Dehalogenimonas alkenigignens sp. nov., a chlorinated-alkane-dehalogenating bacterium isolated from groundwater

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Two strictly anaerobic bacterial strains, designated IP3-3^T and SBP-1, were isolated from groundwater contaminated by chlorinated alkanes and alkenes at a Superfund Site located near Baton Rouge, Louisiana (USA). Both strains reductively dehalogenate a variety of polychlorinated aliphatic alkanes, including 1,2-dichloroethane, 1,2-dichloropropane, 1,1,2,2-tetrachloroethane, 1,1,2-trichloroethane and 1,2,3-trichloropropane, when provided with hydrogen as the electron donor. To clarify their taxonomic position, strains IP3-3^T and SBP-1 were characterized using a polyphasic approach. Both IP3-3^T and SBP-1 are mesophilic, non-spore-forming, non-motile and Gram-stain-negative. Cells of both strains are irregular cocci with diameters of 0.4–1.1 μm. Both are resistant to ampicillin and vancomycin. The genomic DNA G+C contents of strains IP3-3^T and SBP-1 are 55.5±0.4 and 56.2±0.2 mol% (HPLC), respectively. Major cellular fatty acids include $C_{18:1}\omega9c$, $C_{16:0}$, $C_{14:0}$ and $C_{16:1}\omega9c$. 16S rRNA gene sequence based phylogenetic analyses indicated that the strains cluster within the phylum *Chloroflexi* most closely related to but distinct from the species *Dehalogenimonas lykanthroporepellens* (96.2 % pairwise similarity) and *Dehalococcoides mccartyi* (90.6 % pairwise similarity). Physiological and chemotaxonomic traits as well as phylogenetic analysis support the conclusion that these strains represent a novel species within the genus *Dehalogenimonas* for which the name *Dehalogenimonas alkenigignens* sp. nov. is proposed. The type strain is IP3-3^T (=JCM 17062^T=NRRL B-59545^T).

At present, the genus *Dehalogenimonas* contains a single species, *Dehalogenimons lykanthroporepellens*, the type strain of which was isolated from groundwater containing high concentrations of chlorinated solvents (Moe *et al.*, 2009). Strains of this strictly anaerobic species are able to reductively dehalogenate a variety of industrially and environmentally important polychlorinated ethanes and propanes. Due to spills and past inappropriate disposal methods, these chlorinated organic compounds are prevalent contaminants of groundwater and soil (De Wildeman & Verstraete, 2003; Fletcher *et al.*, 2009). PCR-based methods to detect and quantify *Dehalogenimons lykanthroporepellens* indicated that the species had relatively wide spatial distribution and high cell numbers in the groundwater at the site from which it was first isolated (Yan *et al.*, 2009b), even with the presence of dense non-aqueous-phase liquids in the subsurface (Bowman *et al.*, 2006).

To characterize the microbial population present in groundwater of the Scenic Highway portion of the PetroProcessors of Louisiana, Inc. (PPI) Superfund site located near Baton Rouge, LA (USA), 454 pyrosequencing was used to construct 16S rRNA gene libraries from groundwater collected from various wells between January and December 2009. Analysis of the gene libraries revealed that groundwater from two wells contained a large

Abbreviations: 1,2-DCA, 1,2-dichloroethane; 1,2-DCP, 1,2-dichloropropane; DGGE, denaturing gradient gel electrophoresis; SEM, scanning electron microscopy; 1,1,2,2-TeCA, 1,1,2,2-tetrachloroethane; 1,1,2-TCA, 1,1,2-trichloroethane; 1,2,3-TCP, 1,2,3-trichloropropane.

The GenBank/EMBL/DDBJ accession numbers for the16S rRNA gene sequences of strain IP3-3^T (=JCM 17062^T =NRRL B-59545^T) and strain SBP-1 are JQ994266 and JQ994267, respectively.

A supplementary figure and three supplementary tables are available with the online version of this paper.

percentage of sequences (7.7 %, 4617 of 60 334 sequences, and 9.3 %, 3478 of 37 583 sequences) clustering within the class *Dehalococcoidia* (Löffler *et al.*, 2013) of the phylum *Chloroflexi* but with <97 % sequence identity with previously isolated strains with sequences in the public databases. A subsequent effort to isolate representatives of these bacterial groups resulted in recovery of two novel anaerobic bacterial strains, IP3-3^T and SBP-1, which were characterized using a polyphasic approach to clarify their taxonomic position.

Groundwater was collected from PPI Superfund site monitoring wells with locations, contaminant concentrations and geochemistry as summarized in Tables S1 to S3 available in IJSEM Online. Immediately following collection (in sterile glass bottles filled leaving little or no gas headspace), the groundwater was amended with titanium citrate solution (Zehnder & Wuhrmann, 1976) to a final concentration of 1 mM Ti(III) and 2 mM citrate to maintain anaerobic conditions during transport to the laboratory.

An enrichment culture was established from groundwater collected from well ID no. IP-3 by inoculating 10 ml groundwater into a 160 ml serum bottle containing 90 ml defined basal medium prepared as described by Yan *et al.* (2009a) except that titanium citrate [1 mM Ti(III) and 2 mM citrate] replaced sodium sulfide as a reducing agent and 5 mM acetate was replaced by 5 mM each of acetate, pyruvate and lactate. The medium was amended with 1,1,2-trichloroethane (1,1,2-TCA) to reach a final aqueous-phase concentration of 0.5 mM. After four months incubation under dark, static conditions at 30 °C, the enrichment culture was diluted to 10^{-10} using 1,1,2-TCA spiked media as prepared for the enrichment culture. After incubation at 30 °C in the dark for two months, all of the 1,1,2-TCA was transformed to vinyl chloride. Following seven consecutive transfers of the culture [with 2 % (v/v) inoculum] and repeated dilution-to-extinction, strain IP3-3^T was recovered from a 10^{-10} dilution. Strain SBP-1 was recovered using an identical dilution-to-extinction approach but with groundwater collected from well ID no. SBP-017-B. The strains were preserved in anaerobic medium amended with 5 % (v/v) filter-sterilized DMSO and stored at −80 °C.

Culture purity was confirmed by microscopy and observation of single bands in denaturing gradient gel electrophoresis (DGGE) following extraction of genomic DNA and PCR amplification using universal bacterial primers 341f and 907r as described previously (Li & Moe, 2004). PCR employing primers DHC 774 and DHC 1212 (Hendrickson *et al.*, 2002) and primers 582f and 728r (Duhamel *et al.*, 2004) targeting 16S rRNA gene sequences unique to *Dehalococcoides* strains did not produce amplicons. PCR employing primers BL-DC-727f and BL-DC-1020r (Yan *et al.*, 2009b) targeting 16S rRNA gene sequences unique to *Dehalogenimonas lykanthroporepellens* also did not produce amplicons.

Unless stated otherwise, all tests were carried out following strict anaerobic procedures in basal medium prepared as described and used in the isolation of strain IP3-3^T except that acetate, pyruvate and lactate concentrations were lowered from 5 mM to 0.05 mM. Experiments were performed in duplicate, with H_2 : CO_2 : N_2 (10 % : 10 % : 80 %, by vol.) as the headspace gas, a 2 % (v/v) inoculum and incubation in the dark at 30 °C without shaking. Chlorinated solvent concentrations and degradation products in the headspace gas and the aqueous phase were quantified by gas chromatography as described by Yan *et al.* (2009a). *Dehalogenimonas lykanthroporepellens* BL-DC-9^T was included in all testing for comparative purposes.

The potential of strains IP3-3^T and SBP-1 to reductively dehalogenate various chlorinated alkanes [1,2,3-trichloropropane (1,2,3-TCP), 1,2-dichloropropane (1,2-DCP), 1-chloropropane, 2-chloropropane, 1,1,2,2-tetrachloroethane (1,1,2,2-TeCA), 1,1,1-trichloroethane, 1,1,2-trichloroethane (1,1,2-TCA), 1,2-dichloroethane (1,2-DCA), 1,1-dichloroethane, tetrachloromethane (carbon tetrachloride), trichloromethane (chloroform) and dichloromethane (methylene chloride)], chlorinated alkenes (perchloroethene, trichloroethene, *cis*-1,2-dichloroethene, *trans*-1,2-dichloroethene, vinyl chloride), chlorinated benzenes (1,2-dichlorobenzene, chlorobenzene) was determined in serum bottles (25 ml) each containing 15 ml basal medium. Chlorinated solvents were purchased from Sigma–Aldrich except trichloroethene (Mallinckrodt Baker), chloroform (Fisher Scientific) and dichloromethane (Fisher Scientific). Other than 1,1,2-TCA (96 % purity), chloroform [American Chemical Society (ACS) reagent grade] and dichloromethane (pesticide grade), all chemicals used were ⩾98 % purity. Certified gas standards for vinyl chloride (1002 p.p.m. in N_2), ethene (99.5 %) and propene (99 %) were purchased from Sigma–Aldrich. Neat filter-sterilized chlorinated solvents were added to reach approximately 0.5 mM aqueous phase concentration after equilibration, except for vinyl chloride which was supplied via the gas phase to reach a final aqueous phase concentration of 0.03 mM after equilibration. Inoculated serum bottles spiked with 1,1,2-TCA served as positive controls, and uninoculated, chlorinated-solvent-spiked bottles served as negative controls to account for abiotic transformation. After two months incubation, headspace gas and aqueous phases were analysed for chlorinated solvents and potential degradation products by gas chromatography (Yan *et al.*, 2009a).

Strains IP3-3^T, SBP-1 and *Dehalogenimonas lykanthroporepellens* BL-DC-9^T all reductively dehalogenated 1,2-DCA to ethene, 1,2-DCP to propene, 1,1,2-TCA to vinyl chloride and 1,1,2,2-TeCA to a mixture of *cis*- and *trans*-dichloroethene. In the titanium-citrate-reduced medium tested in this study, all three of the strains also reductively dehalogenated 1,2,3-TCP with the production of allyl chloride (3-chloro-1-propene) as an intermediate product. As observed previously (Yan *et al.*, 2009a), allyl chloride is

an unstable compound and underwent hydrolysis to form allyl alcohol. In this study, allyl chloride and allyl alcohol were the only volatile products detected from 1,2,3-TCP dechlorination. Other products reported by Yan *et al.* (2009a) produced from abiotic reactions of allyl chloride with sulfide (e.g. diallyl sulfide, diallyl disulfide) were not detected. This is consistent with the fact that sulfide was not used as a reducing agent in the present study. Neither IP3-3^T, SBP-1 or *Dehalogenimonas lykanthroporepellens* BL-DC-9^T utilized the other chlorinated compounds tested as potential electron acceptors.

To assess whether reductive dechlorination was linked with cell growth, direct cell counts were performed using a DMRXAZ microscope (Leica) equipped with an A4 filter set (360/40 nm excitation, 470/40 nm emission; Leica) and Slidebook 4.1 imaging software (Intelligent Imaging Innovations) after fixing with glutaraldehyde, staining with 4′,6-diamidino-2-phenylindole (DAPI) and collection on 0.2 μm filters (Supor-200, Pall). In bottles amended with 1,2-DCA, 1,2-DCP, 1,2-TCA, 1,1,2,2-TeCA, cell concentrations of strains IP3-3^T and SBP-1 determined after dechlorination was complete ranged from 22 to 52 times higher than those of controls prepared and incubated under identical concentrations but lacking chlorinated solvents. For 1,2,3-TCP, cell concentrations of strains IP3-3^T and SBP-1 determined after dechlorination was complete ranged from five to eight times higher than controls prepared and incubated under identical concentrations but lacking chlorinated solvents.

The ability of strains IP3-3^T and SBP-1 to use acetate (5 mM), butyrate (5 mM), citrate (5 mM), ethanol (5 mM), formate (5 mM), fructose (5 mM), fumarate (5 mM), glucose (5 mM), lactate (5 mM), lactose (5 mM), methanol (5 mM), methyl ethyl ketone (5 mM), propionate (5 mM), pyruvate (5 mM), succinate (5 mM) and yeast extract (0.5 g l^{-1}) as potential electron donors for reductive dechlorination was assessed in 25 ml serum bottles containing 15 ml basal media. Substrates were added from filter-sterilized 100× or 10× stock solutions. Separate replicates were prepared with headspace gas comprised of CO_2 : N_2 (5 % : 95 %, by vol.) and H_2 : CO_2 : N_2 (10 % : 10 % : 80 %, by vol.). Filter-sterilized 1,1,2-TCA (neat) was added to reach a final aqueous-phase concentration of 0.5 mM. Bottles were incubated for two months prior to determination of the concentrations of chlorinated solvent and degradation products.

In the absence of H_2 in the gas headspace, neither IP3-3^T nor SBP-1 dechlorinated 1,1,2-TCA when supplied with any of the potential electron donors tested. In the presence of H_2 supplied in the gas headspace, 1,1,2-TCA dechlorination to vinyl chloride was essentially complete for all compounds tested except for propionate and lactate.

The temperature and pH range for IP3-3^T and SBP-1 to reductively dechlorinate 1,2-DCP was determined in 25 ml serum bottles containing 15 ml anaerobic basal medium amended with 0.5 mM 1,2-DCP and 5 mM acetate in place of 0.05 mM each of acetate, pyruvate and lactate. Temperatures tested were 10, 18, 23.5, 30, 34, 37, 42 and 45 °C. For pH tests, media was adjusted with 1 M NaOH or 2 M HCl to reach pH 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5. Concentrations of 1,2-DCP and the degradation product propene were measured after two weeks and two months incubation. Samples in which more than 5 % of the chlorinated solvent was transformed were scored as positive for dechlorination. Both IP3-3^T and SBP-1 reductively dechlorinated 1,2-DCP in the temperature range from 18–42 °C (optimum 30–34 °C) but not at temperatures $\leqslant$10 °C or $\geqslant$45 °C. Reductive dechlorination occurred in the pH range of 6.0–8.0 (optimum pH 6.5–7.5), but not at pH $\leqslant$5.5 or $\geqslant$8.5.

The ability of IP3-3^T, SBP-1 and *Dehalogenimonas lykanthroporepellens* BL-DC-9^T to reductively dechlorinate 1,2-DCP at various salt concentrations was determined as described above but in media supplemented with NaCl at concentrations of 1, 2 and 3 % (w/v). Strains IP3-3^T and SBP-1 dechlorinated at NaCl concentrations of 1 % (w/v), but not at 2 %. In contrast, *Dehalogenimonas lykanthroporepellens* BL-DC-9^T dechlorinated 1,2-DCP in media containing NaCl at a concentration of 2 % (w/v).

The ability of strains IP3-3^T, SBP-1 and *Dehalogenimonas lykanthroporepellens* BL-DC-9^T to grow in the presence of oxygen was tested as described by Yan *et al.* (2009a). In anaerobically prepared positive controls, reductive dechlorination was complete after two weeks incubation. After two months incubation, no dechlorination occurred in oxygen-purged serum bottles.

To assess the ability to form visible colonies on solid media, strains IP3-3^T, SBP-1 and *Dehalogenimonas lykanthroporepellens* BL-DC-9^T were streaked on 65 ml basal media solidified with agar (15 g l^{-1}) in a 160 ml serum bottle, amended with 0.5 mM 1,2-DCP. No colonies were observed even after two months incubation for any of the strains.

The ability of the strains to reductively dechlorinate in medium containing sulfide rather than titanium citrate as the reducing agent was determined in 25 ml serum bottles containing 15 ml anaerobic basal medium amended with 0.5 mM 1,2-DCP and either 1.5 or 3 mM sodium sulfide with incubation for two months. Identically prepared medium containing titanium citrate served as a positive control. All three strains dechlorinated 1,2-DCP in titanium-citrate-reduced medium and medium containing 1.5 mM sulfide, but only *Dehalogenimonas lykanthroporepellens* BL-DC-9^T dechlorinated 1,2-DCP in medium containing 3 mM sulfide.

Cells of strains IP3-3^T, SBP-1 and *Dehalogenimonas lykanthroporepellens* BL-DC-9^T grown on 1,2-DCP were Gram stained and visualized by light and scanning electron microscopy (SEM). Motility was assessed using phase-contrast light microscopy (Microphot-FXA; Nikon). Cell morphology was determined from SEM in media

filter-sterilized prior to solvent addition and culture inoculation, as described in Yan *et al.* (2009a), but mounted on aluminium specimen stubs and coated with gold : palladium 60 : 40 in an EMS550X sputter coater; and imaged with JSM-6610 high-vacuum-mode SEM. Cultures of strains IP3-3^T, SBP-1 and *Dehalogenimonas lykanthroporepellens* BL-DC-9^T that had completely consumed the 1,2-DCP were incubated for an additional three weeks, then visualized by SEM to check for spore formation. Gram staining and microscopic imaging results showed that cells of strains IP3-3^T and SBP-1 are Gram-negative staining, non-motile, irregular cocci with diameter of 0.4–1.1 μm (Fig. S1). No spores were observed in cultures depleted of chlorinated solvents for three weeks.

Genomic DNA was extracted from strains IP3-3^T and SBP-1 using a Power Max Soil kit (MoBio Laboratories). 16S rRNA gene sequences were determined as described previously (Rainey *et al.*, 1996). The 16S rRNA gene sequences of strains IP3-3^T and SBP-1 were manually aligned against previously determined sequences available from the public databases. Pairwise similarity values were determined using the facilities of EzTaxon (http://eztaxon-e.ezbiocloud.net/; Kim *et al.*, 2012). The phylogenetic dendrogram (Fig. 1) showing the relationships between strains IP3-3^T and SBP-1 and their related taxa was constructed by the neighbour-joining method using the MEGA 4.0 software package (Tamura *et al.*, 2007). The neighbour-joining algorithm was used to build the phylogenetic tree, with Jukes–Cantor correction (Jukes & Cantor, 1969) followed by bootstrap analysis with PHYLIP 3.62 (Felsenstein, 1985).

On the basis of nearly complete 16S rRNA gene sequences, strains IP3-3^T and SBP-1 are closely related (99.9 % pairwise sequence similarity, 1 bp different). Their closest match in the public databases is a soil/sediment slurry clone, Er-MS-91 (EU542454) from a study on polychlorinated biphenyl dechlorination, with 98 % 16S rRNA gene sequence pairwise similarity. Their closest previously cultured phylogenetic relatives are *Dehalogenimonas lykanthroporepellens* strains BL-DC-9^T and BL-DC-8 but with pairwise sequence similarities in the range 96.0 % to 96.2 %. Strains IP3-3^T and SBP-1 are distantly related to *Dehalococcoides mccartyi*, sharing 16S rRNA gene sequence similarity of only ~91 %.

The genomic DNA G+C content of strains IP3-3^T and SBP-1 was determined by HPLC as described by Mesbah *et al.* (1989) following DNA isolation using an UltraClean Water DNA Isolation kit (MoBio Laboratories). The G+C content of genomic DNA of strains IP3-3^T and SBP-1 as determined by HPLC was 55.5 ± 0.4 mol% and 56.2 ± 0.2 mol%, respectively.

Cells for analysis of cellular fatty acids were grown in anaerobic basal medium prepared as described by Moe *et al.* (2009) with titanium citrate solution (Zehnder & Wuhrmann, 1976) as a reducing agent at a final concentration of 1.0 mM Ti(III), 2.0 mM citrate. The medium was supplemented with 0.5 mM 1,1,2-trichloroethane and 5 mM sodium acetate (in place of 0.05 mM each of acetate, pyruvate and lactate). Cells were harvested via centrifugation (10 000 ***g***) during the mid-exponential growth phase following incubation for three weeks. Cellular fatty acids were extracted, saponified and methylated according to the protocol of the Sherlock v. 6.0 Microbial Identification System (MIDI). The fatty acids were analysed by a GC equipped with the Microbial Identification software package (Sasser, 1990) with peak identification using the MOORE 6.0 and TSBA 6.0 libraries. The major cellular fatty acids of both strains IP3-3^T and SBP-1 included $C_{18:1}\omega 9c$, $C_{16:0}$, $C_{14:0}$ and $C_{16:1}\omega 9c$ (Table 1).

Based on 16S rRNA gene sequence comparisons, strains IP3-3^T and SBP-1 cluster with *Dehalogenimonas*

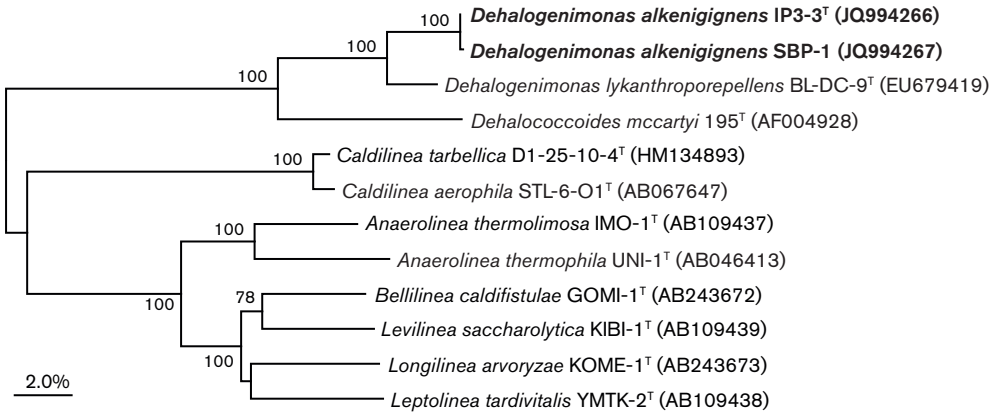

Fig. 1. Neighbour-joining dendrogram based on analysis of 16S rRNA gene sequences showing the phylogenetic relationship of strain IP3-3^T and SBP-1 to taxa of the phylum *Chloroflexi*. Bootstrap values expressed as a percentage of 1000 resamplings are shown at branch points with significant support. Bar, 2 substitutions per 100 nt positions.

Table 1. Comparison of the cellular fatty acids of strains IP3-3^T and SBP-1 (this study) with *Dehalogenimonas lykanthroporepellens* strains BL-DC-9^T and BL-DC-8 (Moe *et al.*, 2009)

Fatty acids occurring at less than 1 % in all strains are not listed. –, Not detected.

PLFA	ECL	Amount of PLFA (% of total)			
		IP3-3^T	SBP-1	BL-DC-9^T	BL-DC-8
UN 11.980	11.980	7.0	4.5	–	–
$C_{12:0}$	12.000	–	–	1.9	1.8
UN 13.768	13.768	2.4	2.0	1.6	1.7
UN 13.937	13.937	1.4	1.1	–	–
$C_{14:0}$	13.999	18.6	16.3	13.0	11.6
UN 15.056	15.056	1.5	0.9	–	–
$C_{16:1}$ $\omega 9c$	15.771	15.7	14.7	22.6	22.6
Summed feature 3*	15.822	–	0.8	2.1	2.7
$C_{16:0}$	16.000	20.8	23.5	20.3	16.8
$C_{18:3}\omega 6c$(6,9,12)	17.577	1.3	0.6	–	–
Summed feature 5†	17.722	–	0.8	6.3	7.7
$C_{18:1}\omega 9c$	17.771	22.7	27.8	22.9	21.4
Summed feature 8‡	17.827	0.8	–	–	4.6
$C_{18:0}$	18.000	6.5	5.7	7.0	7.0

*Summed feature 3: $C_{16:1}\omega 7c$ and/or $C_{16:1}\omega 6c$ and/or $C_{15:0}$ iso 2-OH.
†Summed feature 5: $C_{18:2}\omega 6c$ and/or $C_{18:2}\omega 9c$ and/or $C_{18:0}$ anteiso.
‡Summed feature 8: $C_{18:1}\omega 7c$ and/or $C_{18:1}\omega 6c$.

lykanthroporepellens BL-DC-8 and BL-DC-9^T but represent a distinct lineage (96.0 % to 96.2 % sequence similarity) supported by a high bootstrap value (Fig. 1), within the recently described order *Dehalococcoidales* in the class *Dehalococcoidia* of the phylum *Chloroflexi* (Löffler *et al.*, 2013). The low sequence similarity (~96 %) in 16S rRNA gene sequences of strains IP3-3^T and SBP-1 when compared with *Dehalogenimonas lykanthroporepellens* in combination with phenotypic differences supports classification as a different species.

Additional genomic and chemotaxonomic characteristics differentiate IP3-3^T and SBP-1 from *Dehalogenimonas lykanthroporepellens* strain BL-DC-9^T. The genomic DNA G+C contents of strains IP3-3^T and SBP-1 as determined by HPLC (55.5 ± 0.4 and 56.2 ± 0.2, respectively) are slightly higher than those of *Dehalogenimonas lykanthroporepellens* strains BL-DC-8 and BL-DC-9^T (54.0 and 53.8 mol%, respectively, Moe *et al.*, 2009) determined using the same method.

The major cellular fatty acids determined for strains IP3-3^T and SBP-1 ($C_{18:1}\omega 9c$, $C_{16:0}$, $C_{14:0}$ and $C_{16:1}\omega 9c$) were also present in *Dehalogenimonas lykanthroporepellens* BL-DC-8 and BL-DC-9^T. The proportion of $C_{16:1}\omega 9c$, however, was higher in strains BL-DC-8 and BL-DC-9^T and the proportion of $C_{14:0}$ was higher in IP3-3^T and SBP-1 (Table 1). The proportion of unknown 11.980 reached moderate levels in strains IP3-3^T and SBP-1 (7.0 and 4.5 %, respectively) but was undetectable in *Dehalogenimonas lykanthroporepellens* strains. Summed feature 5 ($C_{18:2}\omega 6c$, $C_{18:2}\omega 9c$ and/or $C_{18:0}$ anteiso) comprised 6.3 % and 7.7 % in *Dehalogenimonas lykanthroporepellens* strains BL-DC-9^T and BL-DC-8 but was undetected in IP3-3^T and was present in only trace amounts (0.8 %) in SBP-1. Unidentified cellular fatty acids with equivalent chain-lengths (ECLs) of 11.980, 13.937 and 15.056, as well as $C_{18:3}\omega 6c$ (6,9,12), were also detected in strains IP3-3^T and SBP-1 but not in *Dehalogenimonas lykanthroporepellens* strains (Table 1). These differences in fatty acid composition clearly distinguish IP3-3^T and SBP-1 as a separate species from *Dehalogenimonas lykanthroporepellens*.

Strains IP3-3^T and SBP-1 share several common phenotypic features with *Dehalogenimonas lykanthroporepellens* strains BL-DC-8 and BL-DC-9^T including strictly anaerobic respiration, requirement for H_2 as an electron donor, use of polyhalogenated alkanes as electron acceptors, coupling of cell growth with reductive dehalogenation, Gram-negative staining, small irregular cell morphology and resistance to the antibiotics ampicillin and vancomycin (Moe *et al.*, 2009). As with *Dehalogenimonas lykanthroporepellens* BL-DC-9^T and BL-DC-8, all reductive dechlorination reactions determined to date for strains IP3-3^T and SBP-1 appear to involve an exclusively dihaloelimination reaction mechanism (involving simultaneous removal of two chlorines from adjacent carbon atoms and formation of a carbon–carbon double bond). However, IP3-3^T and SBP-1 differ from *Dehalogenimonas lykanthroporepellens* strain BL-DC-9^T phenotypically in their inability to reductively dehalogenate in the presence

of 2 % NaCl or in medium containing 3 mM sodium sulfide.

On the basis of genotypic, chemotaxonomic and phenotypic features, the newly isolated strains IP3-3^T and SBP-1 are clearly distinct from other species in the phylum *Chloroflexi*. We propose that strains IP3-3^T and SBP-1 be placed as a new species within the genus *Dehalogenimonas* with the name *Dehalogenimonas alkenigignens* sp. nov.

Description of *Dehalogenimonas alkenigignens* sp. nov.

Dehalogenimonas alkenigignens (al.ke.ni.gi′gnens. N.L. n. *alkenum* alkene; L. part. adj. *gignens* giving birth to, producing; N.L. part. adj. *alkenigignens* producing alkene, because alkenes are produced during anaerobic reductive dehalogenation of chlorinated alkanes).

Cells are Gram-negative staining, non-motile, non-spore-forming, irregular cocci with diameter of 0.4–1.1 μm. Reductively dehalogenates polychlorinated aliphatic alkanes including 1,2-dichloroethane, 1,2-dichloropropane, 1,1,2,2-tetrachloroethane, 1,1,2-trichloroethane and 1,2,3-trichloropropane. Chlorobenzene, chloroform, 1-chloropropane, 2-chloropropane, 1,2-dichlorobenzene, *cis*-1,2-dichloroethene, *trans*-1,2-dichloroethene, 1,1-dichloroethane, dichloromethane, tetrachloroethene, tetrachloromethane, 1,1,1-trichloroethane, trichloroethene, or vinyl chloride do not serve as electron acceptors. Allyl chloride is produced when grown with 1,2,3-trichloropropane as an electron acceptor and titanium citrate as a reducing agent. Utilizes H_2 as an electron donor. Dechlorination is not supported by acetate, butyrate, citrate, formate, fumarate, lactate, propionate, pyruvate, succinate, ethanol, methanol, fructose, glucose, lactose, methyl ethyl ketone, or yeast extract in the absence of H_2. Resistant to ampicillin (1.0 g l^{-1}) and vancomycin (0.1 g l^{-1}). Grows at 18–42 °C (optimum 30–34 °C) and pH 6.0–8.0 (optimum pH 6.5–7.5). Growth does not occur at NaCl concentrations $\geqslant$2 % (w/v). Does not dechlorinate in 3 mM sulphide-reduced media. Major cellular fatty acids include $C_{18:1}\omega9c$, $C_{16:0}$, $C_{14:0}$ and $C_{16:1}\omega9c$.

The type strain, IP3-3^T (=JCM 17062^T=NRRL B-59545^T), was isolated from chlorinated-solvent-contaminated groundwater at the PPI, Superfund Site, located near Baton Rouge, LA (USA). SBP-1, isolated from groundwater at the same site, is a second strain of the species. The G+C content of genomic DNA of the type strain as determined by HPLC is 55.5±0.4 mol%.

Acknowledgements

This research was funded by the Governor's Biotechnology Initiative of the Louisiana Board of Regents grant BOR#015 and NPC Services. The authors thank Jean Euzéby for assistance with the etymology of the new species and Xiao Ying for assistance with microscopy.

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