Desulfitobacterium hafniense sp. nov., an Anaerobic, Reductively Dechlorinating Bacterium

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Strain DCB-2^T (T = type strain) (T. Madsen and D. Licht, *Appl. Environ. Microbiol.* 58:2874–2878, 1992) is an anaerobic, spore-forming bacterium that is capable of reductive dechlorination of chlorophenols. The cells of this strain are rod shaped and 3.3 to 6 µm long by 0.6 to 0.7 µm wide and occur singly and in pairs. Short chains are formed. Spores are terminal. This bacterium is motile, and each cell has one or two terminal flagella. Cells in the exponential and stationary phases are gram negative. This organism does not hydrolyze gelatin and is indole positive and catalase negative, and the guanine-plus-cytosine content of its cellular DNA is 47 mol%. The optimum temperature for growth is 37°C. Only pyruvate and tryptophan are used as substrates. Pyruvate and 2,4,6-trichlorophenol are converted to acetate, CO₂, and 4-chlorophenol by strain DCB-2^T. When grown on pyruvate, this bacterium produces sulfide if thiosulfate or sulfite is added as an electron acceptor. Fe(III) is reduced to Fe(II), but Mn(IV) is not reduced. Sulfate is not reduced to sulfide in the presence of pyruvate or other carbon sources typically used by sulfate-reducing bacteria. Cytochrome *c* is present, but desulfoviridin is not. DCB-2^T reductively dechlorinates 3-chloro-4-hydroxyphenylacetate to 4-hydroxyphenylacetate and conserves energy from the reaction. 16S rRNA sequencing revealed that strain DCB-2^T clusters with the *Clostridium*-*Bacillus* subphylum and groups with *Desulfitobacterium dehalogenans* and *Desulfotomaculum orientis*. *Desulfotomaculum orientis* does not dechlorinate 2,4,6-trichlorophenol. On the basis of the phylogenetic and physiological differences and similarities of strain DCB-2^T, *Desulfitobacterium dehalogenans*, and *Desulfotomaculum orientis*, we concluded that DCB-2^T belongs to the genus *Desulfitobacterium*. We propose that strain DCB-2 is the type strain of *Desulfitobacterium hafniense* sp. nov.

Halogenated hydrocarbons are man-made compounds that, because of their widespread use as pesticides and degreasing and impregnation agents, are common environmental pollutants. The ability to detoxify these compounds is present in many sediments, sludges, and soils when they are incubated in the laboratory (7, 15, 16, 20, 21, 26, 44). Reductive dechlorination is the first step during anaerobic degradation of chlorinated hydrocarbons. Dechlorinating activity has been studied by using various sediment samples obtained from contaminated and noncontaminated sites. It has been shown that the dechlorinating ability of a sample does not always correlate with previous exposure to pollutants (16, 25, 27). Conventional granular sludge that has the ability to degrade pentachlorophenol does not degrade 3-chlorobenzoate (2). However, 3-chlorobenzoate-dechlorinating ability can be introduced into the same sludge by immobilizing *Desulfomonile tiedjei*, a 3-chlorobenzoate-degrading pure culture, on the sludge granules (2). The prospect of using new strains with specific dechlorinating activities is promising, as there is a potential for producing active cell mass which can be used for treating recalcitrant waste and polluted water. Therefore, the availability of and data on cultures that are capable of reductive dechlorination are important as these strains can be used for bioremediation.

Workers have isolated some pure cultures that are capable of aryl reductive dechlorination, including a *Flavobacterium* sp., *Rhodococcus chlorophenolicus*, and a *Rhodococcus* sp. that dechlorinates pentachlorophenol aerobically (6, 19, 37). A facultatively anaerobic, rod-shaped organism that reductively de-

chlorinates 2-chlorophenol was isolated by Cole et al. from an anaerobic culture enriched on acetate and 2-chlorophenol (11). Another facultative anaerobe, *Staphylococcus epidermidis*, dechlorinates 1,2,4-trichlorobenzene, mainly to 1,2-dichlorobenzene and then to monochlorobenzene (45). The following three strictly anaerobic bacteria that dechlorinate chlorinated aromatic compounds have been isolated previously: strain DCB-1 (39), which was named *Desulfomonile tiedjei* (14), *Desulfitobacterium dehalogenans*, and strain DCB-2^T (T = type strain) (31). In this paper we characterize strain DCB-2^T and name this organism *Desulfitobacterium hafniense* sp. nov.

MATERIALS AND METHODS

Medium and conditions for cultivation. The medium which we used was a slightly modified BA medium; 1.00 liter was prepared by adding 10.0 ml of stock solution A, 2.0 ml of stock solution B, 1.0 ml of stock solution C, 1.0 ml of stock solution D, 50.0 ml of a 52.0-g/liter NaHCO₃ solution, and 1.0 g of yeast extract (Difco) to 916 ml of redistilled water. Stock solutions A, B, C, and D have been described previously (5). The medium was gassed with N₂-CO₂ (4:1), adjusted to pH 7.0, and distributed anaerobically into serum vials. The vials were closed with butyl rubber stoppers and aluminum crimps. The medium was autoclaved at 140°C for 20 min. Before use, 10.0 ml of medium was reduced with 0.10 ml of a sterile filtered Ti(III) citrate solution (49) and 0.10 ml of a vitamin solution (catalog no. 141; Deutsche Sammlung von Mikroorganismen und Zellkulturen) (13). Unless indicated otherwise, 18.2 mM pyruvate was added as the carbon source.

The medium used for sulfate reduction experiments was prepared like the medium described for cultivation of *Desulfotomaculum sapomandens*, *Desulfotomaculum geothermicum*, *Desulfotomaculum acetoxidans*, and *Desulfovibrio* sp. (13). In addition, modified BA medium and Postgate's medium were also used (35). All media were prepared without carbon sources and reducing agents before they were autoclaved. Before use, 0.10 ml of the sterile filtered vitamin solution and Ti(III) citrate were added as described above for the modified BA medium.

Modified BA medium was used for the Mn(IV) experiments. For the Fe(III) experiment we used a medium described by Albrechtsen et al. (3) lacking resazurin, Trypticase peptone, yeast extract, sodium sulfide, and L-cysteine hydrochloride.

The control organisms used for the motility, catalase, gelatin liquefaction,

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indole production, and Gram stain reaction tests were grown in Luria-Bertani medium (13).

To test for aerobic growth on blood agar plates, the medium was prepared by autoclaving 30.0 g of tryptone soya broth (Oxoid) and 12.0 g of agar in 950 ml of distilled water at 120°C for 20 min. A 50.0-ml portion of fresh calf blood was added aseptically before the medium was distributed into sterile petri dishes under aerobic conditions.

Desulfovibrio medium was used to test the dechlorinating ability of *Desulfotomaculum orientis* (13).

Organisms and inocula. Strain DCB-2^T was kindly provided by Dorthe Licht (Department of Environmental Science and Engineering, Technical University of Denmark, Lyngby, Denmark). Unless indicated otherwise, the inoculum used in experiments was a culture that was pregrown on modified BA medium supplemented with 40 µM 2,4,6-trichlorophenol and 18.2 mM pyruvate. Organisms with known positive and negative reactions were also included in each test. The control organisms used were *Escherichia coli* MC4100 (for Gram staining and the catalase, gelatin liquefaction, and indole production tests), *Proteus vulgaris* (for the gelatin liquefaction test), *Pseudomonas aeruginosa* (for the indole and catalase tests), and *Lactobacillus lactis* subsp. *cremoris* FD1 (for Gram staining). *Desulfovibrio gigas* and *Desulfotomaculum* sp. were used as control organisms in the desulfovibridin production experiment and were grown as described above.

Tests performed with strain DCB-2^T. Staining experiments were performed with cultures in the exponential and stationary growth phases. Gram staining and the aerobic growth, motility, and catalase tests were performed by using standard methods (18). The gelatin test was done in tubes containing BA medium supplemented with 12% gelatin. Two tubes were inoculated with DCB-2^T, and two tubes were included as uninoculated controls. The indole test was performed by standard methods (18) by growing DCB-2^T in modified BA medium containing 1.0 g of tryptophan per liter. An uninoculated vial containing substrate and an inoculated vial lacking substrate were used as additional controls.

Temperature. The optimum temperature for growth was determined by using 5% DCB-2^T cultures in modified BA medium incubated at 19, 25, 30, 35, 37, 40, 45, and 55°C. Tubes containing substrate but no inoculum or inoculum but no substrate were used as controls. The optical densities at 578 nm of the cultures were measured with time, and the growth rates were calculated from the linear portions of semilogarithmic plots of optical density versus time.

Substrate utilization. Growth with different carbon sources was determined in modified BA media. Most carbon sources were added from sterile stock solutions; the exceptions were the amino acids and esculin, which were autoclaved together with the medium. The final concentration of each carbon source was 1.0 g/liter. Electron acceptors were added at a concentration of 2 mM (thiosulfate) or 100 µM (2,4,6-trichlorophenol) and were also added to the controls. The growth experiments were done in triplicate with two controls. The first control contained substrate but no inoculum, and the second control contained inoculum but no substrate. The optical densities at 578 nm of the cultures were determined before and after inoculation. Positive tubes were defined as those tubes which showed an increase in optical density of more than a factor 4 compared with the control.

Carbon balance. The carbon balance was examined in duplicate vials containing 20 ml of modified BA medium without yeast extract and inoculated with a 5% DCB-2^T culture. 2,4,6-trichlorophenol, pyruvate, and 0.25 g of Na₂S · 7-9H₂O per liter were added to the cultures. Samples were examined at zero time and on day 19. The CO₂, volatile fatty acids, ethanol, and 2,4,6-trichlorophenol contents were determined by gas chromatography as described previously (21, 33, 41). The 2,4-dichlorophenol, 4-chlorophenol, and pyruvate contents were determined by high-performance liquid chromatography (HPLC) as described previously (2, 33).

Electron acceptors. Growth with 2.0 mM Na₂SO₃, 2.0 mM Na₂S₂O₃, 2.0 mM Na₂SO₄, and 2.0 mM NaNO₃ as electron acceptors was examined twice in triplicate by using modified BA medium. The test compounds were added from sterile stock solutions. Growth was determined by measuring the optical density at 578 nm. After growth the tubes were examined for sulfide production by adding 0.10 ml of 0.20 M FeCl₂ · 4H₂O. Tubes were considered positive if FeS precipitated. Fe(III) reduction was examined by measuring the Fe(II) content by the ferrozine method (43). Reduction of Mn(IV)O₂ (insoluble) was examined by measuring the formation of Mn(II) (soluble) by atomic absorption spectrophotometry immediately after sampling (4). Two controls were included; one control preparation did not contain the test compound, and one control preparation did not contain inoculum.

Strain DCB-2^T was examined to determine whether it reduced sulfate with different carbon sources; 2.0 mM Na₂SO₄ and the different carbon sources (each at a concentration of 1.0 g/liter) were added to 20 ml of modified BA medium before the medium was inoculated with an exponential-growth-phase culture of strain DCB-2^T (inoculum size, 5% [vol/vol]). The experiment was done in triplicate. The vials were checked for sulfide production by determining whether FeS precipitated as described above.

Strain DCB-2^T was also checked for sulfate-reducing capacity on the different media described above by using duplicate vials containing 30.0 ml of medium supplemented with 18.2 mM pyruvate or 1.0 g of lactate per liter after autoclaving. The medium was inoculated (inoculum size, 33% [vol/vol]) with a culture that had been pregrown twice on modified BA medium reduced with 0.10 g of thioglycolate per liter instead of Ti(III) citrate. For each medium, one control

FIG. 1. Strain DCB-2^T visualized by indirect immunofluorescence. Bar = 10 µm.

without a carbon source and inoculum was included. After approximately 3 weeks of incubation, the vials were checked for sulfide production (10).

Energy conservation with 3-Cl-4OHPA as the electron acceptor. Modified BA medium was inoculated (inoculum size, 5% [vol/vol]) with a DCB-2^T culture that had been pregrown on medium containing pyruvate and 3-chloro-4-hydroxyphenylacetate (3-Cl-4OHPA). Ti(III) citrate was replaced as the reducing agent by 0.25 g of Na₂S · 7-9H₂O per liter. Pyruvate (20 mM) was added to the vials along with various concentrations of 3-Cl-4OHPA. The experiments were performed in quadruplicate. Protein contents were determined after incubation by using a slightly modified method based on the method of Lowry et al. (28). 3-Cl-4OHPA and 4-hydroxyphenylacetate contents were determined by using the HPLC system described previously (2). A solution containing 0.01% H₃PO₄, 96% ethanol, and methanol (14:3:8) was used as the eluent and was pumped through the system at a rate of 0.7 ml/min. 3-Cl-4OHPA was detected at 275 nm. The samples were diluted 20 times before injection.

G+C content. The guanine-plus-cytosine (G+C) content of the cellular DNA was determined by HPLC (32) at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH.

16S rRNA sequencing. The 16S rRNA sequence of strain DCB-2^T was determined at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH. This sequence was compared with more than 200 sequences of members of the *Clostridium-Bacillus* subphylum. Pairwise evolutionary distances were computed by using the correction factor of Jukes and Cantor (23). A phylogenetic tree was constructed from the distance matrix by using the algorithm of DeSoete (12).

Tests for the presence of cytochromes and desulfovibridin. The presence of cytochromes was determined by recording the reduced-minus-oxidized spectra of the membrane and cytoplasmic fraction as described by Weston and Knowles (47). Whether desulfovibridin was present was checked by obtaining an absorption spectrum (48) and using Postgate's procedure (36).

Antibody probes. Calibrated antibody probes were derived from rabbit antisera against strain DCB-2^T and were used for indirect immunofluorescence tests, as described previously (29, 30).

Determining the dechlorinating ability of *Desulfotomaculum orientis*. The dechlorinating ability of *Desulfotomaculum orientis* DSM 765^T was determined by using 10 µM 2,4,6-trichlorophenol and 1.0 g lactate per liter in triplicate cultures. An uninoculated vial and an inoculated vial without 2,4,6-trichlorophenol were used as controls; 10.0 ml of *Desulfovibrio* medium (13) was used for this experiment.

Nucleotide sequence accession number. The 16S rRNA sequence of strain DCB-2^T has been deposited in the EMBL data library under accession number X94975.

RESULTS

Dechlorinating bacterial strain DCB-2^T is a spore-forming, curved, rod-shaped organism whose cells occur singly, in pairs (31), and in small chains. The cells are 3.3 to 6 µm long and 0.6 to 0.7 µm wide (31). Strain DCB-2^T is motile, and normally each cell has one terminal flagellum. Occasionally, we observed two flagella with fluorescent antibody probes against the bacterium (Fig. 1), which proved to be very useful for visualizing flagella. Flagella were also visualized by using the Leifson staining procedure (data not shown) (18).

FIG. 2. Specific growth rates of strain DCB-2^T at different temperatures. Error bars indicate the standard deviations.

Strain DCB-2^T was catalase negative and did not grow on aerobic blood agar plates. It did not liquefy gelatin and was indole positive. The G+C content was 47 mol%. The optimum temperature for growth was 37°C (Fig. 2). In contrast to the findings of Madsen and Licht (31), cultures in the exponential and stationary phases were gram negative in this study. This finding was verified by using both Hucker's method and Burke's method (18) for Gram staining and both positive and negative control cultures.

We found that DL-tryptophan supported fermentative growth of strain DCB-2^T. Pyruvate supported fermentative growth, as well as growth with all of the electron acceptors tested. L-Arabinose, D-glucose, D-fructose, D-galactose, L-rhamnose, D-ribose, cellobiose, xylose, lactose, maltose, mannose, sucrose, raffinose, trehalose, D-mannitol, benzoate, lactate, acetate, butyrate, propionate, formate, fumarate, sorbitol, salicin, inulin, starch, glycerol, esculin, L-histidine, L-(+)-glutamine, L-threonine, and L-phenylalanine did not support fermentative growth. No growth in the presence of sulfate was observed with the following carbon sources: formate, benzoate, lactate, glycerol, maltose, lactose, mannitol, acetate, glucose, propionate, butyrate, rhamnose, ribose, arabinose, galactose, mannose, and fucose. No growth was observed with thiosulfate added as an electron acceptor with following carbon sources: L-arabinose, D-glucose, D-fructose, D-galactose, L-rhamnose, D-ribose, fucose, mannose, raffinose, D-mannitol, lactate, acetate, butyrate, propionate, and formate. No growth was observed with 2,4,6-trichlorophenol added as an electron acceptor with following carbon sources: L-arabinose, D-glucose, D-galactose, D-ribose, lactose, maltose, glycerol, benzoate, lactate, acetate, butyrate, propionate, and formate.

Sulfide was detected in the cultures grown on pyruvate in the presence of thiosulfate or sulfite but not in the control cultures or any culture containing sulfate. Also, no sulfate reduction was observed with the following carbon sources: formate, benzoate, lactate, glycerol, maltose, lactose, mannitol, acetate, glucose, propionate, butyrate, rhamnose, ribose, arabinose, galactose, mannose, and fucose. Sulfate reduction did not occur in modified BA medium. However, phylogenetically closely related bacteria were sulfate reducers. Therefore, in order to test further for any sulfate-reducing activity, we grew strain DCB-2^T in various media normally used for cultivation of sulfate-reducing bacteria with lactate or pyruvate as the carbon source. No sulfate reduction was detected under these conditions.

The carbon balance experiment showed that pyruvate was degraded to acetate and CO₂ (Table 1). 2,4,6-Trichlorophenol

TABLE 1. Carbon balance with DCB-2^T grown with pyruvate and 2,4,6-trichlorophenol

Expt	Compound	Amt of carbon in vial at ^a :	
		Zero time	Day 19
1	CO ₂	198 (6)	295 (10)
	Acetate	57 (1)	690 (14)
	Pyruvate	767 (55)	0.0 (0.0)
2	4-Chlorophenol	0.0 (0.0)	460 (37)
	2,4-Dichlorophenol	7 (7)	5.7 (0.1)
	2,4,6-Trichlorophenol	469 (26)	0.0 (0.0)

^a The amounts of carbon are expressed in millimoles for experiment 1 and in nanomoles for experiment 2. The numbers in parentheses are standard deviations. The levels of recovery in experiments 1 and 2 were 97% ± 4% and 98% ± 4%, respectively.

was dechlorinated to 4-chlorophenol.

The hydrogen peroxide-oxidized-minus-dithionite-reduced spectrum of the cytoplasmic and membrane fraction had peaks at 550, 520, and 417 nm, which indicated that cytochrome *c* was present (47). A positive reaction was not observed when the Postgate desulfovirdin test was performed, and no absorption peak was observed at 630 nm (48) in the oxidized spectrum. In contrast, when the positive control, *Desulfovibrio gigas*, was used, the Postgate desulfovirdin test was clearly positive, with absorption at 630 nm; when the negative control, a *Desulfotomaculum* sp., was used, the test was negative.

3-Cl-4-OHPA was dechlorinated to 4-hydroxyphenylacetate (data not shown). 3-Cl-4OHPA was recovered as 4-hydroxyphenylacetate after growth had stopped in the eight vials used in the energy conservation experiment. The protein content increased with increasing concentration of 3-Cl-4OHPA (Fig. 3), showing that DCB-2^T conserved energy from the dechlorination reaction.

The phylogenetic relationships of DCB-2^T to other members of the *Clostridium-Bacillus* subphylum are shown in Fig. 4 and Table 2. The levels of similarity between the 16S rRNA of strain DCB-2^T and the 16S rRNAs of the related organisms *Desulfitobacterium dehalogenans* and *Desulfotomaculum orientis* DSM 765^T were 97.4 and 93.9%, respectively. After this relationship was identified, we determined a new *Desulfotomaculum orientis* sequence as the sequence in the database had a large number of undetermined or missing nucleotides. At the present time, complete sequence data are not available for all of the validly described species belonging to the genus

FIG. 3. Protein yield of DCB-2^T grown on 3-Cl-4OHPA and 18.2 mM pyruvate.

FIG. 4. Phylogenetic position of *Desulfotomaculum hafniense* DCB-2^T within the radiation of the *Clostridium-Bacillus* subphylum. Scale bar = 10 changes per 100 nucleotides.

Desulfotomaculum, but a comparison of the strain DCB-2^T and *Desulfotomaculum dehalogenans* sequences with unpublished partial sequences (less than 600 bases) of the other *Desulfotomaculum* species revealed that strain DCB-2^T and *Desulfotomaculum dehalogenans* are unique and group only with

Desulfotomaculum orientis. No dechlorination of 2,4,6-trichlorophenol was observed in tests performed with *Desulfotomaculum orientis*.

DISCUSSION

Information about microorganisms that catalyze reductive dechlorinating reactions is steadily increasing. Several strictly anaerobic bacteria that are capable of reductive dechlorination of chlorinated aliphatic and aromatic compounds have been isolated (14, 22, 38, 39, 46). Strain DCB-2^T, *Desulfomonile tiedjei*, and *Desulfotomaculum dehalogenans* are the only strictly anaerobic bacteria that have been obtained in pure culture that can reductively dechlorinate chlorinated aromatic compounds. Because there are very few isolates available and because of the limited data on these organisms, we wanted to establish the phylogenetic relationships of these organisms.

According to the results of our 16S rRNA sequence analysis, DCB-2^T grouped with *Desulfotomaculum dehalogenans* at a level of similarity of 97.4%, which clearly indicates that DCB-2^T is a member of a new species. However, when we compared morphology as well as physiological differences, we concluded that DCB-2^T belongs to the genus *Desulfotomaculum*. The organism that is most closely related to DCB-2^T and *Desulfotomaculum dehalogenans* is *Desulfotomaculum orientis*, and the levels of similarity (93.9 and 94.1%, respectively) clearly separate the genus containing DCB-2^T and *Desulfotomaculum dehalogenans* from *Desulfotomaculum orientis*. We found no relationship between these organisms and the reductively dechlorinating organism *Desulfomonile tiedjei* (14).

Strain DCB-2^T and *Desulfotomaculum dehalogenans* are capable of reductive dechlorination of most chlorinated phenols at the *ortho* position; the only exception is 2-chlorophenol (31, 46). However, strain DCB-2^T dechlorinates 3,5-dichlorophenol and 2,4,5-trichlorophenol to 3-chlorophenol and 3,4-dichlorophenol, respectively, in contrast to *Desulfotomaculum dehalogenans*, which does not dechlorinate these chlorophenol isomers. Both bacteria catalyze reductive dechlorination of 3-

TABLE 2. Levels of 16S ribosomal DNA similarity for strain DCB-2^T and representatives of the *Clostridium-Bacillus* subphylum

Species	% 16S ribosomal DNA similarity														
	Strain DCB-2 ^T	<i>Desulfotomaculum dehalogenans</i>	<i>Desulfotomaculum orientis</i>	<i>Heliobacterium chlorum</i>	<i>Desulfotomaculum nigrificans</i>	<i>Desulfotomaculum ruminis</i>	<i>Desulfotomaculum australicum</i>	<i>Syntrophomonas wolfei</i>	<i>Syntrophospora bryantii</i>	<i>Moorella thermoacetica</i>	<i>Thermoanaerobacterium thermosulfurigenes</i>	<i>Clostridium oceanicum</i>	<i>Clostridium lituseburense</i>	<i>Sporohalobacter lortetii</i>	<i>Clostridium coccooides</i>
<i>Desulfotomaculum dehalogenans</i>	97.4														
<i>Desulfotomaculum orientis</i>	93.9	94.4													
<i>Heliobacterium chlorum</i>	85.7	86.5	85.6												
<i>Desulfotomaculum nigrificans</i>	84.6	85.0	84.2	83.6											
<i>Desulfotomaculum ruminis</i>	84.8	85.7	84.7	83.6	90.3										
<i>Desulfotomaculum australicum</i>	84.4	84.8	85.4	82.7	85.5	83.8									
<i>Syntrophomonas wolfei</i>	84.9	84.9	86.5	83.0	82.0	82.2	84.7								
<i>Syntrophospora bryantii</i>	85.1	85.3	86.2	83.9	81.7	82.4	84.6	94.0							
<i>Moorella thermoacetica</i>	85.2	85.3	86.4	83.7	85.2	83.1	86.9	86.1	85.4						
<i>Thermoanaerobacterium thermosulfurigenes</i>	82.3	82.4	82.7	82.1	81.6	81.2	83.6	83.3	83.9	86.7					
<i>Clostridium oceanicum</i>	82.9	82.9	83.9	81.4	80.6	80.7	80.3	84.8	84.4	83.3	81.7				
<i>Clostridium lituseburense</i>	81.3	82.1	82.1	80.2	81.2	81.1	81.0	81.4	81.6	83.0	81.1	83.7			
<i>Sporohalobacter lortetii</i>	78.7	78.9	79.8	78.4	78.0	77.9	80.5	79.2	80.7	82.4	79.7	80.5	80.5		
<i>Clostridium coccooides</i>	82.0	82.7	81.7	81.6	80.9	80.6	78.9	81.8	82.8	81.4	80.4	82.0	82.4	78.3	
<i>Megasphaera elsdenii</i>	81.1	81.9	83.0	80.2	80.0	80.6	79.6	80.6	80.0	83.0	80.9	78.6	78.5	78.7	79.6
<i>Propionigenium modestum</i>	78.4	78.9	79.3	77.7	76.6	77.0	76.5	77.6	77.6	78.8	76.5	76.2	76.3	76.3	76.8

TABLE 3. Selected properties of strain DCB-2^T, *Desulfotobacterium dehalogenans*, and *Desulfotomaculum orientis*

Organism	Cell morphology	Spore formation	Gram stain reaction	Fermentative growth with pyruvate	Electron acceptors				G+C content (mol%)
					Sulfate	Nitrate	Sulfite	Thiosulfate	
DCB-2 ^T	Rod	+	—	+	—	+	+	+	47
<i>Desulfotobacterium dehalogenans</i>	Rod	—	+	+	—	+	+	+	45
<i>Desulfotomaculum orientis</i>	Rod	+	—	—	+	—	+	+	45

Cl-4OHPA to 4-hydroxyphenylacetate and are capable of conserving energy by this reaction.

Strain DCB-2^T has an optimum temperature of around 37°C, and the optimum temperature for *Desulfotobacterium dehalogenans* is 38°C (46). Both of these organisms use pyruvate as a carbon and energy source and use nitrate (31), thiosulfate, and sulfite as electron acceptors. Pyruvate and 2,4,6-trichlorophenol are converted to mainly acetate, CO₂, and 4-chlorophenol, and *Desulfotobacterium dehalogenans* also converts pyruvate to acetate and CO₂ when it is grown with 3-Cl-4OHPA. Of the more than 30 other carbon sources tested, the only other substrate used for growth by DCB-2^T was tryptophan. Indole was produced. The organism probably cleaved tryptophan to indole and pyruvate, with the latter serving as a growth substrate. A similar mechanism has been described previously (42). We did not test whether *Desulfotobacterium dehalogenans* is able to utilize tryptophan.

Sulfide is produced from thiosulfate and sulfite by strain DCB-2^T and *Desulfotobacterium dehalogenans* (46). Sulfate was not used as an electron acceptor. Nitrate is reduced to nitrite and ammonia by strain DCB-2^T, while nitrate is reduced to nitrite by *Desulfotobacterium dehalogenans* (46). Fe(III) was reduced by DCB-2^T; this characteristic has not been investigated for *Desulfotobacterium dehalogenans*. When thiosulfate, sulfate, and 2,4,6-trichlorophenol were added as electron acceptors, growth was observed only with pyruvate. Lactate and formate did not support growth when 2,4,6-trichlorophenol was added as an electron acceptor. In contrast, growth of *Desulfotobacterium dehalogenans* was supported by lactate and formate when 3-Cl-4OHPA was added as an electron acceptor.

The cells of both DCB-2^T and *Desulfotobacterium dehalogenans* are slightly curved rods that are motile (31, 46). Each strain DCB-2^T cell has one or two terminal flagella, while each *Desulfotobacterium dehalogenans* cell has one to four flagella. Strain DCB-2^T produces spores, however *Desulfotobacterium dehalogenans* does not. The G+C content of DCB-2^T is 47 mol%, and the G+C content of *Desulfotobacterium dehalogenans* is 45 mol%. DCB-2^T is gram negative, while *Desulfotobacterium dehalogenans* is gram positive.

Desulfotomaculum orientis is a related sulfate-reducing organism that reduces sulfate to sulfide (8, 9, 34). Characteristics of *Desulfotomaculum orientis*, *Desulfotobacterium dehalogenans*, and strain DCB-2^T are shown in Table 3. *Desulfotomaculum orientis* grows fermentatively with lactate (24), whereas pyruvate is used only in the presence of sulfate. DCB-2^T, *Desulfotobacterium dehalogenans*, and *Desulfotomaculum orientis* use sulfite and thiosulfate as electron acceptors. However, *Desulfotomaculum orientis* does not reduce nitrate or reductively dechlorinate chlorophenols. *Desulfotomaculum orientis*, DCB-2^T, and *Desulfotobacterium dehalogenans* are strictly anaerobic, gram-negative, curved, rod-shaped organisms whose cells occur in pairs or in small chains (1). They are motile and have one or more flagella (24). The spores of *Desulfotomaculum orientis* are round and occur centrally or paracentrally; in contrast, DCB-2^T spores are terminal and *Desulfotobacterium de-*

halogenans does not produce spores. The G+C content of *Desulfotomaculum orientis* is 45 mol% (40), which is similar to the *Desulfotobacterium dehalogenans* G+C content but lower than the value determined for strain DCB-2^T (47 mol%). No gelatinase activity and no desulfoviridin were found in *Desulfotomaculum orientis* and DCB-2^T.

On the basis of the phylogenetic and physiological differences of DCB-2^T, *Desulfotobacterium dehalogenans*, and *Desulfotomaculum orientis*, we concluded that DCB-2^T does not belong to the genus *Desulfotomaculum* but belongs to the genus *Desulfotobacterium*. Therefore, we propose that strain DCB-2^T should be placed in a new *Desulfotobacterium* species, *Desulfotobacterium hafniense*.

Description of *Desulfotobacterium hafniense* sp. nov. *Desulfotobacterium hafniense* (haf. ni. en' se. M. L. n. *Hafnia*, Copenhagen; L. suff. -ensis, native of; M. L. adj. *hafniense*, native of Copenhagen, referring to the place of isolation).

Morphology. Cells are rod shaped and gram negative and occur singly, in pairs, and in short chains; they are approximately 3.3 to 6 µm long and 0.6 to 0.7 µm wide and motile and have one or two terminal flagella. Spores are formed, and the spores are terminal.

Physiology. Obligate anaerobe. At pH 7 the optimum growth temperature is near 37°C. Only pyruvate and tryptophan support growth. Acetate, CO₂, 2,4-dichlorophenol, and 4-chlorophenol are formed during growth with pyruvate and 2,4,6-trichlorophenol. L-Arabinose, D-glucose, D-fructose, D-galactose, L-rhamnose, D-ribose, cellobiose, xylose, lactose, maltose, mannose, sucrose, raffinose, trehalose, D-mannitol, benzoate, lactate, acetate, butyrate, propionate, formate, fumarate, sorbitol, salicin, inulin, starch, glycerol, esculin, L-histidine, L-(+)-glutamine, L-threonine, and L-phenylalanine do not support growth. There is no sulfate reduction or growth in the presence of sulfate with the following carbon sources: formate, benzoate, lactate, glycerol, maltose, lactose, mannitol, acetate, glucose, propionate, butyrate, rhamnose, ribose, arabinose, galactose, mannose, and fucose. There is no growth when thiosulfate is added as an electron acceptor with following carbon sources: L-arabinose, D-glucose, D-fructose, D-galactose, L-rhamnose, D-ribose, fucose, mannose, raffinose, D-mannitol, lactate, acetate, butyrate, propionate, and formate. There is no growth when 2,4,6-trichlorophenol is added as an electron acceptor with following carbon sources: L-arabinose, D-glucose, D-galactose, D-ribose, lactose, maltose, glycerol, benzoate, lactate, acetate, butyrate, propionate, and formate. Reduces nitrate to ammonia and nitrite. Thiosulfate, sulfite, and Fe(III) are also reduced in the presence of pyruvate. Does not reduce Mn(IV) or sulfate in the presence of pyruvate. Reductively dechlorinates pentachlorophenol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, 2,4-dichlorophenol, 3,5-dichlorophenol, and 3-Cl-4OHPA. Does not dechlorinate 3,4,5-trichlorophenol, 3,4-dichlorophenol, 2-chlorophenol, 3-chlorophenol, and 4-chlorophenol. Conserves energy from reductive dechlorination of 3-Cl-4OHPA.

Contains cytochrome *c* but no desulfoviridin. Indole positive

and catalase negative. Does not liquify gelatin. The cellular DNA G+C content is 47 mol%. Isolated from municipal sludge.

Desulfotobacterium hafniense DCB-2^T has been deposited in the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH.

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