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Propionicicella superfundia gen. nov., sp. nov., a chlorosolvent-tolerant propionate-forming, facultative anaerobic bacterium isolated from contaminated groundwater

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Abstract

A novel strain, designated as BL-10^T, was characterized using a polyphasic approach after isolation from groundwater contaminated by a mixture of chlorosolvents that included 1,1,2-trichloroethane, 1,2-dichloroethane, and vinyl chloride. Stain BL-10^T is a facultatively anaerobic bacterium able to ferment glucose to form propionate, acetate, formate, lactate, and succinate. Fermentation occurred in the presence of 1,2-dichloroethane and 1,1,2-trichloroethane at concentrations to at least 9.8 and 5.9 mM, respectively. Cells are Gram-positive, rod-shaped, non-motile, and do not form spores. Oxidase and catalase are not produced and nitrate reduction did not occur in PYG medium. Menaquinone MK-9 is the predominant respiratory quinone and *meso*-diaminopimelic acid is present in the cell wall peptidoglycan layer. Major cellular fatty acids are $C_{15:0}$, iso $C_{16:0}$, and anteiso $C_{15:0}$. Genomic DNA G+C content is 69.9 mol%. Phylogenetic analysis based on 16S rRNA gene sequence comparisons showed strain BL-10^T to fall within the radiation of genera *Propionicimonas* and *Micropruina*. On the basis of the results obtained in this study, it is proposed that strain BL-10^T should be classified as a novel taxon, for which the name *Propionicicella superfundia* gen. nov., sp. nov. is proposed. The type strain of *Propionicicella superfundia* is BL-10^T (=ATCC BAA-1218^T, =LMG 23096^T).[1]

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Keywords: *Propionicicella superfundia*; Facultative anaerobe; Propionate; Chlorosolvent tolerant; DNAPL; Dechlorination

Introduction

From 1969 to 1980, petrochemical wastes including free-phase chlorinated solvents were disposed of by direct discharge to earthen ponds at the Brooklawn site, one of two areas that comprise what is now known as the Petro-Processors of Louisiana, Inc. (PPI) EPA

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[1]*Nucleotide sequence accession number*. Nucleotide sequence data reported are available in the DDBJ/EMBL/GenBank databases under the accession number DQ176646.

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Superfund Site, located approximately 10 miles north of Baton Rouge, LA (USA). Portions of the Brooklawn area were capped in the early 1990s, and an array of wells was installed to recover organic contaminants present in the subsurface as dense non-aqueous-phase liquid (DNAPL) [9]. Residual DNAPL remains in the subsurface, and groundwater within the DNAPL source zone contains high concentrations of chlorinated organics including 1,1,2,2-tetrachloroethane, 1,1,2-trichloroethane, 1,2-dichloroethane, 1,2-dichloropropane, hexachloro-1,3-butadiene, and vinyl chloride.

During a study aimed at characterizing the microbial population present within the DNAPL source zone at the PPI site in order to assess the potential for in situ bioremediation, we isolated a novel bacterium designated as strain BL-10^T. To determine its taxonomic position, a polyphasic approach, including phylogenetic, chemotaxonomic, and phenotypic assessment was conducted. Results indicate that strain BL-10^T should be classified as a novel genus and novel species, for which the name *Propionicicella superfundia*, gen. nov, sp. nov. is proposed.

Materials and methods

Isolation and bacterial strains

Groundwater samples were collected from well W-1024-1 located in the DNAPL source zone at the Brooklawn portion of the PPI site. Sterile, 1.0 L glass sample collection bottles were filled with groundwater leaving little or no headspace, and placed on ice during transport to the laboratory (approximately 1 h). After serial dilution with a potassium phosphate buffer (100 mM, pH 7.0) in an anaerobic chamber (Coy Laboratory Products Inc., MI), 0.1 mL aliquots were spread on Columbia Anaerobic Sheep Blood agar plates (CASB, BBL). Plates were incubated in the anaerobic chamber (headspace 90% N_2, 5% CO_2, and 5% H_2) at 30 °C for up to 5 weeks. Isolated colonies were subcultured on CASB agar and purity verified by microscopy. *Propionicimonas paludicola* strains Wd^T and Wf were kindly provided by Professor Atsuko Ueki of the Yamagata University, Japan. These reference strains were maintained on CASB agar or PYG agar [4] in the anaerobic chamber. The isolates were stored at −80 °C in PYG with 15% (v/v) glycerol and 5% (v/v) dimethylsulfoxide.

Growth, morphological, biochemical, and tolerance characteristics

Growth of the organisms was examined in CASB and PYG media. Cell morphology was observed using differential interference contrast (DIC) microscopy (Nikon microphot-Fxa) or transmission electron microscopy (JEOL 1000CX TEM). For TEM, cells were negatively stained using uranyl acetate (2%, v/v). Spore formation and motility were observed using phase contrast microscopy (Nikon Optiphot).

Gram staining was performed using a Gram Stain Kit (Difco) according to the manufacturer's recommended protocol. Catalase activity was assessed as described by Smibert and Krieg [36] using 3% hydrogen peroxide added to cells grown anaerobically on PYG agar plates. Oxidase tests were performed using the tetramethyl-*p*-phenylenediamine dihydrochloride filter paper technique [36]. Aerobic growth was examined on several complex media including nutrient agar (Difco), PYG agar [4], plate count agar (Difco) and R2A (Difco) to which no reducing agent was added. Nitrate reducing ability in PYG cultures containing 10 mM sodium nitrate was determined according to Smibert and Krieg [36]. Additional biochemical and physiological characterization was conducted using Rapid ID 32A kits (bioMérieux) with preparation and incubation performed in the anaerobic chamber. The cell suspension used to inoculate Rapid ID 32A kit was produced by flooding a CASB agar plate grown anaerobically at 30 °C for 14 days with API Suspension Medium provided in the kit.

Carbon utilization tests were performed in 16-mL Hungate tubes containing 10 mL of PY medium [4] and a headspace of N_2 and CO_2 (95:5, v/v). Each substrate was added at a concentration of 5 or 10 g L^{-1} for mono-, di-, and polysaccharides and sugar alcohols. Organic acids were added at a concentration of 15 or 30 mM. Cultures were inoculated using a 1% (v/v) of an exponentially growing seed culture. Tubes showing an increase in OD_{660} of more than 0.1 OD unit above the controls without added carbon were recorded as positive. The pH range for growth was evaluated over the range pH 3.0–10.0 using buffers comprised of 100 mM acetate buffer for pH 3.0–6.0, 100 mM potassium phosphate buffer for pH 6.0–8.0, and Tris buffer for pH 8.0–10.0. Growth in the NaCl concentration range of 0–5% (w/v) and the temperature range 10–40 °C was determined in Hungate tubes containing 10 mL of PYG.

The ability to use chlorosolvents as electron acceptors was examined in 25-mL serum bottle containing 10 mL of anaerobic basal medium [39] or in 1/10 strength PY broth. The anaerobic medium was amended with acetate, lactate, and pyruvate (each 2 mM) and one of the following chlorosolvents: 1,1,2-trichloroethane, 1,2-dichloroethane, tetrachloroethene, trichloroethene, *cis*-1,2-dichloroethene, or 1,2-dichloropropane (each 2.0 μmol). Serum bottle headspace gas consisted of N_2, CO_2, and H_2 (80:10:10, v/v/v). The ability to degrade aromatic compounds was also tested in serum bottles.

Each bottle was amended with 0.5 μmol of benzene, toluene, ethylbenzene, or *p*-xylene. Serum bottle headspace gas consisted of N_2, CO_2, and H_2 (80:10:10, v/v/v) for anaerobic basal medium and N_2 and CO_2 (95:5, v/v) for PY/10. Serum bottles were sealed with butyl rubber stoppers and aluminum seals. Uninoculated bottles were used as negative controls. Incubation was at 30 °C.

The ability of strain BL-10 to grow in the presence of 1,2-dichloroethane (1,2-DCA) or 1,1,2-trichloroethane (1,1,2-TCA) was examined in 120-mL serum bottles containing 80 mL of PYG supplemented with various concentrations (ranging to 9.8 mM in the aqueous phase) of 1,2-DCA or 1,1,2-TCA. The headspace was filled with a mixture of N_2 and CO_2 (95:5%, v/v). Growth based on OD was measured using a Smart Spec™ Plus spectrophotometer (BioRAD) at 660 nm.

16S rRNA gene sequence, phylogenetic analysis, and determination of G + C content of DNA

The extraction of genomic DNA, PCR amplification, and sequencing of the 16 rRNA gene was carried out as described previously [30]. Purified sequencing-reaction products were electrophoresed using a model 3100 DNA sequencer (Applied Biosystems). The 16S rRNA gene sequence of strain BL-10^T was aligned against previously determined Actinobacteria sequences available from the public databases using the BioEdit program version 4.7.8 (http://www.mbio.ncsu.edu/BioEdit/page2.html) [17]. Phylogenetic analyses were performed using ARB (http://www.arb-home.de/) [37]. The neighbor-joining algorithm was used to build the phylogenetic tree, with Jukes–Cantor correction [21].

The G + C content of DNA was determined by HPLC as described by Mesbah et al. [27] following DNA isolation as described by Nielsen et al. [28].

Chemotaxonomic characterization

Cellular fatty acids were extracted from exponential-phase PYG-grown cells using the modified method of Bligh and Dyer as described by White et al. [43]. The total lipid extract was fractionated by silicic acid chromatography, the polar lipid fraction was transesterified to fatty acid methyl esters and analyzed by gas chromatography/mass spectrometry (GC/MS) [16].

Analysis of respiratory lipoquinones was carried out by the Identification Services of the DSMZ, Braunschweig, Germany. Quinones were extracted from 100 mg of freeze-dried cell material and separated by thin layer chromatography and analyzed by HPLC as described by Tindall [40,41].

The presence of diaminopimelic acid in the cell wall and the peptidoglycan type were determined as described by Schleifer and Kandler [33], using TLC on cellulose plates using the solvent system of Rhuland et al. [31], at DSMZ.

Analytical methods

Fermentation products from PYG grown cultures were analyzed by ion chromatography (IC) using a Metrohm peak 761 Compact IC equipped with a Metrosep Organic Acid Column (25 cm × 7.8 mm) and a conductivity detector. Isocratic elution was performed with 0.5 mM H_2SO_4 at a flow rate of 0.5 mL min^{-1} and constant temperature of 25 °C. Retention times and peak areas of products were compared to those of authentic standards that included acetic, butyric, formic, lactic, isovaleric, propionic, pyruvic, and succinic acids.

Concentrations of chlorosolvents and aromatic compounds were analyzed using a gas chromatograph (HP6890, HP) equipped with a capillary column (GS-Gaspro, 60 m × 0.32 mm ID, J&W P/N 113-4362) and flame ionization detector. Helium was used as carrier gas at a flow rate of 45 mL min^{-1}. Injector and detector temperatures were 225 °C. Oven temperature increased from 50 to 200 °C at a rate of 50 °C min^{-1}.

Results

Morphological, phenotypic, and physiological characteristics

When grown on PYG plates incubated for 3 days at 30 °C under anaerobic conditions, strain BL-10^T formed white, circular, convex and smooth surface colonies 2–3 mm in diameter. Cells were Gram-positive, rod-shaped, 0.5 × 1.0–2.5 μm, and flagella were not observed (Fig. 1). Spore formation and cell motility were not observed when viewed using phase contrast microscopy.

Strain BL-10^T was oxidase and catalase negative. Under aerobic conditions weak growth occurred on nutrient agar (Difco), R2A (Difco) and PYG agar; much better growth was observed under anaerobic conditions. Vitamin B12 was not required as a growth factor for anaerobic growth in PYG. The optimal temperature for growth was 30 °C and growth occurred within the range 15–37 °C. The optimal pH for growth was pH 6.5 with growth occurring in the range 4.5–8.5.

Strain BL-10^T utilized adonitol, erythritol, fructose, glucose, glycerol, lactate, maltose, mannitol, mannose, pyruvate, sucrose, sorbitol, and xylose. Acetate, arabinose, crystalline cellulose, ducitol, ethanol, fucose, fumarate, galactose, lactose, malate, meliobiose, methanol, raffinose, starch, and succinate were not utilized.

The major end products of glucose fermentation were propionic acid, acetic acid and formic acid. Lactic acid and succinic acid were produced at trace levels as minor

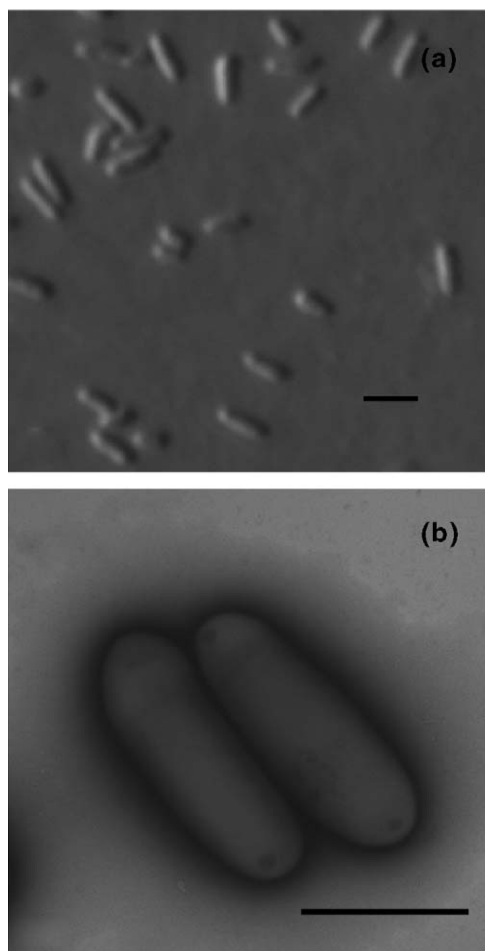

Fig. 1. Morphological features of strain BL-10^T observed by (a) differential interference contrast microscopy (DIC) and (b) transmission electron microscopy (TEM). Cells were grown on PYG at 30 °C for 3 days. Bars in DIC and TEM represent 2 and 1 μm, respectively.

products. This strain did not utilize 1,1,2-TCA, 1,2-DCA, tetrachloroethene, trichloroethene, *cis*-1,2-dichloroethene, or 1,2-dichloropropane as electron acceptors after 80 days incubation. Benzene, toluene, ethylbenzene, and *p*-xylene were not utilized as substrates under anaerobic conditions after 52 days incubation.

Using the Rapid ID 32A kits (bioMérieux) as described above, strain BL-10^T showed a positive result for α-glucosidase, β-glucosidase, glycine arylamidase, histidine arylamidase, proline arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, and alanine arylamidase. Negative results were observed for glutamyl glutamic acid arylamidase alkaline phosphatase, α-fucosidase, glutaminc acid decarboxylase, arginin dehydrogenase, urease, α-galactosidase, and β-galactosidase. Other physiological characteristics are summarized in Table 1.

Phylogenetic characteristics

An almost complete 16S rRNA gene sequence comprising 1469 nucleotide positions was determined for stain BL-10^T. Phylogenetic comparisons with members of the domain bacteria showed strain BL-10^T to represent a lineage within the Actinobacteria phylum (Fig. 2). Strain BL-10^T formed an independent phylogenetic branch close to the species of the genera *Propionicimonas* and *Micropruina*.

The closest bacterial strain to strain BL-10^T was *Propionicimonas* sp. F6 with a sequence similarity of 97.3%, which was isolated from a low-temperature biodegraded Canadian oil reservoir (AY570689). The 16S rRNA gene sequence similarity with the type strains *Propionicimonas paludicola* DSM 15597^T [4] and *Micropruia glycogenica* JCM 10248^T [34] were 97.5% and 95.4%, respectively. A neighbor-joining tree was constructed using the most closely related 16S rRNA gene sequences available from the public databases (Fig. 2)

Chemotaxonomic characteristics

Strain BL-10^T has anteiso $C_{15:0}$ (54.3%), $C_{15:0}$ (16.3%), iso $C_{16:0}$ (12.8%), anteiso $C_{17:0}$ (5.4%), iso $C_{14:0}$ (5.3%), $C_{17:0}$ (2.9%), and $C_{16:0}$ (1.1%). Minor fractions less than 1.0% were $C_{13:0}$, $C_{14:0}$, $C_{16:1}$ ω9c, $C_{18:1}$ ω9c, and iso $C_{15:0}$. The predominant respiratory quinone was MK-9. The peptidoglycan type was A1γ with *meso*-diaminopimelic (*meso*-DAP) in the cell wall.

Tolerance to chlorosolvents

Although strain BL-10^T was not able to utilize 1,2-DCA or 1,1,2-TCA as electron acceptors, it was able to grow in the presence of both compounds (Fig. 3). Strain BL-10 ^{T} grew at all 1,2-DCA concentrations tested in this study (range 0–9.8 mM); however, it grew only in the range of 0–5.9 mM of 1,1,2-TCA. Both chlorosolvents adversely affected growth of strain BL-10^T in terms of the observed specific growth rate, maximum cell concentrations attained, and duration of the lag phase, with adverse effects increasing as chlorosolvent concentrations increased. 1,1,2-TCA more adversely affected growth than did 1,2-DCA.

To determine whether these chlorosolvents affect the fermentation pathways, samples amended with 7.84 mM 1,2-DCA (aqueous concentration), 3.93 mM 1,1,2-TCA, and no chlorosolvent were collected after 18 days incubation and analyzed for fermentation products. Propionate, acetate, and formate were the dominant fermentation products both in the presence and absence of chlorosolvents. Fermentation product concentrations

Table 1. Characterisitics of strains BL-10 T and related taxa: 1, strain BL-10[T]; 2, *Propionicimonas paludicola* Wd[T] (= DSM 15597[T], data from Akasaka et al. [4] except for cellular fatty acids [this study]); 3, *Propionicimonas paludicola* Wf (= DSM 15598, data from Akasaka et al. [4] except for cellular fatty acids [this study]); 4, *Micropruina glycogenica* Lg2[T] (= JCM 10248[T], data from Shintani et al. [34])

Characteristic	1	2	3	4
Origin	Groundwater	Plant residue in paddy soil	Plant residue in paddy soil	Activated sludge reactor
Cell shape (μm)	Rods (0.5 × 1.7)	Irregular rods (0.4–0.5 × 1.8–2.0)	Irregular rods (0.4–0.5 × 1.4–2.2)	Coccoid (0.5–2.2)
Growth temperature (°C)				
Optimum	30	35	35	30
Range	15–37	10–40	10–40	20–30
Growth pH				
Optimum	6.5	6.5	6.5	7.0
Range	4.5–8.5	4.5–7.5	4.5–7.5	6.0–8.0
NaCl range for growth (%)	0–4	0–2	0–2	0–3
O_2 requirement	Facultative anaerobic	Facultative anaerobic	Facultative anaerobic	Aerobic
Cobalamin requirement	–	+	–	ND
Catalase/oxidase	–/–	–/–	–/–	+/+
Nitrate reduction	–	–	–	+
DNA G+C mol%	69.9	68.7	67.4	70.5
Major quinone	MK-9	MK-9(H_4), MK-10(H_4)	MK-9(H_4), MK-10(H_4)	MK-9(H_4)
Peptidoglycan	*meso*-DAP	*meso*-DAP	*meso*-DAP	*meso*-DAP
Cellular fatty acid (%)[a]				
$C_{13:0}$	0.2	2.3	0.7	
$C_{14:0}$	0.5	0.7	1.1	3.7
$C_{15:0}$	16.3	33.6	41.7	5.9
$C_{16:0}$	1.1	1.5	1.2	12.9
$C_{17:0}$	2.9	4.5	1.7	
$C_{18:0}$				4.6
$C_{16:1}$ ω9c	0.3	0.5	0.5	
$C_{18:1}$ ω9c	0.3	0.3	0.4	
iso $C_{14:0}$	5.3	10.4	12.6	14.1
iso $C_{15:0}$	0.2	0.7	0.5	2.2
iso $C_{16:0}$	12.8	5.5	3.0	10.8
anteiso $C_{15:0}$	54.3	41.7	36.9	37.1
anteiso $C_{17:0}$	5.4	0.4	0.1	
Utilization of[b]:				
Adonitol	+	–	–	ND
Arabinose	–	+	+	+
Cellobiose	–	+	+	+
meso-Erythritol	+	–	–	ND
Galactose	–	+	+	+
Sorbitol	+	–	–	ND
Products from glucose fermentation (mM) with/without B12				
Acetic acid	11.20/11.5	8.06/2.46	13.8/8.12	ND
Formic acid	2.59/2.50	–	–	ND
Lactic acid	0.83/1.13	3.21/4.26	6.01/15.2	ND

Table 1. (*continued*)

Characteristic	1	2	3	4
Propionic acid	29.1/29.9	17.7/0.59	24.7/12.3	ND
Succinic acid	0.59/0.57	0.89/2.45	0.27/0.53	ND

[a]Cellular fatty acid composition of strains Wd^T and Wf was analyzed in this study from cells cultured under the same conditions used for strain $BL\text{-}10^T$.

[b]Substrate utilization results reported for strains $BL\text{-}10^T$, Wd^T, and Wf are for anaerobic conditions with incubation in liquid PY medium supplement with each of the various substrates. Substrate utilization results reported for $Lg2^T$ are for aerobic conditions using the BIOLOG system. Under anaerobic conditions, strains $BL\text{-}10^T$, Wd^T, and Wf all utilized fructose, D-glucose, glycerol, lactate, maltose, D-mannitol, D-mannose, pyruvate, D- sucrose, and xylose, but not cellulose, dulcitol, ethanol, L-fucose, fumarate, glycogen, D-lactose, D,L-malate, meliobiose, methanol, D-raffinose, L-rhamnose, soluble starch, succinate, or xylan.

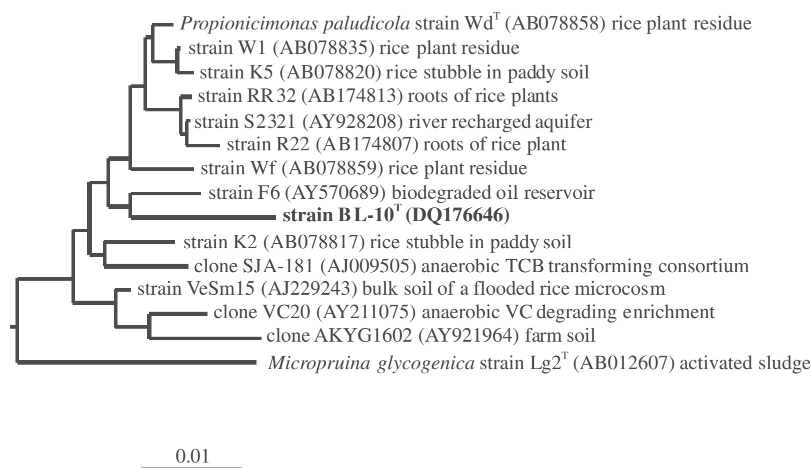

Fig. 2. 16S rRNA gene sequence based phylogeny showing the relationships of strain $BL\text{-}10^T$ and related taxa. The dendrogram was constructed from distance matrices using the neighbor-joining method. Bar represents 1 substitution per 100 nucleotide positions.

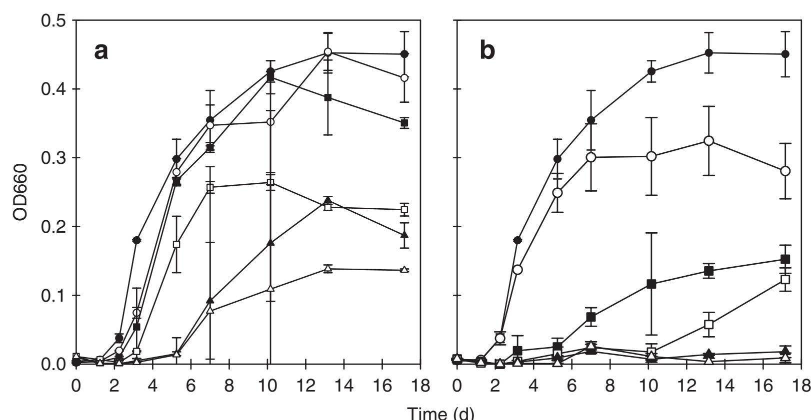

Fig. 3. Growth of strain $BL\text{-}10^T$ in the presence of (a) 1,2-dichloroethane (1,2-DCA) and (b) 1,1,2-trichloroethane (1,1,2-TCA) at aqueous-phase chlorosolvent concentrations of ●, 0 mM; ○, 1.96 mM; ■, 3.92 mM; □, 5.89 mM; ▲, 7.84 mM, △, 9.80 mM.

in microcosms amended with high concentrations of 1,2-DCA or 1,1,2-TCA were proportionally lower when compared to control microcosms without chlorosolvents, consistent with the fact that less cell growth was observed.

Discussion

Biodegradation of chlorosolvents by indigenous microbial populations (i.e., natural attenuation) or by externally supplied consortia (i.e., bioaugmentation) is

increasingly applied as an in situ remediation approach for contaminated soil and groundwater [1,2,12,20,22,24,25]. Although strain BL-10^T was unable to directly biotransform chlorinated solvents under the conditions reported here, the strain nevertheless has the potential to play an important role in chlorosolvent biodegradation. To sustain the reductive dechlorination process (in which chlorosolvents serve as terminal electron acceptor under anaerobic conditions) a source of reducing equivalents (i.e., an electron donor) must be provided. The range of electron donors that may be used by dechlorinating microorganisms is generally quite limited and can include fermentation products such as acetate, lactate, or pyruvate [15,32,38,39] or may be limited to only H_2 [3,18,26]. Chlororespiring populations, thus, depend on the activity of fermentative organisms to convert complex organic materials into suitable electron donors (e.g., acetate or H_2) in the absence of a readily available external supply [7,8,19]. The ability of strain BL-10^T to produce acetate, a compound directly used as an electron donor by many dechlorinating bacteria species, and propionate, a compound that can be further fermented to H_2 that is also used as an electron donor by dechlorinating bacteria species, may play an important role in bioremediation at the PPI site. Further research, however, is needed to draw such an unequivocal conclusion. Because propionate results in slow production of hydrogen, it may be particularly advantageous because it allows a larger fraction of reducing equivalents to be directed toward dechlorination [5,13,14,19,23,35].

The fact that strain BL-10^T was able to grow fermentatively even in the presence of very high concentrations of 1,2-DCA (9.8 mM) or 1,1,2-TCA (5.9 mM) likely explains why the organism was able to survive in the DNAPL source zone of the PPI Superfund Site. Until recently, microbial degradation of chlorosolvents in zones where DNAPLs are present was generally assumed to be negligible due to the toxicity of high concentrations of chlorinated compounds [44,45]. Recent laboratory-scale experiments using tetrachloroethene (PCE) as a model compound have demonstrated that some microbial populations can reductively dechlorinate in the presence of chlorosolvent DNAPL [6,10,11,29,44,45]. This suggests that in situ bioremediation may be a feasible clean-up strategy for DNAPL source zones when applied directly [1,2] or as a polishing step following more aggressive source zone removal techniques (e.g., co-solvent or surfactant flushing) [7]. Presence of microorganisms like strain BL-10^T able to grow fermentatively in the presence of very high concentrations of chlorosolvents may syntrophically interact with dechlorinators through intraspecies transfer of electron donors.

Phylogenetic analysis results indicate that strain BL-10^T represents a distinct lineage within the radiation of the genera *Propionicimona*s and *Micropruina* (Fig. 2). The most closely related taxa are strains that have been isolated from a number of sources. Many of these strains have not been fully characterized but assigned to the species *Propionicimona*s *paludicola* based on their proximity to the type strain Wd^T. Currently, the genus *Propionicimona*s contains one species with a validly published name, *Propionicimona*s *paludicola* Wd^T (= DSM 15597^T), another strain of this species, strain Wf (= DSM 15598, [4]), as well as unidentified bacterial strains *Propionicimonas* sp. F6, K5, K2, RR22, RR32, W1, and S2321. An uncultured environmental clone SJA-181 from an anaerobic microbial consortium in a trichlorobenzene-transforming bioreactor [42] is also included in this generic group. The strains and 16S rRNA clones show sequence similarity values in the 97.1–97.5% range. *Micropruina*, containing the single species *Micropruina glycogenica* [34], is the most closely related validly described taxon to *Propionicimona*s *paludicola*, but with a 16S rRNA gene sequence similarity of 95.4% is not closely related to strain BL-10^T.

Strain BL-10^T shares some common features with the genus *Propionicimona*s: facultative anaerobic trait, producing propionate as a major fermentation product, and catalase, oxidase and nitrate reduction negative, as well as having *meso*-DAP as the cell wall petidoglycan layer (Table 1). However, strain BL-10^T is clearly distinguished from genus *Propionicimona*s on the basis of other characteristics. Morphologically, strain BL-10^T is characterized by regular rod-shaped cells (Fig. 1) while *Propionicimonas paludicola* Wd^T, type species of this genus, forms irregular rods with irregular V- or crescent-shapes [4]. Strain BL-10^T has MK-9 as the predominant menaquinone which distinguishes it from the genus *Propionicimona*s with MK-9(H_4) and MK-10(H_4). Cellular fatty acids analysis also differentiates strain BL-10^T from *Propionicimonas paludicola* strains Wd^T and Wf. The three strains have the same fatty acids, but differ significantly in the proportions of the individual components. For example, the amounts of $C_{15:0}$ (33.6–41.7%) and iso $C_{14:0}$ (10.4–12.6%) of strains Wd^T and Wf are two times higher than those of strain BL-10^T (16.3% and 5.3%) while the proportions of iso $C_{16:0}$ (12.8%) and anteiso $C_{17:0}$ (5.4%) of strain BL-10^T are higher by comparison to those of strains Wd^T and Wf (3.0–5.5% and 0.1–0.4%, respectively). Besides the chemotaxonomic features, strain BL-10^T can be differentiated from *Propionicimonas paludicola* based on substrate utilization patterns (Table 1) as well as end products of fermentation. Formic acid, an end product of glucose fermentation for strain BL-10^T, was not detected in the fermentation products of strains Wd^T or Wf [4]. Strain BL-10^T can differentiated from *Micropruina glycogenica* on the basis of aerobic metabolism, coccoid morphology, catalase- and oxidase positive

reactions, and the nitrate reduction positive reaction of the later taxon. *Micropruina glycogenica* is also differentiated from strain BL-10^T based on chemotaxonomic features, menaquinone MK-9(H_4) and fatty acid composition.

On the basis of phylogenetic, chemotaxonomic, and phenotypic features obtained in this study, strain BL-10^T is assigned to a novel genus, *Propionicicella* gen. nov., with *Propionicicella superfundia* sp. nov. as the type species.

Description of *Propionicicella* gen. nov. (Rainey, da Costa, and Moe)

Propionicicella (Pro.pi.o.ni.ci.cel'la, N.L. n. *acidum propionicum*, propionic acid; L. fem. n. *cella*, a storeroom, chamber and in biology a cell; N.L. fem. n. *Propionicicella*, a propionic acid producing cells). *Propionicicella* cells are Gram-positive and rod-shaped, mesophilic, facultively anaerobic, chemo-heterotrophic. Propionate and acetate are produced when grown anaerobically on glucose. Cell wall peptidoglycan is *meso*-DAP. Major cellular fatty acids are $C_{15:0}$, iso $C_{16:0}$ and anteiso $C_{15:0}$. MK-9 is the dominant menaquinone. Sugars and organic acids are used as carbon and energy sources. The G+C content of the type species is 69.9 mol%. On the basis of 16S rRNA gene sequences, BL-10^T belongs to the class Actinobacteria. The genera *Propionicimonas* and *Micropruina* are the most closely related genera. The type species is *Propionicicella superfundia*.

Description of *Propionicicella superfundia* sp. nov. (Rainey, da Costa, and Moe)

Propionicicella superfundia (su.per.fun' di.a. L. prep. *super*, above/on top, L. masc. n. fundus, land owned by someone, referring to land designated as an Environmental Protection Agency Superfund Site). *Propionicicella superfundia* cells are non-motile, non-spore forming, and 0.5 μm in diameter $\times$ 1.0–2.5 μm in length. Colonies are white, circular, convex, smooth and 2–3 mm in diameter on PYG agar. Growth occurs between 15 and 37 °C; the optimum growth temperature is about 30 °C. Growth occurs between pH 4.5 and 8.5; the optimum pH for growth is 6.5. Grows in the presence of up to 4.0% (w/v) NaCl. Catalase, oxidase, and nitrate reduction is negative. Formic acid, acetic acid, propionic acid, and succinic acid are fermentation products from glucose. Cells grow on adonitol, erythritol, fructose, glucose, glycerin, lactate, maltose, mannitol, mannose, pyruvate, sorbitol, sucrose, and xylose but did not utilize acetate, arabinose crystalline cellulose, dulcitol, ethanol, fucose, galactose, lactose, malate, meliobiose, methanol, raffinose, rhamnose, starch, succinate, and fumarate. This bacterium was isolated from the Brooklawn site, Baton Rouge, LA (USA). The type strain is BL-10^T and has been deposited in the American Type Culture Collection, Manassas, Virginia, USA as strain ATCC BAA-1218 and in the BCCM/LMG Bacteria Collection, Ghent, Belgium as strain LMG 23096.

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