka et al. 2006; Kim et al. 2012). Both strains Y51 and DCB-2 have a functional Embden-Meyerhof-Parnas pathway. On the other hand, the tricarboxylic acid cycle is characterized by the absence of 2-oxoglutarate dehydrogenase and the anaplerotic glyoxylate bypass, indicating an incomplete cycle. The reductive acetyl-CoA pathway (Wood-Ljungdahl pathway) for carbon dioxide fixation has also been found in both strains Y51 and DCB-2. Moreover, autotrophic growth of strain DCB-2 in a carbon dioxide fixation medium was confirmed (Kim et al. 2012).

Genome analyses of *D. hafniense* DCB-2 confirmed the presence of the complete synthetic pathways for the cofactors flavin adenine dinucleotide, nicotinamide adenine dinucleotide, menaquinone, heme, and cobalamin (Kim et al. 2012). The cobalamin biosynthetic pathway has also been identified in *D. hafniense* strains Y51 and TCE1 (Nonaka et al. 2006; Choudhary et al. 2013). Genomic analyses also revealed the presence of diverse cobalamin riboswitches that may be involved in regulating corrinoid metabolism in these *D. hafniense* strains (Choudhary et al. 2013). In addition, 14 putative ABC-type corrinoid transporter-encoding genes were identified in the genome of *D. hafniense* Y51 (Nonaka et al. 2006), but exogenous corrinoid may not be incorporated into the PceA precursor in strain Y51 (Reinhold et al. 2012). Menaquinone is believed to serve as an electron carrier in organohalide respiration. Electron paramagnetic resonance spectroscopy, visible spectroscopy, and proteomic analyses of *D. dehalogenans* JW/IU-DC1 suggested that electrons are transferred from menaquinones to RDase CprA via an as yet unidentified membrane complex and potentially by an extracellular flavoprotein (Kruse et al. 2014b).

9.7.3 Protein Characterization Based on Genome Data

Genomic data have also enabled other "omics" studies. For example, high expression of the unusual rhodanese protein PhsE during respiration with PCE in *D. hafniense* TCE1 as revealed by a proteomic study led to the characterization of this protein (Prat et al. 2011, 2012). PhsE was shown to have two unusual rhodanese domains that usually function to bind sulfane sulfur and catalyze sulfur transfer. The expression of PhsE was found to be induced in the presence of sulfide in the medium and is believed to play a role in sulfur homeostasis. In addition, genomic studies have also opened the door to the discovery of new scientific knowledge from perspectives other than organohalide respiration. For example, the crystal structure of Dhaf4260 from *D. hafniense* DCB-2 was determined as the first representative of the Pfam family PF04016 (DUF364) (DUF: domains of unknown function) in the course of a study aimed at enhancing knowledge regarding the structures of proteins of unknown biological function (Miller et al. 2010). Moreover, DSY3156 (MtgB) from *D. hafniense* Y51 was characterized as a non-pyrrolysine member of the widely distributed trimethylamine methyltransferase family and was the first glycine betaine:corrinoid methyltransferase characterized (Ticak et al. 2014).

Desulfitobacterium strains are among the ideal model microorganisms for the study of organohalide respiration. Molecular biology tools will become increasingly important in future studies of *Desulfitobacterium* spp. Transposon mutagenesis using Tn*916* and gene disruption using a thermosensitive plasmid have been used in studies of *D. dehalogenans* JW/IU-DC1, and functional heterologous expression of RDase in *D. hafniense* strains Y51 and DCB-2 in the Gram-negative gamma-proteobacterium *Shimwellia blattae* ATCC 33,430 has been demonstrated using molecular tools (Smidt et al. 1999, 2001; Mac Nelly et al. 2014). Recent sequencing efforts of further *Desulfitobacterium* genomes will also attract researchers to the study of *Desulfitobacterium* species from perspectives other than organohalide respiration. Combining molecular biology tools with current genomic information will reveal novel insights into the genus *Desulfitobacterium*.

References

Amos BK, Christ JA, Abriola LM, Pennell KD, Löffler FE (2007) Experimental evaluation and mathematical modeling of microbially enhanced tetrachloroethene (PCE) dissolution. Environ Sci Technol 41:963–970. doi:10.1021/es061438n

Beaudet R, Lévesque MJ, Villemur R, Lanthier M, Chénier M, Lépine F, Bisaillon JG (1998) Anaerobic biodegradation of pentachlorophenol in a contaminated soil inoculated with a methanogenic consortium or with *Desulfitobacterium frappieri* strain PCP-1. Appl Microbiol Biotechnol 50:135–141. doi:10.1007/s002530051268

Bisaillon A, Beaudet R, Lépine F, Déziel E, Villemur R (2010) Identification and characterization of a novel CprA reductive dehalogenase specific to highly chlorinated phenols from *Desulfitobacterium hafniense* strain PCP-1. Appl Environ Microbiol 76:7536–7540. doi:10.1128/AEM.01362-10

Bouchard B, Beaudet R, Villemur R, McSween G, Lépine F, Bisaillon JG (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

Boyanov MI, Fletcher KE, Kwon MJ, Rui X, O'Loughlin EJ, Löffler FE, Kemner KM (2011) Solution and microbial controls on the formation of reduced U(IV) species. Environ Sci Technol 45:8336–8344. doi:10.1021/es2014049

Boyer A, Pagé-BéLanger R, Saucier M, Villemur R, Lépine F, Juteau P, Beaudet R (2003) Purification, cloning and sequencing of an enzyme mediating the reductive dechlorination of 2,4,6-trichlorophenol from *Desulfitobacterium frappieri* PCP-1. Biochem J 373:297–303. doi:10.1042/BJ20021837

Breitenstein A, Saano A, Salkinoja-Salonen M, Andreesen JR, Lechner U (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

Cervantes FJ, de Bok FAM, Duong-Dac T, Stams AJM, Lettinga G, Field JA (2002) Reduction of humic substances by halorespiring, sulphate-reducing and methanogenic microorganisms. Environ Microbiol 4:51–57

Chang YC, Hatsu M, Jung K, Yoo YS, Takamizawa K (2000) Isolation and characterization of a tetrachloroethylene dechlorinating bacterium, *Clostridium bifermentans* DPH-1. J Biosci Bioeng 89:489–491

Choudhary PK, Duret A, Rohrbach-Brandt E, Holliger C, Sigel RKO, Maillard J (2013) Diversity of cobalamin riboswitches in the corrinoid-producing organohalide respirer *Desulfitobacterium hafniense*. J Bacteriol 195:5186–5195. doi:10.1128/JB.00730-13

Christiansen N, Ahring BK (1996a) *Desulfitobacterium hafniense* sp. nov., an anaerobic, reductively dechlorinating bacterium. Int J Syst Bacteriol 46:442–448. doi:10.1099/00207713-46-2-442

Christiansen N, Ahring BK (1996b) Introduction of a de novo bioremediation activity into anaerobic granular sludge using the dechlorinating bacterium DCB-2. Antonie Van Leeuwenhoek 69:61–66. doi:10.1007/BF00641612

Christiansen N, Ahring BK, Wohlfarth G, Diekert G (1998) Purification and characterization of the 3-chloro-4-hydroxy-phenylacetate reductive dehalogenase of *Desulfitobacterium hafniense*. FEBS Lett 436:159–162. doi:10.1016/S0014-5793(98)01114-4

Cupples AM, Sanford RA, Sims GK (2005) Dehalogenation of the herbicides bromoxynil (3,5-dibromo-4-hydroxybenzonitrile) and ioxynil (3,5-diiodino-4-hydroxybenzonitrile) by *Desulfitobacterium chlororespirans*. Appl Environ Microbiol 71:3741–3746. doi:10.1128/AEM.71.7.3741-3746.2005

Davis JW, Odom JM, DeWeerd KA, Stahl DA, Fishbain SS, West RJ, Klecka GM, DeCarolis JG (2002) Natural attenuation of chlorinated solvents at Area 6, Dover Air Force Base: characterization of microbial community structure. J Contam Hydrol 57:41–59. doi:10.1016/S0169-7722(01)00217-0

De Wildeman S, Diekert G, Van Langenhove H, Verstraete W (2003) Stereoselective microbial dehalorespiration with vicinal dichlorinated alkanes. Appl Environ Microbiol 69:5643–5647

De Wildeman S, Linthout G, Van Langenhove H, Verstraete W (2004) Complete lab-scale detoxification of groundwater containing 1,2-dichloroethane. Appl Microbiol Biotechnol 63:609–612. doi:10.1007/s00253-003-1363-y

Dennie D, Gladu I, Lepine F, Villemur R, Bisaillon J, Beaudet R (1998) Spectrum of the reductive dehalogenation activity of *Desulfitobacterium frappieri* PCP-1. Appl Environ Microbiol 64:4603–4606

DeWeerd K, Mandelco L, Tanner R, Woese C, Suflita J (1990) *Desulfomonile tiedjei* gen. nov. and sp. nov., a novel anaerobic, dehalogenating, sulfate-reducing bacterium. Arch Microbiol. doi:10.1007/BF00249173

Ding C, Zhao S, He J (2014) A desulfitobacterium sp. strain PR reductively dechlorinates both 1,1,1-trichloroethane and chloroform. Environ Microbiol 11:3387–97. doi:10.1111/1462-2920.12387

Dowideit K, Scholz-Muramatsu H, Miethling-Graff R, Vigelahn L, Freygang M, Dohrmann AB, Tebbe CC (2010) Spatial heterogeneity of dechlorinating bacteria and limiting factors for in situ trichloroethene dechlorination revealed by analyses of sediment cores from a polluted field site. FEMS Microbiol Ecol 71:444–459. doi:10.1111/j.1574-6941.2009.00820.x

Duret A, Holliger C, Maillard J (2012) The physiological opportunism of *Desulfitobacterium hafniense* strain TCE1 towards organohalide respiration with tetrachloroethene. Appl Environ Microbiol 78:6121–6127. doi:10.1128/AEM.01221-12

El Fantroussi S, Mahillon J, Naveau H, Agathos SN (1997) Introduction of anaerobic dechlorinating bacteria into soil slurry microcosms and nested-PCR monitoring. Appl Environ Microbiol 63:806–811

Finneran KT, Forbush HM, VanPraagh CVG, Lovley DR (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

Fletcher KE, Ritalahti KM, Pennell KD, Takamizawa K, Löffler FE (2008) Resolution of culture *Clostridium bifermentans* DPH-1 into two populations, a *Clostridium* sp. and tetrachloroethene-dechlorinating *Desulfitobacterium hafniense* strain JH1. Appl Environ Microbiol 74:6141–6143. doi:10.1128/AEM.00994-08

Fletcher KE, Boyanov MI, Thomas SH, Wu Q, Kemner KM, Löffler FE (2010) U(VI) reduction to mononuclear U(IV) by *Desulfitobacterium* species. Environ Sci Technol 44:4705–4709. doi:10.1021/es903636c

Furukawa K, Suyama A, Tsuboi Y, Futagami T, Goto M (2005) Biochemical and molecular characterization of a tetrachloroethene dechlorinating *Desulfitobacterium* sp. strain Y51: a review. J Ind Microbiol Biotechnol 32:534–541. doi:10.1007/s10295-005-0252-z

Futagami T, Tsuboi Y, Suyama A, Goto M, Furukawa K (2006a) Emergence of two types of non-dechlorinating variants in the tetrachloroethene-halorespiring *Desulfitobacterium* sp. strain Y51. Appl Microbiol Biotechnol 70:720–728. doi:10.1007/s00253-005-0112-9

Futagami T, Yamaguchi T, Nakayama S-I, Goto M, Furukawa K (2006b) Effects of chloromethanes on growth of and deletion of the *pce* gene cluster in dehalorespiring *Desulfitobacterium hafniense* strain Y51. Appl Environ Microbiol 72:5998–6003. doi:10.1128/AEM.00979-06

Gábor K, Hailesellasse Sene K, Smidt H, de Vos WM, van der Oost J (2008) Divergent roles of CprK paralogues from *Desulfitobacterium hafniense* in activating gene expression. Microbiology 154:3686–3696. doi:10.1099/mic.0.2008/021584-0

Gauthier A, Beaudet R, Lépine F, Juteau P, Villemur R (2006) Occurrence and expression of *crdA* and *cprA5* encoding chloroaromatic reductive dehalogenases in *Desulfitobacterium* strains. Can J Microbiol 52:47–55. doi:10.1139/w05-111

Gerritse J, Renard V, Visser J, Gottschal JC (1995) Complete degradation of tetrachloroethene by combining anaerobic dechlorinating and aerobic methanotrophic enrichment cultures. Appl Microbiol Biotechnol 43:920–928

Gerritse J, Renard V, Pedro Gomes TM, Lawson PA, Collins MD, Gottschal JC (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

Gerritse J, Kloetstra G, Borger A, Dalstra G, Alphenaar A, Gottschal JC (1997) Complete degradation of tetrachloroethene in coupled anoxic and oxic chemostats. Appl Microbiol Biotechnol 48:553–562

Gerritse J, Drzyzga O, Kloetstra G, Keijmel M, Wiersum LP, Hutson R, Collins MD, Gottschal JC (1999) Influence of different electron donors and acceptors on dehalorespiration of tetrachloroethene by *Desulfitobacterium frappieri* TCE1. Appl Environ Microbiol 65:5212–5221

Goris T, Hornung B, Kruse T, Reinhold A, Westermann M, Schaap PJ, Smidt H, Gabriele Diekert G (2015) Draft genome sequence and characterization of *Desulfitobacterium hafniense* PCE-S. Stand Genomic Sci 10:15. doi:10.1186/1944-3277-10-15

Holliger C, Wohlfarth G, Diekert G (1998) Reductive dechlorination in the energy metabolism of anaerobic bacteria. FEMS Microbiol Rev 22:383–398. doi:10.1016/S0168-6445(98)00030-8

Huang D, Becker JG (2011) Dehalorespiration model that incorporates the self-inhibition and biomass inactivation effects of high tetrachloroethene concentrations. Environ Sci Technol 45:1093–1099. doi:10.1021/es102729v

Hug LA, Maphosa F, Leys D, Löffler FE, Smidt H, Edwards EA, Adrian L (2013) Overview of organohalide-respiring bacteria and a proposal for a classification system for reductive dehalogenases. Philos Trans R Soc Lond B Biol Sci 368:20120322. doi:10.1098/rstb.2012.0322

Kaksonen AH, Plumb JJ, Robertson WJ, Franzmann PD, Gibson JAE, Puhakka JA (2004) Culturable diversity and community fatty acid profiling of sulfate-reducing fluidized-bed reactors treating acidic, metal-containing wastewater. Geomicrobiol J 21:469–480. doi:10.1080/01490450490505455

Kalan L, Ebert S, Kelly T, Wright GD (2009) Noncanonical vancomycin resistance cluster from *Desulfitobacterium hafniense* Y51. Antimicrob Agents Chemother 53:2841–2845. doi:10.1128/AAC.01408-08

Kim S-H, Harzman C, Davis JK, Hutcheson R, Broderick JB, Marsh TL, Tiedje JM (2012) Genome sequence of *Desulfitobacterium hafniense* DCB-2, a Gram-positive anaerobe capable of dehalogenation and metal reduction. BMC Microbiol 12:21. doi:10.1186/1471-2180-12-21

Kranzioch I, Stoll C, Holbach A, Chen H, Wang L, Zheng B, Norra S, Bi Y, Schramm K-W, Tiehm A (2013) Dechlorination and organohalide-respiring bacteria dynamics in sediment samples of the Yangtze Three Gorges Reservoir. Environ Sci Pollut Res Int 20:7046–7056. doi:10.1007/s11356-013-1545-9

Krasotkina J, Walters T, Maruya KA, Ragsdale SW (2001) Characterization of the B_{12}- and iron-sulfur-containing reductive dehalogenase from *Desulfitobacterium chlororespirans*. J Biol Chem 276:40991–40997. doi:10.1074/jbc.M106217200

Kreher S, Schilhabel A, Diekert G (2008) Enzymes involved in the anoxic utilization of phenyl methyl ethers by *Desulfitobacterium hafniense* DCB2 and *Desulfitobacterium hafniense* PCE-S. Arch Microbiol 190:489–495. doi:10.1007/s00203-008-0400-8

Kruse T, Levisson M, de Vos WM, Smidt H (2014a) *vanI*: a novel D-Ala-D-Lac vancomycin resistance gene cluster found in *Desulfitobacterium hafniense*. Microb Biotechnol 7:456–466. doi:10.1111/1751-7915.12139

Kruse T, van de Pas BA, Atteia A, Krab K, Hagen WR, Goodwin L, Chain P, Boeren S, Maphosa F, Schraa G, de Vos WM, van der Oost J, Smidt H, Stams AJM (2014b) Genomic, proteomic and biochemical analysis of the organohalide respiratory pathway in *Desulfitobacterium dehalogenans*. J Bacteriol JB.02370-14-. doi:10.1128/JB.02370-14

Kunapuli U, Jahn MK, Lueders T, Geyer R, Heipieper HJ, Meckenstock RU (2010) *Desulfitobacterium aromaticivorans* sp. nov. and *Geobacter toluenoxydans* sp. nov., iron-reducing bacteria capable of anaerobic degradation of monoaromatic hydrocarbons. Int J Syst Evol Microbiol 60:686–695. doi:10.1099/ijs.0.003525-0

Lanthier M, Villemur R, Lépine F, Bisaillon JG, Beaudet R (2000) Monitoring of *Desulfitobacterium frappieri* PCP-1 in pentachlorophenol-degrading anaerobic soil slurry reactors. Environ Microbiol 2:703–708. doi:10.1046/j.1462-2920.2000.00144.x

Lanthier M, Villemur R, Lépine F, Bisaillon JG, Beaudet R (2001) Geographic distribution of *Desulfitobacterium frappieri* PCP-1 and *Desulfitobacterium* spp. in soils from the province of Quebec. Canada. FEMS Microbiol Ecol 36:185–191

Lanthier M, Tartakovsky B, Villemur R, DeLuca G, Guiot SR (2002) Microstructure of anaerobic granules bioaugmented with *Desulfitobacterium frappieri* PCP-1. Appl Environ Microbiol 68:4035–4043. doi:10.1128/AEM.68.8.4035-4043.2002

Lanthier M, Juteau P, Lépine F, Beaudet R, Villemur R (2005) *Desulfitobacterium hafniense* is present in a high proportion within the biofilms of a high-performance pentachlorophenol-degrading, methanogenic fixed-film reactor. Appl Environ Microbiol 71:1058–1065. doi:10.1128/AEM.71.2.1058-1065.2005

Lecouturier D, Godon JJ, Lebeault JM (2003) Phylogenetic analysis of an anaerobic microbial consortium deiodinating 5-amino-2,4,6-triiodoisophthalic acid. Appl Microbiol Biotechnol 62:400–406. doi:10.1007/s00253-003-1278-7

Lee T, Tokunaga T, Suyama A, Furukawa K (2001) Efficient dechlorination of tetrachloroethylene in soil slurry by combined use of an anaerobic *Desulfitobacterium* sp. strain Y-51 and zero-valent iron. J Biosci Bioeng 92:453–458. doi:10.1016/S1389-1723(01)80295-4

Lie TJ, Godchaux W, Leadbetter ER (1999) Sulfonates as terminal electron acceptors for growth of sulfite-reducing bacteria (*Desulfitobacterium* spp.) and sulfate-reducing bacteria: effects of inhibitors of sulfidogenesis. Appl Environ Microbiol 65:4611–4617

Löffler F, Sanford R, Tiedje J (1996) Initial characterization of a reductive dehalogenase from *Desulfitobacterium chlororespirans* Co23. Appl Envir Microbiol 62:3809–3813

Löffler FE, Ritalahti KM, Tiedje JM (1997) Dechlorination of chloroethenes is inhibited by 2-bromoethanesulfonate in the absence of methanogens. Appl Environ Microbiol 63:4982–4985

Lovley DR, Fraga JL, Blunt-Harris EL, Hayes LA, Phillips EJP, Coates JD (1998) Humic substances as a mediator for microbially catalyzed metal reduction. Acta Hydrochim Hydrobiol 26:152–157. doi:10.1002/(SICI)1521-401X(199805)26:3<152:AID-AHEH152>3.0.CO;2-D

Luijten ML, Weelink SA, Godschalk B, Langenhoff AA, van Eekert MH, Schraa G, Stams AJ (2004) Anaerobic reduction and oxidation of quinone moieties and the reduction of oxidized metals by halorespiring and related organisms. FEMS Microbiol Ecol 49:145–150. doi:10.1016/j.femsec.2004.01.015

Lupa B, Wiegel J (2009) Genus IV: *Desulfitobacterium*. In: Vos P, Garity G, Jones D, Krieg NR, Ludwig W, Rainey FA, Schleifer K-H, Whitman W (eds) Bergey's manual of systematic bacteriology, vol 3, 2nd edn. Springer, New York, pp 975–982

Mac Nelly A, Kai M, Svatoš A, Diekert G, Schubert T (2014) Functional heterologous production of reductive dehalogenases from *Desulfitobacterium hafniense* strains. Appl Environ Microbiol 80:4313–4322. doi:10.1128/AEM.00881-14

Mackiewicz M, Wiegel J (1998) Comparison of energy and growth yields for *Desulfitobacterium dehalogenans* during utilization of chlorophenol and various traditional electron acceptors. Appl Environ Microbiol 64:352–355

Madsen T, Licht D (1992) Isolation and characterization of an anaerobic chlorophenol-transforming bacterium. Appl Environ Microbiol 58:2874–2878

Maes A, Van Raemdonck H, Smith K, Ossieur W, Lebbe L, Verstraete W (2006) Transport and activity of *Desulfitobacterium dichloroeliminans* strain DCA1 during bioaugmentation of 1,2-DCA-contaminated groundwater. Environ Sci Technol 40:5544–5552. doi:10.1021/es060953i

Maillard J, Schumacher W, Vazquez F, Regeard C, Hagen WR, Holliger C (2003) Characterization of the corrinoid iron-sulfur protein tetrachloroethene reductive dehalogenase of *Dehalobacter restrictus*. Appl Environ Microbiol 69:4628–4638

Maillard J, Regeard C, Holliger C (2005) Isolation and characterization of *Tn*-Dha1, a transposon containing the tetrachloroethene reductive dehalogenase of *Desulfitobacterium hafniense* strain TCE1. Environ Microbiol 7:107–117. doi:10.1111/j.1462-2920.2004.00671.x

Maillard J, Genevaux P, Holliger C (2011) Redundancy and specificity of multiple trigger factor chaperones in Desulfitobacteria. Microbiology 157:2410–2421. doi:10.1099/mic.0.050880-0

Miller E, Wohlfarth G, Diekert G (1997) Comparative studies on tetrachloroethene reductive dechlorination mediated by *Desulfitobacterium* sp. strain PCE-S. Arch Microbiol 168:513–519

Miller E, Wohlfarth G, Diekert G (1998) Purification and characterization of the tetrachloroethene reductive dehalogenase of strain PCE-S. Arch Microbiol 169:497–502

Miller MD, Aravind L, Bakolitsa C, Rife CL, Carlton D, Abdubek P, Astakhova T, Axelrod HL, Chiu HJ, Clayton T, Deller MC, Duan L, Feuerhelm J, Grant JC, Han GW, Jaroszewski L, Jin KK, Klock HE, Knuth MW, Kozbial P, Krishna SS, Kumar A, Marciano D, McMullan D, Morse AT, Nigoghossian E, Okach L, Reyes R, van den Bedem H, Weekes D, Xu Q, Hodgson KO, Wooley J, Elsliger MA, Deacon AM, Godzik A, Lesley SA, Wilson IA (2010) Structure of the first representative of Pfam family PF04016 (DUF364) reveals enolase and Rossmann-like folds that combine to form a unique active site with a possible role in heavy-metal chelation. Acta Crystallogr, Sect F: Struct Biol Cryst Commun 66:1167–1173. doi:10.1107/S1744309110007517

Milliken CE, May HD (2007) Sustained generation of electricity by the spore-forming, Gram-positive, *Desulfitobacterium hafniense* strain DCB2. Appl Microbiol Biotechnol 73:1180–1189. doi:10.1007/s00253-006-0564-6

Milliken CE, Meier GP, Sowers KR, May HD (2004a) Chlorophenol production by anaerobic microorganisms: transformation of a biogenic chlorinated hydroquinone metabolite. Appl Environ Microbiol 70:2494–2496

Milliken CE, Meier GP, Watts JEM, Sowers KR, May HD (2004b) Microbial anaerobic demethylation and dechlorination of chlorinated hydroquinone metabolites synthesized by basidiomycete fungi. Appl Environ Microbiol 70:385–392

Mingo FS, Studenik S, Diekert G (2014) Conversion of phenyl methyl ethers by *Desulfitobacterium* spp. and screening for the genes involved. FEMS Microbiol Ecol 90:783–790. doi:10.1111/1574-6941.12433

Mohn WW, Tiedje JM (1992) Microbial reductive dehalogenation. Microbiol Rev 56:482–507

Morita Y, Futagami T, Goto M, Furukawa K (2009) Functional characterization of the trigger factor protein PceT of tetrachloroethene-dechlorinating *Desulfitobacterium hafniense* Y51. Appl Microbiol Biotechnol 83:775–781. doi:10.1007/s00253-009-1958-z

Neumann A, Engelmann T, Schmitz R, Greiser Y, Orthaus A, Diekert G (2004) Phenyl methyl ethers: novel electron donors for respiratory growth of *Desulfitobacterium hafniense* and *Desulfitobacterium* sp. strain PCE-S. Arch Microbiol 181:245–249. doi:10.1007/s00203-004-0651-y

Newman LA, Reynolds CM (2004) Phytodegradation of organic compounds. Curr Opin Biotechnol 15:225–230. doi:10.1016/j.copbio.2004.04.006

Niggemyer A, Spring S, Stackebrandt E, Rosenzweig RF (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. doi:10.1128/AEM.67.12.5568-5580.2001

Nonaka H, Keresztes G, Shinoda Y, Ikenaga Y, Abe M, Naito K, Inatomi K, Furukawa K, Inui M, Yukawa H (2006) Complete genome sequence of the dehalorespiring bacterium *Desulfitobacterium hafniense* Y51 and comparison with *Dehalococcoides ethenogenes* 195. J Bacteriol 188:2262–2274. doi:10.1128/JB.188.6.2262-2274.2006

Patel BK, Love CA, Stackebrandt E (1992) Helix 6 of the 16S rRNA of the bacterium *Desulfotomaculum australicum* exhibits an unusual structural idiosyncrasy. Nucleic Acids Res 20:5483

Peng X, Yamamoto S, Vertès AA, Keresztes G, Inatomi K, Inui M, Yukawa H (2012) Global transcriptome analysis of the tetrachloroethene-dechlorinating bacterium *Desulfitobacterium hafniense* Y51 in the presence of various electron donors and terminal electron acceptors. J Ind Microbiol Biotechnol 39:255–68. doi:10.1007/s10295-011-1023-7

Prat L, Maillard J, Grimaud R, Holliger C (2011) Physiological adaptation of *Desulfitobacterium hafniense* strain TCE1 to tetrachloroethene respiration. Appl Environ Microbiol 77:3853–3859. doi:10.1128/AEM.02471-10

Prat L, Maillard J, Rohrbach-Brandt E, Holliger C (2012) An unusual tandem-domain rhodanese harbouring two active sites identified in *Desulfitobacterium hafniense*. FEBS J 279:2754–2767. doi:10.1111/j.1742-4658.2012.08660.x

Puyol D, Mohedano AF, Rodriguez JJ, Sanz JL (2011) Effect of 2,4,6-trichlorophenol on the microbial activity of adapted anaerobic granular sludge bioaugmented with *Desulfitobacterium* strains. N Biotechnol 29:79–89. doi:10.1016/j.nbt.2011.06.011

Reinhold A, Westermann M, Seifert J, von Bergen M, Schubert T, Diekert G (2012) Impact of vitamin B_{12} on formation of the tetrachloroethene reductive dehalogenase in *Desulfitobacterium hafniense* strain Y51. Appl Environ Microbiol 78:8025–8032. doi:10.1128/AEM.02173-12

Rogosa M (1971) *Peptococcaceae*, a new Family to include the Gram-positive, anaerobic cocci of the Genera *Peptococcus*, *Peptostreptococcus*, and *Ruminococcus*. Int J Syst Bacteriol 21:234–237. doi:10.1099/00207713-21-3-234

Rouzeau-Szynalski K, Maillard J, Holliger C (2011) Frequent concomitant presence of *Desulfitobacterium* spp. and "*Dehalococcoides*" spp. in chloroethene-dechlorinating microbial communities. Appl Microbiol Biotechnol 90:361–368. doi:10.1007/s00253-010-3042-0

Sanford RA, Cole JR, Löffler FE, Tiedje JM (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

Shelobolina ES, VanPraagh CG, Lovley DR (2003) Use of ferric and ferrous iron containing minerals for respiration by *Desulfitobacterium frappieri*. Geomicrobiol J 20:143–156. doi:10.1080/01490450390193255

Shelton DR, Tiedje JM (1984) Isolation and partial characterization of bacteria in an anaerobic consortium that mineralizes 3-chlorobenzoic Acid. Appl Environ Microbiol 48:840–848

Smidt H, de Vos WM (2004) Anaerobic microbial dehalogenation. Annu Rev Microbiol 58:43–73. doi:10.1146/annurev.micro.58.030603.123600

Smidt H, Song D, van Der Oost J, de Vos WM (1999) Random transposition by Tn*916* in *Desulfitobacterium dehalogenans* allows for isolation and characterization of halorespiration-deficient mutants. J Bacteriol 181:6882–6888

Smidt H, van Leest M, van der Oost J, de Vos WM (2000) Transcriptional regulation of the *cpr* gene cluster in *ortho*-chlorophenol-respiring *Desulfitobacterium dehalogenans*. J Bacteriol 182:5683–5691

Smidt H, van der Oost J, de Vos WM (2001) Development of a gene cloning and inactivation system for halorespiring *Desulfitobacterium dehalogenans*. Appl Environ Microbiol 67:591–597. doi:10.1128/AEM.67.2.591-597.2001

Spring S, Rosenzweig F (2006) The genera *Desulfitobacterium* and *Desulfosporosinus*: taxonomy. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K, Stackebrandt E (eds) The prokaryotes, Volume 4: bacteria: firmicutes, cyanobacteria. Springer, New York, pp 771–786. doi:10.1007/0-387-30744-3_240

Studenik S, Vogel M, Diekert G (2012) Characterization of an *O*-demethylase of *Desulfitobacterium hafniense* DCB-2. J Bacteriol 194:3317–3326. doi:10.1128/JB.00146-12

Sutcliffe IC (2010) A phylum level perspective on bacterial cell envelope architecture. Trends Microbiol 18:464–470. doi:10.1016/j.tim.2010.06.005

Suyama A, Iwakiri R, Kai K, Tokunaga T, Sera N, Furukawa K (2001) Isolation and characterization of *Desulfitobacterium* sp. strain Y51 capable of efficient dehalogenation of tetrachloroethene and polychloroethanes. Biosci Biotechnol Biochem 65:1474–1481. doi:10.1271/bbb.65.1474

Suyama A, Yamashita M, Yoshino S, Furukawa K (2002) Molecular characterization of the PceA reductive dehalogenase of *Desulfitobacterium* sp. strain Y51. J Bacteriol 184:3419–3425

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729. doi:10.1093/molbev/mst197

Tartakovsky B, Levesque MJ, Dumortier R, Beaudet R, Guiot SR (1999) Biodegradation of pentachlorophenol in a continuous anaerobic reactor augmented with *Desulfitobacterium frappieri* PCP-1. Appl Environ Microbiol 65:4357–4362

Thibodeau J, Gauthier A, Duguay M, Villemur R, Lépine F, Juteau P, Beaudet R (2004) Purification, cloning, and sequencing of a 3,5-dichlorophenol reductive dehalogenase from *Desulfitobacterium frappieri* PCP-1. Appl Environ Microbiol 70:4532–4537. doi:10.1128/AEM.70.8.4532-4537.2004

Ticak T, Kountz DJ, Girosky KE, Krzycki JA, Ferguson DJ (2014) A nonpyrrolysine member of the widely distributed trimethylamine methyltransferase family is a glycine betaine methyltransferase. Proc Natl Acad Sci USA 111:E4668–E4676. doi:10.1073/pnas.1409642111

Tront JM, Amos BK, Löffler FE, Saunders FM (2006) Activity of *Desulfitobacterium* sp. strain Viet1 demonstrates bioavailability of 2,4-dichlorophenol previously sequestered by the aquatic plant *Lemna minor*. Environ Sci Technol 40:529–535. doi:10.1021/es0514545

Tsukagoshi N, Ezaki S, Uenaka T, Suzuki N, Kurane R (2006) Isolation and transcriptional analysis of novel tetrachloroethene reductive dehalogenase gene from *Desulfitobacterium* sp. strain KBC1. Appl Microbiol Biotechnol 69:543–553. doi:10.1007/s00253-005-0022-x

Utkin I, Woese C, Wiegel J (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, Dalton DD, Wiegel J (1995) Specificity of reductive dehalogenation of substituted ortho-chlorophenols by *Desulfitobacterium dehalogenans* JW/IU-DC1. Appl Environ Microbiol 61:346–351

van de Pas BA, Smidt H, Hagen WR, van der Oost J, Schraa G, Stams AJ, de Vos WM (1999) Purification and molecular characterization of ortho-chlorophenol reductive dehalogenase, a key enzyme of halorespiration in *Desulfitobacterium dehalogenans*. J Biol Chem 274:20287–20292

van de Pas BA, Gerritse J, de Vos WM, Schraa G, Stams AJ (2001a) Two distinct enzyme systems are responsible for tetrachloroethene and chlorophenol reductive dehalogenation in *Desulfitobacterium* strain PCE1. Arch Microbiol 176:165–169

van de Pas BA, Harmsen HJ, Raangs GC, de Vos WM, Schraa G, Stams AJ (2001b) A *Desulfitobacterium* strain isolated from human feces that does not dechlorinate chloroethenes or chlorophenols. Arch Microbiol 175:389–394

Villemur R (2013) The pentachlorophenol-dehalogenating *Desulfitobacterium hafniense* strain PCP-1. Philos Trans R Soc Lond B Biol Sci 368:20120319. doi:10.1098/rstb.2012.0319

Villemur R, Lanthier M, Beaudet R, Lépine F (2006) The *Desulfitobacterium* genus. FEMS Microbiol Rev 30:706–733. doi:10.1111/j.1574-6976.2006.00029.x

Villemur R, Constant P, Gauthier A, Shareck M, Beaudet R (2007) Heterogeneity between 16S ribosomal RNA gene copies borne by one *Desulfitobacterium* strain is caused by different 100-200 bp insertions in the 5′ region. Can J Microbiol 53:116–128. doi:10.1139/w06-111

Wiegel J, Zhang X, Wu Q (1999) Anaerobic dehalogenation of hydroxylated polychlorinated biphenyls by *Desulfitobacterium dehalogenans*. Appl Environ Microbiol 65:2217–2221

Woo PCY, Teng JLL, Leung K-W, Lau SKP, Woo GKS, Wong ACY, Wong MKM, Yuen K-Y (2005) *Anaerospora hongkongensis* gen. nov. sp. nov., a novel genus and species with ribosomal DNA operon heterogeneity isolated from an intravenous drug abuser with pseudobacteremia. Microbiol Immunol 49:31–39

Yang Y, Pesaro M, Sigler W, Zeyer J (2005) Identification of microorganisms involved in reductive dehalogenation of chlorinated ethenes in an anaerobic microbial community. Water Res 39:3954–3966. doi:10.1016/j.watres.2005.07.010

Ye L, Schilhabel A, Bartram S, Boland W, Diekert G (2010) Reductive dehalogenation of brominated ethenes by *Sulfurospirillum multivorans* and *Desulfitobacterium hafniense* PCE-S. Environ Microbiol 12:501–509. doi:10.1111/j.1462-2920.2009.02093.x

Yoshida N, Asahi K, Sakakibara Y, Miyake K, Katayama A (2007) Isolation and quantitative detection of tetrachloroethene (PCE)-dechlorinating bacteria in unsaturated subsurface soils contaminated with chloroethenes. J Biosci Bioeng 104:91–97. doi:10.1263/jbb.104.91

Zhao S, Ding C, He J (2015) Detoxification of 1,1,2-trichloroethane to ethene by desulfitobacterium and identification of its functional reductase gene. PLoS ONE 10:e0119507. doi:10.1371/journal.pone.0119507