Dehalogenimonas formicexedens sp. nov., a chlorinated alkane-respiring bacterium isolated from contaminated groundwater

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Abstract

A strictly anaerobic, Gram-stain-negative, non-spore-forming bacterium designated NSZ-14^T, isolated from contaminated groundwater in Louisiana (USA), was characterized using a polyphasic approach. Strain NSZ-14^T reductively dehalogenated a variety of polychlorinated aliphatic alkanes, producing ethene from 1,2-dichloroethane, propane from 1,2-dichloropropane, a mixture of cis- and trans-1,2-dichloroethene from 1,1,2,2-tetrachloroethane, vinyl chloride from 1,1,2-trichloroethane and allyl chloride (3-chloro-1-propene) from 1,2,3-trichloropropane. Formate or hydrogen could both serve as electron donors. Dechlorination occurred between pH 5.5 and 7.5 and over a temperature range of 20–37°C. Major cellular fatty acids included C_{18:1ω9c}, C_{14:0} and C_{16:0}. 16S rRNA gene sequence-based phylogenetic analysis indicated that the strain clusters within the class Dehalococcoida of the phylum Chloroflexi, most closely related to but distinct from type strains of the species Dehalogenimonas alkenigignens (97.63 % similarity) and Dehalogenimonas lykanthroporepellens (95.05 %). A complete genome sequence determined for strain NSZ-14^T revealed a DNA G+C content of 53.96 mol%, which was corroborated by HPLC (54.1 ±0.2 mol% G+C). Genome-wide comparisons based on average nucleotide identity by orthology and estimated DNA–DNA hybridization values combined with phenotypic and chemotaxonomic traits and phylogenetic analysis indicate that strain NSZ-14^T represents a novel species within the genus Dehalogenimonas, for which the name Dehalogenimonas formicexedens sp. nov. is proposed. The type strain is NSZ-14^T (=HAMBI 3672^T=JCM 19277^T=VKM B-3058^T). An emended description of Dehalogenimonas alkenigignens is also provided.

At the time of writing, the genus Dehalogenimonas contains two recognized species, Dehalogenimonas lykanthroporepellens and Dehalogenimonas alkenigignens. The type strains of both species were isolated from groundwater contaminated by chlorinated solvents at a Superfund site located near Baton Rouge, Louisiana (USA) [1, 2]. Strains of both strictly anaerobic species are able to reductively dehalogenate a variety of polychlorinated ethanes and propanes that occur as widespread anthropogenic groundwater pollutants [1, 2]. In an effort to further characterize the microbial community present in contaminated groundwater near where the type strains of both Dehalogenimonas lykanthroporepellens and Dehalogenimonas alkenigignens were first discovered, a novel bacterial strain designated as NSZ-14^T was isolated and characterized using a polyphasic approach.

Groundwater from which strain NSZ-14^T was isolated was collected from a monitoring well with location, groundwater contaminant concentrations and geochemistry as summarized in Tables S1–S3 (available in the online Supplementary Material). Immediately following collection (in a sterile glass bottle filled leaving little or no gas headspace), the groundwater sample was amended with titanium citrate solution [3] 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 by inoculating 1.5 ml of groundwater into a glass serum bottle (25 ml) containing 15 ml of defined basal medium prepared as described by Bowman et al. [2] sealed with a butyl rubber stopper and aluminium crimp cap. Headspace gas comprised 10 % H_2.
10 % CO₂ and 80 % N₂ (vol%). The medium was amended with 1,1,2 trichloroethane (1,1,2-TCA) to reach a final aqueous-phase concentration of 0.5 mM as well as the antibiotics ampicillin (1.0 g l⁻¹) and vancomycin (0.1 g l⁻¹) prior to inoculation. Sodium pyruvate, sodium acetate and lactic acid (0.05 mM each) were provided as potential carbon sources. After 2.5 months of incubation at 30 °C without mixing, dechlorination of 1,1,2-TCA to vinyl chloride was observed via GC. Following repeated dilution-to-extinction (10-fold serial dilutions, incubation time 2 months at 30 °C) in medium prepared as above that was amended with 1,1,2 trichloroethane (1,1,2-TCA) to reach a final aqueous concentration of 10⁻⁷ dilution. The strain was preserved in anaerobic medium amended with 5 % (v/v) filter-sterilized DMSO and stored at −80 °C.

Culture purity was supported by microscopy and observation of a single band in denaturing gradient gel electrophoresis following extraction of genomic DNA and PCR amplification using universal bacterial primers 341f and 907r as described previously [4]. PCR targeting 16S rRNA gene sequences unique to Dehalococcoides strains using primer combinations 582f and 728r [5] and DHC 774 and DHC 1212 [6] did not produce amplicons. PCR employing primer sets designated as M, N, O, Q, S, T, V, W and Y [7] targeting 16S rRNA gene sequences unique to Dehalogenimonas lykanthroporepellens also did not produce amplicons [8].

The 16S rRNA gene sequence of strain NSZ-14T was determined as described by Rainey et al. [9] following genomic DNA extraction using an UltraClean Microbial DNA Isolation Kit (MoBio Laboratories). The 16S rRNA gene sequence of strain NSZ-14T was manually aligned against previously determined sequences available from the public databases. Pairwise similarity values were determined using the EzBioCloud service [10]. The phylogenetic dendrogram (Fig. 1) showing the relationship between strain NSZ-14T and related taxa was reconstructed by the neighbour-joining method using the MEGA 4.0 software package [11]. The neighbour-joining algorithm was used to build the phylogenetic tree, with Jukes–Cantor correction [12] followed by bootstrap analysis with PHYLP 3.62 [13].

On the basis of nearly complete 16S rRNA gene sequences, the type strains most closely related to strain NSZ-14T are Dehalogenimonas alkenigenigens IP3-3T (pairwise similarity 97.63 %) and Dehalogenimonas lykanthroporepellens BL-DC-9T (95.05 %). Pairwise sequence similarity between strain NSZ-14T and the uncultured trans-dichloroethene-respiring bacterium referred to as Dehalogenimonas sp. WBC-2 [14, 15] was 96.36 %. Strain NSZ-14T is distantly related to Dehalococcoides mccartyi 195T [16], sharing 16S rRNA gene sequence similarity of only 90.05 %.

Unless stated otherwise, all phenotypic tests were carried out following strict anaerobic procedures in basal medium prepared as described above for the isolation of strain NSZ-14T with a mixture of 10 % H₂, 10 % CO₂ and 80 % N₂ (vol%) as the headspace gas. Experiments were performed in at least duplicate with 2 % (v/v) inoculum and incubation in the dark at 30 °C without shaking. Samples in which more than 5 % of the chlorinated solvent was transformed (after accounting for transformation in uninoculated abiotic controls) were scored as positive for dechlorination. Dehalogenimonas alkenigenigens IP3-3T was included in testing for comparative purposes. For formate utilization experiments, Dehalogenimonas lykanthroporepellens BL-DC-9T was also included in testing.

The potential of strain NSZ-14T to reductively dehalogenate chlorobenzene, 1-chloropropane, 2-chloropropane, 1,2-dichlorobenzene, 1,1-dichloroethane, 1,2-dichloroethene (1,2-DCA), cis-1,2-dichloroethene, trans-1,2-dichloroethene, dichloromethane (methylene chloride), 1,2-DCP, 1,1,2,2-tetrachloroethane (1,1,2,2-TeCA), tetrachloroethene, tetrachloromethane (carbon tetrachloride), trichloromethane (chloroform), 1,2,3-trichloropropane (1,2,3-TCP), 1,1,1-trichloroethane, 1,1,2-TCA, trichloroethene and vinyl chloride was determined in serum bottles (25 ml) each containing 15 ml of basal medium. Except for chloroform (Fisher Scientific), dichloromethane (Fisher Scientific), trichloroethene (Mallinckrodt Baker) and vinyl chloride (Scott Specialty Gases), all chlorinated organics were purchased from Sigma-Aldrich. Other than 1,1,2-TCA (96 % purity) and chloroform (ACS reagent grade), all chemicals used were ≥98 % purity. Neat, filter-sterilized chlorinated solvents were added to reach 0.5 mM aqueous phase concentration after equilibration. Inoculated serum bottles spiked with 1,2-DCP served as positive controls and uninoculated, chlorinated solvent spiked bottles served as negative controls. Headspace gas and aqueous phases were analysed for chlorinated solvents and potential degradation products using an Agilent model 7820A gas chromatograph equipped with a flame ionization detector and a DB-624 capillary column (60 m × 0.32 mm × 1.8 µm). Headspace gas samples collected in 100 ml gas-tight glass syringes were introduced to the chromatograph via direct injection. Aqueous samples were introduced to the chromatograph via a Teledyne Tekmar Stratum purge and trap concentrator.

Strain NSZ-14T and Dehalogenimonas alkenigenigens IP3-3T both 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, both 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 [17], allyl chloride is an unstable compound that undergoes abiotic 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. [17] 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
NSZ-14\textsuperscript{T} nor \textit{Dehalogenimonas alkenigignign} \textsuperscript{}IP-3-3\textsuperscript{T} utilized the other chlorinated compounds tested as potential electron acceptors even after an incubation time of 2 months.

In an additional test, ion chromatography performed as described previously \cite{18} was used to measure chloride concentration when strain NSZ-14\textsuperscript{T} was grown in 1,2-DCP-amended low-chloride medium prepared as described above but with chloride-containing salts replaced with bromide-containing salts. Mass balance calculations revealed that the increase in chloride concentration was roughly twice the increase in propene on a molar basis, consistent with the stoichiometric release of two moles of chloride and one mole of propene per mole of 1,2-DCP completely dechlorinated via a dihaloelimination reaction pathway (Fig. S1).

To assess whether reductive dechlorination was linked with cell growth, quantitative real-time PCR (qPCR) was performed using primer set DHG-2 targeting 16S rRNA gene sequences unique to the genus \textit{Dehalogenimonas} as described previously \cite{8}. In bottles amended with 1,2-DCA, 1,2-DCP, 1,2-TCA, 1,1,2,2-TeCA and 1,2,3-TCP, the 16S rRNA gene copies of strain NSZ-14\textsuperscript{T} determined after dechlorination was complete ranged from 13 to 44 times higher than controls prepared and incubated under identical concentrations but lacking chlorinated solvents.

To determine if formate could replace H\textsubscript{2} as an electron donor for dechlorination by strain NSZ-14\textsuperscript{T}, experiments were conducted using medium supplemented with sodium formate (added from a 100 mM, filter-sterilized stock solution) to a final concentration of 1.0 mM in bottles containing a gas headspace of CO\textsubscript{2}/N\textsubscript{2} (5\%:95 \%, v/v) and an initial aqueous-phase 1,2-DCP concentration of 0.5 mM. Abiotic negative controls consisted of identically prepared serum bottles without microbial inoculation. Serum bottles without formate but with H\textsubscript{2} supplied in the gas headspace (10 \%, v/v) served as positive controls. Medium containing 1.0 mM formate but no H\textsubscript{2} or 1,2-DCP was included as an additional control to assess whether growth occurred with formate in the absence of a chlorinated alkane as an electron acceptor. 1,2-DCP and propene concentrations were measured using GC, formate concentrations were measured using ion chromatography and cell growth was assessed by qPCR as described above.

When strain NSZ-14\textsuperscript{T} was supplied with 1.0 mM formate and 0.5 mM 1,2-DCP in bottles with CO\textsubscript{2}/N\textsubscript{2} in the gas headspace (i.e. without exogenous H\textsubscript{2}), dechlorination of 1,2-DCP was essentially complete within 12 days with near stoichiometric production of the daughter product propene. Concurrently, the formate concentration decreased and 16S rRNA gene copies increased. The fraction of electrons from the electron donor (f\textsubscript{e}) used to reduce 1,2-DCP was estimated by plotting the micromoles of formate consumed versus micromoles of propene produced in bottles sacrificed at various time intervals. The slope of the resulting regression line (R\textsuperscript{2}=0.95), which is equivalent to the f\textsubscript{e}, was 0.76 (Fig. S2). The yield for strain NSZ-14\textsuperscript{T} grown with formate as the electron donor, (3.6±2.1)\times10\textsuperscript{8} 16S rRNA gene copies/umole propene produced, was essentially the same as the yield with H\textsubscript{2} as the electron donor, (3.8±2.0)\times10\textsuperscript{8} 16S rRNA gene copies/umole propene produced. There was no statistically significant decrease in formate concentration or increase in 16S rRNA gene copies in inoculated serum bottles amended with formate when 1,2-DCP was omitted. Likewise, there was no statistically significant increase in 16S rRNA gene copies when both H\textsubscript{2} and formate were omitted. In uninoculated abiotic controls, <0.5 \% of the starting 1,2-DCP was recovered as propene, indicating minimal abiotic 1,2-DCP transformation. Sequential transfer in medium amended with formate and 1,2-DCP without exogenous supply of H\textsubscript{2} demonstrated that strain NSZ-14\textsuperscript{T} could achieve essentially complete (>99 \%) dechlorination over at least five transfers.

![Neighbour-joining tree based on analysis of 16S rRNA gene sequences showing the phylogenetic relationship of strain NSZ-14\textsuperscript{T} to taxa of the phylum Chloroflexi.](image-url)
Dehalogenimonas alkenigignens IP3-3<sup>T</sup> and Dehalogenimonas lykanthroporepellens BL-DC-9<sup>T</sup> were also found to dechlorinate 1,2-DCP to propene with a concomitant increase in 16S rRNA gene copies in formate-amended medium lacking H<sub>2</sub> in the gas headspace. The yields for Dehalogenimonas alkenigignens IP3-3<sup>T</sup> and Dehalogenimonas lykanthroporepellens BL-DC-9<sup>T</sup> with formate as the electron donor, (2.3±1.2)×10<sup>6</sup> and (1.5±0.6)×10<sup>8</sup> 16S rRNA gene copies/µmole propene produced, respectively, were also similar to the yields with H<sub>2</sub> as the electron donor, (5.0±4.5)×10<sup>6</sup> and (2.0±1.0)×10<sup>8</sup> 16S rRNA gene copies/µmole propene produced, respectively. Both Dehalogenimonas alkenigignens IP3-3<sup>T</sup> and Dehalogenimonas lykanthroporepellens BL-DC-9<sup>T</sup> could also achieve essentially complete (>99%) dechlorination over at least five sequential transfers in formate-amended medium lacking H<sub>2</sub> in the gas headspace.

It should be noted that Bowman et al. [2] reported that Dehalogenimonas alkenigignens IP3-3<sup>T</sup> did not dechlorinate using formate as an electron donor, albeit in medium supplied with 1,1,2-TCA rather than 1,2-DCP as an electron acceptor and with higher concentrations of the potential carbon sources acetate, lactate and pyruvate (5 mM each). Here, we conclusively demonstrated that Dehalogenimonas alkenigignens IP3-3<sup>T</sup> can in fact use formate as an electron donor, at least for reductive dechlorination of 1,2-DCP.

The ability of strain NSZ-14<sup>T</sup> to use citrate (5 mM), methyl ethyl ketone (5 mM), propionate (5 mM), pyruvate (5 mM), starch (5 mM), succinate (5 mM) and yeast extract (0.5 g l<sup>−1</sup>) as potential electron donors for reductive dechlorination was assessed in 25 ml serum bottles containing 15 ml of basal medium. Substrates were added from filter-sterilized (0.2 µm syringe filters; Fischer Scientific) 100× or 10× stock solutions. Separate replicates were prepared with headspace gas comprising CO<sub>2</sub>/N<sub>2</sub> (5.95%, v/v) and H<sub>2</sub>/CO<sub>2</sub>/N<sub>2</sub> (10:10:80%, by vol.). Filter sterilized 1,2-DCP ( neat) was added to reach a final aqueous-phase concentration of 0.5 mM. Bottles were incubated for at least 1 month prior to determination of 1,2-DCP and propene concentrations via GC. None of these additional compounds tested as potential electron donors resulted in more than 5% transformation of the starting 1,2-DCP to propene when H<sub>2</sub> was omitted from the gas headspace.

The temperature and pH range for strains to reductively dechlorinate 1,2-DCP was determined in 25 ml serum bottles containing 15 ml anaerobic basal medium. Temperatures tested were 4, 15, 20, 22.5, 30, 34, 37, 42 and 45 °C. For pH tests, media were adjusted with 1 M NaOH or 2 M HCl to reach pH 5.0, 5.5, 6.0, 6.5, 7.0, 7.2, 7.5, 8.0 and 8.5. Concentrations of 1,2-DCP and the dechlorination product propene were measured after 2 weeks and 2 months of incubation. Strain NSZ-14<sup>T</sup> reductively dechlorinated 1,2-DCP in the temperature range 20–37 °C (optimum 30–34 °C) but not at temperatures ≤15 °C or ≥42 °C. In contrast, Dehalogenimonas alkenigignens IP3-3<sup>T</sup> dechlorinated 1,2-DCP in media incubated at 15 and 42 °C. For strain NSZ-14, reductive dechlorination occurred in the pH range 5.5–7.5 (optimum pH 7.0–7.2), but not at pH ≤5.0 or ≥8.0. In contrast, Dehalogenimonas alkenigignens IP3-3<sup>T</sup> dechlorinated 1,2-DCP in media at a pH of ≤8.5.

The ability of NSZ-14<sup>T</sup> and Dehalogenimonas alkenigignens IP3-3<sup>T</sup> to reductively dechlorinate 1,2-DCP at various salt concentrations was determined in media supplemented with NaCl to final concentrations of 0.1, 1.1, 2.1, 3.1 and 4.1% (w/v). Strain NSZ-14<sup>T</sup> dechlorinated at NaCl concentrations of 0.1% (w/v), but not at ≥1.1%. In contrast, Dehalogenimonas alkenigignens IP3-3<sup>T</sup> dechlorinated 1,2-DCP in media containing NaCl at a concentration of 1.1% (w/v).

The ability of strain NSZ-14<sup>T</sup> and D. alkenigignens IP3-3<sup>T</sup> to grow in the presence of oxygen was tested as described by Yan et al. [17]. In anaerobically prepared positive controls, reductive dechlorination was complete after 2 weeks of incubation. After 2 months of incubation, no dechlorination occurred in oxygen-purged serum bottles or serum bottles exposed briefly to oxygen. Samples with oxygen in the headspace remained aerobic throughout the incubation period, as indicated by resazurin.

To assess the ability to form visible colonies on solid media, strain NSZ-14<sup>T</sup> and Dehalogenimonas alkenigignens IP3-3<sup>T</sup> were streaked on 65 ml basal medium solidified with agar (15 g l<sup>−1</sup>) in a 160 ml serum bottle, amended with 0.5 mM 1,2-DCP. No colonies were observed even after 2 months of incubation for either strain.

The ability of the strains to reductively dechlorinate in medium containing 1.5 mM sodium 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 with incubation for 2 months. Identically prepared medium containing titanium citrate served as a positive control. Both strains dechlorinated 1,2-DCP in medium containing 1.5 mM sulfide.

The ability of strain NSZ-14<sup>T</sup> and Dehalogenimonas alkenigignens IP3-3<sup>T</sup> to reductively dechlorinate in the presence of high concentrations of chlorinated solvents [19] was determined in media amended with various initial 1,2-DCA aqueous-phase concentrations as high as 13.6±0.3 mM. Concentrations of the 1,2-DCA dechlorination product ethene were measured after 2 months of incubation. Dechlorination was essentially complete after 2 months of incubation for initial aqueous phase 1,2-DCA concentrations of 5.5±0.2 mM and lower for both strain NSZ-14<sup>T</sup> and Dehalogenimonas alkenigignens IP3-3<sup>T</sup>. For initial aqueous phase 1,2-DCA concentrations above 6.5±0.1 and 5.5±0.2 mM for strain NSZ-14<sup>T</sup> and Dehalogenimonas alkenigignens IP3-3<sup>T</sup>, respectively, the quantity of ethene produced decreased as the initial 1,2-DCA concentration increased, indicating that at sufficiently high concentration, 1,2-DCA became inhibitory (Fig. S3). 1,2-DCA reductive dechlorination, however, was observed in samples with initial aqueous phase 1,2-DCA concentrations as high as 6.5±0.1 and 9.3±0.2 mM for strain NSZ-14<sup>T</sup> and Dehalogenimonas alkenigignens IP3-3<sup>T</sup>.
detected at higher initial aqueous phase 1,2-DCA concentrations, but not in amounts statistically different from abiotic negative controls.

Cells of strain NSZ-14T and *Dehalogenimonas alkenigignens* IP3-3T 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 (Leica DM RXA2). Cell morphology was determined by SEM in medium filter sterilized prior to solvent addition and culture inoculation, as described in Yan et al. [17], but mounted on aluminium specimen stubs and coated with gold/palladium (60:40) in an EMS550X sputter coater and imaged with a JSM-6610 high vacuum mode scanning electron microscope. Cultures of strain NSZ-14T and *Dehalogenimonas alkenigignens* IP3-3T that had completely consumed the 1,2-DCP were incubated for an additional 3 weeks, then visualized by SEM to check for spore formation. Gram staining and microscopic imaging results showed that cells of strain NSZ-14T were Gram-stain-negative, non-motile, irregular cocci with diameters of 0.4–1.1µm (Fig. S4). No spores were observed in cultures depleted of chlorinated solvents for 3 weeks.

Cells for analysis of cellular fatty acids were grown in anaerobic basal medium prepared as described by Moe et al. [1] with titanium citrate solution [3] as a reducing agent at a final concentration of 1.0 mM Ti(III) and 2.0 mM citrate. The medium was supplemented with 0.5 mM 1,1,2-TCA and 5 mM sodium acetate (in place of 0.05 mM each of acetate, pyruvate and lactate). Cells were harvested via centrifugation (30 min at 3500 g) during the mid-exponential growth phase. 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 using a gas chromatograph equipped with the Microbial Identification software package with peak identification using the TSBA 6.0 libraries [20]. The major cellular fatty acids of strain NSZ-14T included C18:1ω9c, C14:0 and C16:0 (Table 1).

To facilitate further phylogenetic comparison, the genome of strain NSZ-14T was sequenced using a combination of PacBio and Illumina platforms. Following growth in anaerobic basal medium as described above supplemented with 2 mM 1,2-DCP, cells were harvested via centrifugation (3500 g, 30 min, 4 °C) and total DNA was extracted using a GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich) following the manufacturer’s recommended protocol. Genomic DNA was fragmented to 20kb and cleaned using a PowerClean DNA Clean-up kit (MoBio) prior to PacBio SMRTbell library construction at the University of Florida Interdisciplinary Center for Biotechnology Research (ICBR, Gainesville, FL, USA). The library was size-selected on the BluePippin (Sage Science) for optimum long-insert sequencing using P5/C3 on the PacBio. A paired-end library with 2×150bp reads was constructed using the Illumina TruSeq preparation protocol and sequenced with a sequencer (Illumina NextSeq) at the University of Georgia Genomics Facility (GGF, Athens, GA, USA).

Two SMRT cells were used for long-read genome sequencing according to the manufacturer’s recommendations (Pacific Biosciences). The complete genome sequence was obtained by combining 1.1 Gb of short- and 332 Mb of long-read sequencing data.

Genes were identified using Prodigal v. 2.6.3 and were functionally annotated using the JGI microbial annotation pipeline [21] followed by manual curation. Genome statistics for strain NSZ-14T in comparison with the type strains of other species in the class *Dehalococcoidia* are shown in Table 2.

**In silico** calculation of estimated genomic DNA–DNA hybridization (DDH) values between strain NSZ-14T and related type strains (Table S4) was performed using GGDC 2.1 [22–24] with BLAST+ at http://ggdc.dsmz.de. Average nucleotide identity by orthology (OrthoANI, Table S5) was calculated using the algorithm of Lee et al. [25] using OAT v0.93 (www.ezbiocloud.net/sw/oat).

The genomic DNA G+C content of strain NSZ-14T determined by HPLC as described by Mesbah et al. [26] following DNA isolation using a GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich) was 54.1±0.2 mol%. This range as measured by HPLC is consistent with the 53.96 mol% G+C content determined from genome sequencing.

Consistent with the experimental observation that strain NSZ-14T could use formate as an electron donor, the annotated genome contains a gene (locus tag Dform_00419) that putatively encodes an selenocysteine-containing formate dehydrogenase. This gene has selenocysteine-containing homologues in the genomes of *Dehalogenimonas alkenigignens* IP3-3T (DEALK_19115) and *Dehalogenimonas lykanthroporepellens* BL-DC-9T (BK009976), with inferred amino acid sequences sharing 53.5 % identity with strain NSZ-14T. *Dehalococcoides* strains with genomes sequenced to date also contain homologues of this gene; however, their inferred amino acid sequence identities are lower (52–53 %) and they are predicted to encode proteins containing serine rather than selenocysteine at a critical location (Fig. S5), a feature speculated to render *Dehalococcoides* strains unable to utilize formate [27, 28]. An operon associated with selenocysteine synthesis and insertion (selCDAB) is present in the genome of strain NSZ-14T (Dform_02176–02180) as well as *Dehalogenimonas alkenigignens* IP3-3T [29] and *Dehalogenimonas lykanthroporepellens* BL-DC-9T [30] but not in *Dehalococcoides mccartyi* genomes sequenced to date.

Consistent with the fact that strain NSZ-14T dechlorinates 1,2-DCP to propene, the genome sequence for NSZ-14T contains a gene (locus tag Dform_01463) encoding a predicted 482 aa protein sharing 96 % sequence identity with the 1,2-dichloropropane-to-propene dehalogenase (DcpA) identified in *Dehalogenimonas lykanthroporepellens* BL-DC-9T (gene locus tag Dehly_1524) [31]. An adjacent, downstream gene (Dform_01464) encodes a putative membrane anchoring protein with multiple predicted transmembrane helices that shares 97 % sequence identity with the putative membrane anchoring protein (Dehly_1525) for the DcpA
identified in *Dehalogenimonas lykanthroporepellens* BL-DC-9\(^T\). An additional 24 putative reductive dehalogenase homologous genes (*rdhA*) and a total of eight genes encoding putative reductive dehalogenase membrane anchoring proteins (*rdhB*) found in the strain NSZ-14\(^T\) genome are most closely related to those found in the genomes of *Dehalogenimonas alkenigignens* IP3-3\(^T\), *Dehalogenimonas lykanthroporepellens* BL-DC-9\(^T\), *Dehalogenimonas* sp. WBC-2, *Dehalococcoides mccartyi* strains, or environmental sequences (Tables S6 and S7). As with the genomes of *Dehalogenimonas alkenigignens* IP3-3\(^T\) and *Dehalogenimonas lykanthroporepellens* BL-DC-9\(^T\), a majority of the *rdhA* genes in strain NSZ-14\(^T\) lack cognate *rdhB* genes (Table S6).

Based on 16S rRNA gene sequence comparisons, strain NSZ-14\(^T\) clusters with *Dehalogenimonas alkenigignens* IP3-3\(^T\) and *Dehalogenimonas lykanthroporepellens* BL-DC-9\(^T\) but represents a distinct lineage supported by a 100 % bootstrap value (Fig. 1), within the recently described order *Dehalococcoides* in the class *Dehalococcoidia*, family *Dehalococcoidaceae*, phylum *Chloroflexi* [32]. The low 16S rRNA gene sequence similarity of strain NSZ-14\(^T\) when compared with *Dehalogenimonas alkenigignens* IP3-3\(^T\) (97.63 %) and *Dehalogenimonas lykanthroporepellens* BL-DC-9\(^T\) (95.05 %) in combination with phenotypic differences supports its classification within a different species.

Additional genomic characteristics differentiate strain NSZ-14\(^T\) from *Dehalogenimonas alkenigignens* IP3-3\(^T\) and *Dehalogenimonas lykanthroporepellens* BL-DC-9\(^T\). The genome of strain NSZ-14\(^T\) (2 092 789 bp, 53.96 mol% G+C) is considerably larger and lower in G+C content than the genome sequences of *Dehalogenimonas alkenigignens* IP3-3\(^T\) (1 849 792 bp, 55.88 mol% G+C) and *Dehalogenimonas lykanthroporepellens* BL-DC-9\(^T\) (1 686 510 bp, 55.04 mol% G+C) (Table 2). *In silico* DDH values calculated based on genome sequences of strain NSZ-14\(^T\) and the related type strains (Table S4) were well below the 60 % cut-off for delineating species by this approach [22–24]. The OrthoANI value from the comparison between strain NSZ-14\(^T\) and *Dehalogenimonas alkenigignens* IP3-3\(^T\) was 75.31 % and that between strain NSZ-14\(^T\) and *Dehalogenimonas lykanthroporepellens* BL-DC-9\(^T\) was 69.72 % (Table S5), well below the 95–96 % range previously recommended for species demarcation [25, 33, 34].

Strain NSZ-14\(^T\) shares several common phenotypic features with *Dehalogenimonas alkenigignens* IP3-3\(^T\) and *Dehalogenimonas lykanthroporepellens* BL-DC-9\(^T\) including strictly anaerobic respiration, small irregular cell morphology, Gram-negative staining, resistance to the antibiotics vancomycin and ampicillin, the use of polychlorinated alkanes as electron acceptors, the use of H\(_2\) and formate as electron donors and the coupling of cell growth with reductive dechlorination [1, 2]. As with *Dehalogenimonas alkenigignens* IP3-3\(^T\) and *Dehalogenimonas lykanthroporepellens* BL-DC-9\(^T\), all reductive dechlorination reactions determined to date for strain NSZ-14\(^T\) appear to involve a

Table 1. Comparison of the cellular fatty acids of strain NSZ-14\(^T\) and related species

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>ECL(^*)</th>
<th>Amount of fatty acid (% of total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NSZ-14(^T)</td>
<td>D. alkenigignens IP3-3(^T)</td>
</tr>
<tr>
<td>UN 11.980</td>
<td>11.980</td>
<td>7.0</td>
</tr>
<tr>
<td>C(_{12})</td>
<td>12.000</td>
<td>2.3±0.2</td>
</tr>
<tr>
<td>UN 13.768</td>
<td>13.768</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>UN 13.937</td>
<td>13.937</td>
<td>–</td>
</tr>
<tr>
<td>C(_{14})</td>
<td>14.000</td>
<td>23.8±0.5</td>
</tr>
<tr>
<td>UN 15.056</td>
<td>15.056</td>
<td>–</td>
</tr>
<tr>
<td>C(<em>{16});6(</em>{9})c</td>
<td>15.773</td>
<td>5.7±0.2</td>
</tr>
<tr>
<td>Summed feature 3†</td>
<td>15.817</td>
<td>1.4±0.1</td>
</tr>
<tr>
<td>C(_{16});0</td>
<td>16.000</td>
<td>21.7±0.4</td>
</tr>
<tr>
<td>Summed feature 9†</td>
<td>16.434</td>
<td>2.0±0.2</td>
</tr>
<tr>
<td>C(<em>{18});6(</em>{9})c(6, 9, 12)</td>
<td>17.577</td>
<td>–</td>
</tr>
<tr>
<td>Summed feature 5†</td>
<td>17.720</td>
<td>1.0±0.1</td>
</tr>
<tr>
<td>C(<em>{18});6(</em>{9})c</td>
<td>17.770</td>
<td>33.5±0.5</td>
</tr>
<tr>
<td>Summed feature 8†</td>
<td>17.825</td>
<td>1.5±0.1</td>
</tr>
<tr>
<td>C(_{18});0</td>
<td>18.000</td>
<td>5.5±0.2</td>
</tr>
</tbody>
</table>

\(*ECL*, equivalent chain-length.
†Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI System: summed feature 3 comprises C\(_{16}\);6\(_{9}\)c and/or C\(_{18}\);6\(_{9}\)c and/or anteiso-C\(_{18}\);0; summed feature 5 comprises iso-C\(_{17}\);1\(_{0}\)9c and/or C\(_{16}\);1\(_{0}\)methyl.

Results are percentages of the total fatty acids (mean ± s.d of two analyses) with data for *Dehalogenimonas alkenigignens* IP3-3\(^T\) [2] and *Dehalogenimonas lykanthroporepellens* BL-DC-9\(^T\) [1]. Fatty acids occurring at least 1 % in all strains are not listed. –, Not detected.
Table 2. Genome statistics for strain NSZ-14\(^T\) and related type strains in the class *Dehalococcoidia*

Data from strain NSZ-14\(^T\) (this study), *Dehalogenimonas alkenigignens* IP3-3\(^T\) [29], *Dehalogenimonas lykanthroporepellens* BL-DC-9\(^T\) [30] and *Dehalococcoides mccartyi* 195\(^T\) [35].

<table>
<thead>
<tr>
<th>Property</th>
<th>NSZ-14(^T)</th>
<th><em>Dehalogenimonas alkenigignens</em> IP3-3(^T)</th>
<th><em>Dehalogenimonas lykanthroporepellens</em> BL-DC-9(^T)</th>
<th><em>Dehalococcoides mccartyi</em> 195(^T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GenBank accession no.</td>
<td>CP018258</td>
<td>LFDV000000000</td>
<td>CP002084</td>
<td>CP000007</td>
</tr>
<tr>
<td>Genome size (bp)</td>
<td>2 092 789</td>
<td>1 849 792</td>
<td>1 686 510</td>
<td>1 469 720</td>
</tr>
<tr>
<td>G+C content (genome) (mol%)</td>
<td>53.96</td>
<td>55.88</td>
<td>55.04</td>
<td>48.85</td>
</tr>
<tr>
<td><strong>Total genes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>tRNA genes</strong></td>
<td>2210</td>
<td>1988</td>
<td>1771</td>
<td>1640</td>
</tr>
<tr>
<td><strong>tRNA genes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rRNA genes (23S : 16S : 5S)</td>
<td>(1:1:1)</td>
<td>(1:1:1)</td>
<td>(1:1:1)</td>
<td></td>
</tr>
<tr>
<td>rDNA coding genes</td>
<td>2156</td>
<td>1936</td>
<td>1720</td>
<td>1591</td>
</tr>
<tr>
<td>DNA coding (%)</td>
<td>89.07</td>
<td>90.17</td>
<td>87.73</td>
<td>90.5</td>
</tr>
</tbody>
</table>

**DESCRIPTION OF *DEHALOGENIMONAS FORMICEXEDENS* SP. NOV.**

*Dehalogenimonas formicexedens* (for.mic.ex.e'dens. N.L. n. *acidum* *foricum* formic acid; L. part. adj. *exedens* eating up; N.L. part. adj. *formicexedens* eating up formate).

Grows at 20–37 °C (30–34 °C optimum) and pH 5.5–7.5 (7.0–7.2 optimum). Chemotrophic. Utilizes formate and H\(_2\) as electron donors. Reductively dehalogenates 1,2-DCA to ethene, 1,2-DCP to propine, 1,1,2,2-TeCA to dichloroethenes, 1,1,2-TCA to vinyl chloride and 1,2,3-TCP to allyl chloride. Able to dechlorinate 1,2-DCA at concentrations at least as high as 6.5±0.1 mM. Resistant to ampicillin (1.0 g l\(^{-1}\)) and vancomycin (0.1 g l\(^{-1}\)). Major cellular fatty acids include C\(_{18:1}\)ω9c, C\(_{14:0}\) and C\(_{16:0}\).

The type strain, NSZ-14\(^T\) (=HAMBI 3672\(^T\)=JCM 19277\(^T\)=VKM B-3058\(^T\)), was isolated from chlorinated solvent contaminated groundwater at a site located near Baton Rouge, LA (USA). The G+C content of the genomic DNA of the type strain as determined from genome sequencing is 53.96 mol%.

**EMENDED DESCRIPTION OF *DEHALOGENIMONAS ALKENIGIGNENS* BOWMAN ET AL. 2013**

The description is as given by Bowman et al. [2] with the following modification. Dechlorination can be supported by formate in the absence of H\(_2\).

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Conflicts of interest

The authors declare that there are no conflicts of interest.

References


18. Moe WM, Stebbing RE, Rao JU, Bowman KS, Nobre MF et al. Pelo- sinus defluvi sp. nov., isolated from chlorinated solvent-

19. Maness AD, Bowman KS, Yan J, Rainey FA, Moe WM. Dehalogeni- monas spp. can reductively dehalogenate high concentrations of 1,2-dichloroethane, 1,2-dichloropropane and 1,1,2-trichloroethane. AMB Express 2012;2:54.


