Pelosinus defluvii sp. nov., isolated from chlorinated solvent-contaminated groundwater, emended description of the genus *Pelosinus* and transfer of *Sporotalea propionica* to *Pelosinus propionicus* comb. nov.

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Two anaerobic bacterial strains, designated SHI-1^T and SHI-2, were isolated from chlorinated solvent-contaminated groundwater. They were found to be identical in phenotypic properties and shared high (98.5–99.8%) pairwise 16S rRNA gene sequence similarity. Multiple 16S rRNA genes were found to be present in the isolates as well as *Pelosinus fermentans* DSM 17108^T and *Sporotalea propionica* DSM 13327^T. Strains SHI-1^T and SHI-2 could be differentiated from their closest phylogenetic relatives, *P. fermentans* DSM 17108^T and *S. propionica* DSM 13327^T, on the basis of their phenotypic and phylogenetic properties. The isolates were Gram-negative, spore-forming, motile rods with peritrichous flagella. Growth occurred at 10–42 °C and pH 5.5–8.5. Fermentative growth was observed on Casamino acids, fructose, fumarate, glucose, glycerol, pyruvate and yeast extract. The major organic acids produced from glucose and glycerol fermentation were propionate and acetate. The major organic acids produced from fermentation of fumarate were propionate, acetate and succinate. The major cellular fatty acids were summed feature 4 (consisting of C_{15:1}ω8c and/or C_{15:2}), summed feature 8 (consisting of C_{17:1}ω8c and/or C_{17:2}) and C_{14:0} dimethyl aldehyde. The polar lipids comprised aminophospholipids, including phosphatidylethanolamine and phosphatidylserine, and an unknown phospholipid. The genomic DNA G + C content was 39.2 mol%. We propose that strains SHI-1^T and SHI-2 are assigned to a novel species of the genus *Pelosinus*, with the name *Pelosinus defluvii* sp. nov. (type strain SHI-1^T = NRRL Y-59407^T = LMG 25549^T). The description of the genus *Pelosinus* is emended. We also propose the transfer of *S. propionica* to the genus *Pelosinus* as *Pelosinus propionicus* comb. nov. (type strain TmPN3^T = DSM 13327^T = ATCC BAA-626^T), on the basis of phylogenetic, chemotaxonomic and phenotypic properties.

The genus *Pelosinus* was described by Shelobolina *et al.* (2007) to accommodate a bacterium belonging to the *Sporomusa-Pectinatus-Selenomonas* group of the phylum *Firmicutes* (Strömpl *et al.*, 1999). This group is a heterogeneous assemblage of organisms with Gram-negative cell

walls and is also referred to as clostridial cluster IX (Collins *et al.*, 1994) and the family *Veillonellaceae* (Rogosa, 1971). At present, the genus *Pelosinus* is represented by a single species, *Pelosinus fermentans*, the type strain of which was first isolated from a subsurface kaolin deposit in Russia (Shelobolina *et al.*, 2007). The genus *Sporotalea* contains the single species *Sporotalea propionica* and was described by Boga *et al.* (2007) to accommodate a propionigenic bacterium isolated from the intestinal tract of the soil-feeding termite *Thoracotermes macrothorax*. At the time of initial publication, the closest phylogenetic relative reported for both *P. fermentans* R7^T (Shelobolina *et al.*,

Abbreviation: DMA, Dimethyl aldehyde.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains SHI-1^T, SHI-2, DSM 17108^T and DSM 13327^T are JF750003–JF750008, JF750009–JF750014, JF749997–JF750002 and JF749991–JF749996, respectively.

A supplementary figure is available with the online version of this paper.

2007) and *S. propionica* TmPN3^T (Boga *et al.*, 2007) was *Acetonea longum* DSM 6540^T, which was first isolated from the hindgut of the wood-feeding termite *Pterotermes occidentis* (Kane & Breznak, 1991).

Because the descriptions of *P. fermentans* and *S. propionica* as novel taxa occurred in close succession (Shelobolina *et al.*, 2007; Boga *et al.*, 2007), the two closely related species were assigned to separate genera. Here, we present results from a study to clarify the taxonomic relationships between *P. fermentans* (Shelobolina *et al.*, 2007) and *S. propionica* (Boga *et al.*, 2007) as well as two strains isolated from contaminated groundwater at the Scenic Highway portion of the PetroProcessors of Louisiana Superfund Site located near Baton Rouge, Louisiana, USA.

The groundwater from which the isolates were obtained was moderately acidic (pH 5.8) and was contaminated with several halogenated solvents, including chlorinated alkanes (l⁻¹: 285 µg 1,1,2,2-tetrachloroethane, 5540 µg 1,1,2-trichloroethane, 8890 µg 1,2-dichloroethane, 4170 µg 1,2-dichloropropane) and chlorinated alkenes (4640 µg tetrachloroethene, 4440 µg trichloroethene, 983 µg vinyl chloride). The groundwater also contained (l⁻¹) 3.32 mg ferrous iron and 59.3 mg sulfate, but not nitrite or nitrate (<0.01 mg N). One isolate, designated SHI-1^T, was isolated on anaerobic medium described by Sekiguchi *et al.* (2000) supplemented with 5 mM ribose but with Na₂S replaced with 0.5 g l-cysteine hydrochloride l⁻¹ as the reducing agent and the pH adjusted to pH 7. A second isolate, designated SHI-2, was isolated on 1/10 R2A agar (Difco) supplemented with (l⁻¹) 0.5 g l-cysteine hydrochloride as the reducing agent and 1.0 mg resazurin as the redox indicator, with incubation at 30 °C in an anaerobic chamber. The purity of the strains was verified by microscopy. Strains were preserved at -80 °C in peptone-yeast extract-glucose (PYG) broth (Holdeman *et al.*, 1977) supplemented with 15 % (v/v) glycerol and 5 % (v/v) DMSO. In addition to the media used for initial isolation, strains SHI-1^T and SHI-2 also grew well on anaerobic PYG agar (15 g agar l⁻¹). For comparative purposes, *P. fermentans* DSM 17108^T and *S. propionica* DSM 13327^T were obtained from the DSMZ, Braunschweig, Germany. Unless stated otherwise, all tests were carried out in duplicate and the procedures followed strict anaerobic protocols as described by Holdeman *et al.* (1977).

16S rRNA gene sequences were determined as described previously (Rainey *et al.*, 1996a). Initial attempts to sequence the 16S rRNA gene of strains SHI-1^T and SHI-2 resulted in a mixed sequence for the 5' end. Inspection of the sequence of *P. fermentans* R7^T (accession number DQ145536) indicated that the 16S rRNA gene sequence started at *Escherichia coli* position 130 and not at the 5' end. To obtain complete and clean sequences of strains SHI-1^T and SHI-2, *P. fermentans* DSM 17108^T and *S. propionica* DSM 13327^T, PCR products were cloned and sequenced as described previously (Rainey *et al.*, 1996b). Sequencing of the clones revealed that all four strains had multiple 16S rRNA genes, which varied in size because of

gene copies containing intervening sequences in variable region I. Two lengths of intervening sequences were observed, one comprising 17 nt and the other 115 nt. The short intervening sequences for strains SHI-1^T, SHI-2 and *P. fermentans* DSM 17108^T were identical but those for *S. propionica* DSM 13327^T differed in three positions. The long intervening sequences showed variability both within and between strains. The intervening sequences were excluded in sequence comparisons for the calculation of pairwise similarity values between strains and between cloned sequences of each strain. Complete 16S rRNA gene sequences were determined for six clones of each strain, three with the short and three with the long intervening sequences. A phylogenetic dendrogram was reconstructed by the neighbour-joining method using MEGA version 4.0.

Strains SHI-1^T and SHI-2, *P. fermentans* DSM 17108^T and *S. propionica* DSM 13327^T clustered within the family Veillonellaceae of the order Clostridiales and were most closely related to 'Psychrosinus fermentans' FCF9 (Sattley *et al.*, 2008) and *A. longum* DSM 6540^T (Kane & Breznak, 1991). For *P. fermentans* DSM 17108^T, the pairwise sequence similarities between the different copies of the 16S rRNA gene ranged from 98.7 to 99.6 % and similarities with the original sequence of *P. fermentans* R7^T (GenBank accession no. DQ145536) ranged from 98.9 to 99.4 %. For *S. propionica* DSM 13327^T, the pairwise sequence similarities between the different copies of the 16S rRNA gene ranged from 99.3 to 99.9 % and similarities with the original sequence of *S. propionica* TmPN3^T (AM258975) ranged from 98.2 to 98.5 %. The relatively low pairwise similarity between the cloned sequences and those determined in the original descriptions of *P. fermentans* and *S. propionica* by direct sequencing of PCR products (Shelobolina *et al.*, 2007; Boga *et al.*, 2007) is a result of the heterogeneity in the original sequences due to the multiple gene copies. The multiple 16S rRNA gene sequences of strains SHI-1^T and SHI-2 shared 98.5–99.8 % pairwise similarity and were shown to be interrelated in the phylogenetic analysis (Fig. 1). This was in contrast to the cloned sequences of *P. fermentans* DSM 17108^T and *S. propionica* DSM 13327^T, which clustered separately. Strains SHI-1^T and SHI-2 shared 97.3–97.9 % sequence similarity with *S. propionica* DSM 13327^T and 97.0–98.3 % sequence similarity with *P. fermentans* DSM 17108^T. The low 16S rRNA gene sequence similarities as well as the phylogenetic positions (Fig. 1) supported the distinct single-species status of strains SHI-1^T and SHI-2.

The finding that 16S rRNA genes with intervening sequences of varying lengths were present in strains SHI-1^T and SHI-2 as well as *P. fermentans* DSM 17108^T and *S. propionica* DSM 13327^T, which were isolated from very different environments and geographically distinct regions, is interesting. Such intervening sequences were also detected in the phylogenetically related strain UFO1 (Fig. 1), which was isolated from sediments at the Oak Ridge Integrated Field Research Challenge site in Tennessee, USA (Ray *et al.*, 2010). Intervening sequences at variable

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the relationships of strains SHI-1^T and SHI-2 with *S. propionica* DSM 13327^T, *P. fermentans* DSM 17108^T and related strains. Bootstrap values (>70 %) for 1000 replicates are shown at branching nodes. Bar, 1 substitution per 100 nucleotide positions.

region I are not uncommon in the class *Clostridia* and have been observed in *Clostridium paradoxum* (Rainey *et al.*, 1996b) and the distantly related taxa *Desulfotomaculum* (Stackebrandt *et al.*, 1997), *Desulfobacterium* (Villemur *et al.*, 2007) and *Carboxydotherrnus hydrogeniformans* (Wu *et al.*, 2005).

Colony morphology was observed after growth on anaerobic PYG agar in an anaerobic chamber (headspace gas N₂/CO₂/H₂, 90:5:5, v/v) at 33 °C for 5 days. Colonies of strains SHI-1^T and SHI-2 were circular, 1–3 mm in diameter, buff to off-white, translucent, convex and smooth with entire margins and a butyrous texture. Cell size, shape and morphology were observed by differential interference contrast microscopy (Microphot-FXA; Nikon) after 24 h in anaerobic PYG broth at 30 °C. Cells of strains SHI-1^T and SHI-2 were observed to be motile, straight to slightly curved rods (0.6–1 × 2–7 µm). Peritrichous flagella were observed in cell preparations negatively stained with uranyl acetate and imaged by transmission electron microscopy (100CX; JEOL) as described by Bae *et al.* (2007). Cells of SHI-1^T and SHI-2 were Gram-negative with both the BD kit and the KOH test (Powers, 1995). After 8 days under the same conditions, both strains exhibited terminal, spherical, bulging spores.

The temperature range for growth of the isolates and the reference strains was assessed in PYG broth with a headspace gas comprised of N₂/CO₂ (80:20, v/v) at 4, 7, 10, 15,

22, 26, 30, 33, 37, 40, 42 and 50 °C. The pH range for growth at 33 °C was evaluated at pH 5.0–10.5 (at intervals of 0.5 pH) in anaerobic PYG broth, prepared without NaHCO₃ and supplemented with 10 mM phosphate-citrate buffer (pH 5.0), MES buffer (pH 5.5–6.5), HEPES buffer (pH 7.0–8.0), TAPS buffer (pH 8.5) or CAPSO buffer (pH 9.0–10.5). The headspace gas was N₂. Growth was scored as positive if OD₆₀₀ increased by >0.05 absorbance units within 7 days in comparison to uninoculated controls. Strains SHI-1^T and SHI-2 and *P. fermentans* DSM 17108^T grew at 10–42 °C (optimum 30–37 °C), but not at 7 or 50 °C. In comparison, *S. propionica* DSM 13327^T grew at 15–37 °C (optimum 30–37 °C), but not at 10 or 40 °C. All four test strains grew at pH 5.5–8.5. The optimum pH for strains SHI-1^T, SHI-2 and *P. fermentans* DSM 17108^T was pH 7.0–7.5, while that for *S. propionica* DSM 13327^T was pH 6.5–7.0.

The ability to utilize various substrates for growth was assessed in 25 ml glass serum bottles containing 15 ml anaerobic medium [R] medium, 1⁻¹: 0.348 g K₂HPO₄, 0.227 g KH₂PO₄, 0.50 g NH₄Cl, 0.415 g MgCl₂·6H₂O, 0.25 g CaCl₂·2H₂O, 2.25 g NaCl, 1.0 g yeast extract, 1.0 mg resazurin, 1.0 ml trace element solution SL10 (Widdel *et al.*, 1983), 10 ml vitamin solution (Wolin *et al.*, 1963) and 0.5 g L-cysteine hydrochloride], prepared with a headspace gas comprised of N₂/CO₂ (80:20, v/v) and adjusted to pH 7.2 prior to autoclaving. Each bottle was supplemented with (5 g l⁻¹) adonitol, amygdalin, arabinose,

2,3-butanediol, butyrate, Casamino acids, cellobiose, dulcitol, erythritol, ethanol, formate, fructose, fucose, fumarate, glucose, glutamic acid, glycerol, glycogen, inulin, lactate, lactose, malonate, maltose, mannitol, melezitose, methanol, *myo*-inositol, propionate, pyrogallol, pyruvate, raffinose, rhamnose, salicin, salicylic acid, sorbitol, starch, succinate, sucrose, trehalose, xylan or yeast extract. Tests to assess the ability of the strains to utilize $H_2 + CO_2$ were performed in anaerobic broth with the composition described above but with 15 ml liquid volume in 60 ml serum bottles with a headspace gas comprised of H_2/CO_2 (80:20, v/v). Serum bottles were inoculated with 2% (v/v) exponential growth-phase cultures grown in 1/10 peptone-yeast extract (PY) broth (Holdeman *et al.*, 1977) and incubated at 30 °C for 3 days. Cultures exhibiting an increase in $OD_{600} > 0.05$ in comparison with controls not supplemented with respective substrates were scored as positive. The results for strains SHI-1^T and SHI-2 were identical and are shown in Table 1 and the species description. It should be noted that Shelobolina *et al.* (2007) reported that *P. fermentans* R7^T did not ferment glycerol in anaerobic freshwater medium, but the present study found that *P. fermentans* DSM 17108^T could grow on glycerol in RJ medium and formed propionate as the dominant product.

Table 1. Characteristics that differentiate strain SHI-1^T from related taxa

Strains: 1, *Pelosinus defluvii* sp. nov. SHI-1^T; *Pelosinus fermentans* DSM 17108^T; 3, *Sporotalea propionica* DSM 13327^T. All data were taken from this study. The results for strain SHI-2 were identical to those for strain SHI-1^T. +, Positive; –, negative.

Characteristic	1	2	3
Cell dimensions (µm)			
Width	0.6–1	0.6	0.5–0.7
Length	2–7	2–6	2.2–12
DNA G + C content (mol%, HPLC)	39.2	39.4	38.6
Temperature for growth (°C)	10–42	10–42	15–37
Optimum pH for growth	7.0–7.5	7.0–7.5	6.5–7.0
Growth on:			
Amygdalin	–	+	–
Arabinose	–	+	+
Cellobiose	–	+	+
Erythritol	–	+	–
Mannitol	–	+	+
<i>myo</i> -Inositol	–	+	–
Rhamnose	–	–	+
Salicin	–	+	–
Sorbitol	–	+	–
Organic acids from fermentation of:			
Fumarate	P, A, S	P, A	S, A
Glycerol	P, A	P	P

*Major products listed in stoichiometric order: A, acetate; P, propionate; S, succinate.

The organic acids produced from cultures grown in RJ medium supplemented with fumarate, glucose, glycerol and $H_2 + CO_2$ were measured using an ion chromatograph (ICS-2000; Dionex) equipped with an IonPac AS11-HC column and ASRS-ULTRA II (4 mm) suppressor and conductivity detector. The sample injection volume was 100 µl. Elution was performed with KOH (1.5 mM for 10 min then ramping to 30 mM at a rate of 1.6 mM min⁻¹) at a flow rate of 1.5 ml min⁻¹ and at 30 °C. Retention times and peak areas of fermentation products were compared with those of standards, which included acetate, butyrate, formate, lactate, propionate, pyruvate and succinate. Hydrogen concentrations in the headspace gas were analysed by gas chromatography as described by Van Ginkel *et al.* (2001). From glucose fermentation, all four test strains produced propionate and acetate. From glycerol fermentation, strains SHI-1^T and SHI-2 produced propionate and acetate while *P. fermentans* DSM 17108^T and *S. propionica* DSM 13327^T produced only propionate. From fumarate fermentation, strains SHI-1^T and SHI-2 produced propionate, acetate and succinate while *P. fermentans* DSM 17108^T produced only propionate and acetate and *S. propionica* DSM 13327^T produced only succinate and acetate (Table 1). None of the strains tested produced acetate from $H_2 + CO_2$. H_2 was not detected at >1% (v/v) in the headspace of serum bottles supplied with fumarate, glucose or glycerol as substrates, indicating that it is not a major product of fermentation.

The ability to reduce thiosulfate (10 mM), sulfite (10 mM), sulfate (10 mM), Fe(III) citrate (45 mM) and Fe(III) nitriloacetic acid (10 mM) was assessed in Hungate tubes containing anaerobic PY broth supplemented with 10 mM acetate. The headspace gas comprised N_2/CO_2 (80:20, v/v). Following inoculation (exponential growth-phase culture grown in PY broth amended with 10 mM glucose, 2%, v/v), tubes were incubated at 33 °C for 7 days with shaking at 120 r.p.m. The ability to reduce sulfite, sulfate and thiosulfate was assessed by measuring sulfide accumulation relative to uninoculated controls, as described by Kelly & Wood (1998). The ability to reduce Fe(III) citrate and Fe(III) nitriloacetic acid was assessed by measuring Fe(II) accumulation relative to uninoculated controls, as described by Lovley & Phillips (1986). Strains SHI-1^T, SHI-2, *P. fermentans* DSM 17108^T and *S. propionica* DSM 13327^T did not reduce thiosulfate, sulfite or sulfate to sulfide, but they reduced Fe(III) citrate, with Fe(II) accumulating to concentrations of 4, 3, 4 and 2 mM, respectively, and reduced Fe(III) nitriloacetic acid, with Fe(II) accumulating to concentrations of 1.3, 2.5, 2.1 and 2.5 mM, respectively. Shelobolina *et al.* (2007) observed reduction of Fe(III) citrate, but not Fe(III) nitriloacetic acid, by *P. fermentans* R7^T; however, their medium did not contain a fermentable substrate, whereas the PY broth used here contained yeast extract, which is a fermentable substrate for this strain.

The ability to grow aerobically was assessed in aerobic PYG broth lacking cysteine hydrochloride as a reducing agent and without $NaHCO_3$. The inoculum was 1% (v/v) and

the cultures were incubated at 33 °C with shaking (150 r.p.m.) for 8 days. None of the strains grew under aerobic conditions. Good growth was observed in positive controls, which consisted of the same broth made anaerobically (headspace gas N₂, reduced with cysteine hydrochloride) and inoculated and incubated at the same time.

The ability to use chlorinated solvents as electron acceptors was examined in 25 ml serum bottles containing 10 ml of titanium citrate and cysteine reduced anaerobic basal medium (Moe *et al.*, 2009) amended with acetate, lactate, pyruvate and ribose (each 2 mM) and also in 1/10 PY broth. The following chlorinated solvents (0.5 mM concentration in the aqueous phase) were each tested separately: 1,1,2-trichloroethane, 1,2-dichloroethane, tetrachloroethene, trichloroethene, *cis*-1,2-dichloroethene and 1,2-dichloropropane. The headspace gas consisted of N₂/CO₂/H₂ (80:10:10, v/v). Incubation was at 30 °C for 6 weeks. Analysis of chlorinated solvents and potential dechlorination products was determined by GC as described by Yan *et al.* (2009). None of the strains transformed any of the chlorinated solvents under the conditions tested.

Because the cellular morphology and substrate utilization profiles for strains SHI-1^T and SHI-2 were identical and the 16S rRNA gene sequences shared high similarity, the cellular fatty acids, polar lipids, G+C content and DNA–DNA relatedness were determined for only strain SHI-1^T, *P. fermentans* DSM 17108^T and *S. propionica* DSM 13327^T.

For cellular fatty acid analysis, cells grown in PYG broth for 24 h at 30 °C were harvested in mid-exponential growth phase by centrifugation at 3500 g for 15 min. Fatty acids were extracted, saponified and methylated at the BCCM/LMG (Ghent, Belgium) using the Microbial Identification System (Sherlock version 3.10; MIDI) and analysed by GC (Sasser, 1990) using a 6890N gas chromatograph (Agilent Technologies). Compounds were identified using the MIDI MOORE library version 5.0 (for VPI broth-grown anaerobes in PYG broth). Although differing somewhat in relative proportions, the major cellular fatty acids in strain SHI-1^T, *P. fermentans* DSM 17108^T and *S. propionica* DSM 13327^T were identical (Table 2). With the exception of iso-C_{13:0}, which was not detected in strain SHI-1^T but comprised 0.3 and 1.0% of the total fatty acids in *P. fermentans* DSM 17108^T and *S. propionica* DSM 13327^T, respectively, all of the other cellular fatty acids detected were common to all strains. The fatty acids comprised straight-chain saturates, terminally branched saturates, monoenoics, hydroxy fatty acids and dimethyl aldehydes.

For polar lipid analysis, cells grown in PYG broth for 48 h at 30 °C were harvested by centrifugation at 3500 g for 15 min at 4 °C. The polar lipids were analysed as described by Ross & Grant (1985). The polar lipids of all strains were practically identical and contained aminophospholipids, including phosphatidylethanolamine and phosphatidylserine, as well as at least one other unidentified aminophospholipid and an unknown phospholipid (Fig. S1, available in IJSEM Online).

Table 2. Comparison of cellular fatty acid compositions of strain SHI-1^T and related taxa

Strains: 1, *Pelosinus defluvii* sp. nov. SHI-1^T; *Pelosinus fermentans* DSM 17108^T; 3, *Sporotalea propionica* DSM 13327^T. All data were taken from this study. Values are percentages of total gas chromatogram peak area. Fatty acids consisting of <1% in all strains are not shown.

Fatty acid (%)	1	2	3
C _{9:0}	1.7	1.4	0.3
C _{10:0}	1.8	2.8	0.9
iso-C _{11:0}	4.0	3.6	3.5
C _{11:0}	3.6	4.9	3.3
Unknown*	6.4	5.2	9.1
iso-C _{13:0}	—	0.3	1.0
C _{13:0}	0.5	1.0	1.4
Summed feature 2†	3.9	5.1	5.9
C _{14:0}	0.7	1.9	1.4
iso-C _{13:0} 3-OH	4.6	1.2	5.6
C _{14:1ω7c} DMA	1.3	0.9	0.5
C _{14:0} DMA‡	10.0	12.4	15.2
iso-C _{15:0}	0.4	0.5	1.3
Summed feature 4†	25.8	24.8	15.0
C _{15:1ω6c}	1.4	0.8	0.6
C _{15:0}	5.9	5.5	8.3
C _{16:1ω9c}	2.4	4.7	3.1
C _{16:1ω7c}	2.3	2.2	1.0
C _{16:0}	1.3	1.9	2.4
Summed feature 8†	12.5	6.0	7.5
C _{17:1ω6c}	1.2	0.7	0.8
C _{17:0}	1.3	0.5	2.1
C _{18:2ω6,9c}	0.3	0.9	0.4

*Constituents with an equivalent chain-length of 12.44 were identified as C_{11:0} 3-OH using the MIDI TSBA 50.0 (rev. 5.0) peak naming table.

†Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 2 consisted of C_{12:0} 3-OH and/or C_{13:0} DMA. Summed feature 4 consisted of C_{15:1ω8c} and/or C_{15:2}. Summed feature 8 consisted of C_{17:1ω8c} and/or C_{17:2}.

‡Constituents with an equivalent chain-length of 14.47 were identified as a summed feature comprising one or more of C_{13:0} 3-OH, iso-C_{15:1} I and iso-C_{15:1} H in the MIDI TSBA 50.0 (rev. 5.0) peak naming table.

For determination of DNA G+C content and DNA–DNA relatedness, genomic DNA was extracted from PYG-grown cells according to a modification of the procedure of Gevers *et al.* (2001). Genomic DNA G+C content was determined at the BCCM/LMG using the HPLC technique of Mesbah *et al.* (1989). Table 1 gives the means of three independent analyses of the same DNA sample. Shelo-bolina *et al.* (2007) reported the G+C content of *P. fermentans* R7^T to be 41.0 mol%, which is slightly higher than the value of 39.4 mol% determined in the present study. This difference may be due to the use of different methods for the determination of G+C content.

DNA–DNA hybridization was performed at the BCCM/LMG at 38 °C in a solution containing 50 % (v/v) formamide using a modification (Goris *et al.*, 1998; Cleenwerck *et al.*, 2002) of the microplate method described by Ezaki *et al.* (1989). Four replicate hybridizations were performed and the averages were calculated. Reciprocal reactions were performed. The variation observed was within the limits of the method (Goris *et al.*, 1998). DNA–DNA relatedness between strain SHI-1^T and *P. fermentans* DSM 17108^T and *S. propionica* DSM 13327^T was low (20 and 16 %, respectively; reciprocal 22 and 21 %, respectively). DNA–DNA relatedness between *P. fermentans* DSM 17108^T and *S. propionica* DSM 13327^T was also low (44 %; reciprocal 44 %). These results supported the classification of strain SHI-1^T to a species distinct from the previously described members of the genera *Pelosinus* and *Sporotalea*. Likewise, the results supported the classification of *P. fermentans* DSM 17108^T to a species separate from *S. propionica* DSM 13327^T.

Strains SHI-1^T and SHI-2 shared many similarities with *P. fermentans* and *S. propionica*. All were obligate anaerobes with cells that were straight or slightly curved rods and motile by means of peritrichous flagella. All formed endospores. They shared common cellular fatty acids (Table 2) and polar lipids and had similar G+C contents (Table 1). All produced propionate from the fermentation of glucose. Strains SHI-1^T and SHI-2 could be differentiated from *P. fermentans* on the basis that they did not grow on amygdalin, arabinose, cellobiose, erythritol, mannitol, *myo*-inositol, salicin or sorbitol (Table 1) and could be differentiated from *S. propionica* on the basis that they did not grow on arabinose, cellobiose, mannitol or rhamnose. Additionally, strains SHI-1^T and SHI-2 produced both propionate and acetate from fermentation of glycerol while *P. fermentans* and *S. propionica* produced only propionate. Products from fermentation of fumarate also served as a differentiating feature, with strains SHI-1^T and SHI-2 producing propionate, acetate and succinate, *P. fermentans* producing only propionate and acetate and *S. propionica* producing only succinate and acetate. The temperature range for growth of strains SHI-1^T and SHI-2 (10–42 °C) was greater than that of *S. propionica* (15–37 °C).

The name *S. propionica* appeared on IJSEM Validation List no. 115 (Euzéby, 2007) 5 months after the valid publication of the name *P. fermentans* (Shelobolina *et al.*, 2007). As such, the name *Pelosinus* has priority in the nomenclature. On the basis of the phylogenetic, chemotaxonomic and phenotypic properties determined in the present study, we propose that *S. propionica* (Boga *et al.*, 2007) is renamed as *Pelosinus propionicus* comb. nov.

Hansel *et al.* (2008) reported that gene sequences most closely related to *P. fermentans* comprised a large percentage (49–100 %) of 16S rRNA clone libraries constructed from Fe(III)-reducing, lactate-amended enrichment cultures from three geochemically contrasting soils in Tennessee (pH ranging from 4.5 to 7). The 16S rRNA gene sequences that clustered most closely with members of the genera *Pelosinus*

and *Sporotalea* and the related '*Psychrosinus fermentans*' (Sattley *et al.*, 2008) comprised 0.83 % (918/110 697) of sequences in a pyrosequencing library constructed from DNA extracted from groundwater at the Scenic Highway site from April to December 2009 in an area where molasses was injected into the subsurface to stimulate bioremediation of chlorinated solvents (unpublished data). This group of 16S rRNA gene sequences comprised a much smaller percentage [(0.019 %, (7/37 583)] of sequences in a library constructed from DNA extracted from groundwater collected from the nearby well SBP-017-B, from which the isolates were recovered and which was not impacted by subsurface injection of molasses. This suggests that addition of fermentable carbohydrates in bioremediation approaches may serve to increase the relative abundances of these microbial groups.

Emended description of the genus *Pelosinus*

The description is based on Shelobolina *et al.* (2007), Boga *et al.* (2007) and this study. Cells are Gram-stain-variable, motile, straight or slightly curved rods. Spore-forming. Sporangia are distinctly swollen. Obligately anaerobic. Mesophilic. Does not produce acetate from H₂ + CO₂. Chemo-organotrophic with fermentative metabolism. Propionate is a major product of glucose fermentation. The major cellular fatty acids (>10 %, MIDI MOORE version 5.0 database) are summed feature 4 (comprising C_{15:1}ω8c and/or C_{15:2}) and C_{14:0} dimethyl aldehyde (DMA). Aminophospholipids, including phosphatidylethanolamine and phosphatidylserine, and other phospholipids are present. The DNA G+C content is 38–41 mol%. The type species is *Pelosinus fermentans*.

Description of *Pelosinus defluvii* sp. nov.

Pelosinus defluvii (de.flu'vi.i. L. gen. n. *defluvii* of waste, because the first isolation source was groundwater from a site contaminated with hazardous waste).

After incubation for 5 days at 33 °C on anaerobic PYG agar, colonies are circular, 1–3 mm in diameter, buff to off-white, translucent, convex and smooth with entire margins. Cells are Gram-negative, straight or curved rods (0.6–1 × 2–7 µm). Motile by peritrichous flagella. Grows at 10–42 °C (optimum 30–37 °C) and at pH 5.5–8.5 (optimum pH 7.0–7.5). Grows on Casamino acids, fructose, fumarate, glucose, glycerol, pyruvate and yeast extract, but not on adonitol, acetate, amygdalin, arabinose, 2,3-butanediol, butyrate, cellobiose, dulcitol, erythritol, ethanol, formate, fucose, glutamic acid, glycogen, H₂ + CO₂, inulin, lactate, lactose, malonate, maltose, mannitol, melezitose, methanol, *myo*-inositol, propionate, pyrogallol, raffinose, rhamnose, salicin, salicylic acid, sorbitol, starch, succinate, sucrose, trehalose or xylan. The major products of glucose and glycerol fermentation are propionate and acetate. The major products of fumarate fermentation are propionate, acetate and succinate. The major cellular fatty acids (>5 %, MIDI MOORE version 5.0 database) are

summed feature 4 (comprising C_{15:1}ω8c and/or C_{15:2}), summed feature 8 (comprising C_{17:1}ω8c and/or C_{17:2}) C_{14:0} DMA and C_{15:0}. The DNA G + C content of the type strain is 39.2 mol%.

The type strain, SHI-1^T (=NRRL Y-59407^T =LMG 25549^T), was isolated from chlorinated solvent-contaminated groundwater.

Description of *Pelosinus propionicus* comb. nov.

Pelosinus propionicus (pro.pi.o'ni.cus. N.L. neut. n. *acidum propionicum* propionic acid; N.L. masc. adj. *propionicus* pertaining to propionic acid, which the organism produces from various substrates).

Basonym: *Sporotalea propionica* Boga *et al.* 2007.

The emended description is based on Boga *et al.* (2007) and this study. Cells are long rods (0.5–0.7 × 2.2–12 μm). Cells appear singly or in pairs. Forms heat-resistant endospores. Motile by peritrichous flagella. Gram-staining-positive but Gram-negative with the KOH test. Catalase-negative. Ferments cellobiose, glucose, fructose, lactose, citrate, lactate, mannitol, oxaloacetate, glutamate and glycerol to propionate, acetate and CO₂. L-Malate is fermented to acetate, succinate and propionate. Fumarate and aspartate are fermented to succinate and acetate. Does not utilize trehalose, methanol, ethanol, succinate, malonate, propionate, formate, acrylate, glyoxylate, acetate, vanillate, syringate or 3,4,5-trimethoxybenzoate. Cells possess B-type cytochromes. Does not form acetate from H₂ and CO₂. Nitrate and sulfate are not reduced. Resting cells reduce oxygen. Grows at 15–37 °C (optimum 30–37 °C) and at pH 5.5–8.5 (optimum pH 6.5–7.0). The major cellular fatty acids (>5 %, MIDI MOORE version 5.0 database) are C_{14:0} DMA, summed feature 4 (comprising C_{15:1}ω8c and/or C_{15:2}), C_{15:0}, summed feature 8 (comprising C_{17:1}ω8c and/or C_{17:2}), summed feature 2 (comprising C_{12:0} 3-OH and/or C_{13:0} DMA) and iso-C_{13:0} 3-OH.

The type strain, TmPN3^T (=DSM 13327^T =ATCC BAA-626^T), was isolated from the intestinal tract of the termite *T. macrothorax*. The DNA G + C content of the type strain is 38.6 mol%.

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