Sporosalibacterium faouarense gen. nov., sp. nov., a moderately halophilic bacterium isolated from oil-contaminated soil

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A novel strictly anaerobic, moderately halophilic and mesophilic bacterium, designated strain SOL3f37^T, was isolated from a hydrocarbon-polluted soil surrounding a deep petroleum environment located in south Tunisia. Cells of strain SOL3f37^T stained Gram-positive and were motile, straight and spore-forming. Strain SOL3f37^T had a typical Gram-positive-type cell-wall structure, unlike the thick, multilayered cell wall of its closest relative *Clostridiisalibacter paucivorans*. The major fatty acids were iso-$C_{15:0}$ (41 %), iso-$C_{14:0}$ 3-OH and/or iso-$C_{15:0}$ dimethyl acetal (21.6 %), iso-$C_{13:0}$ (4.4 %), anteiso-$C_{15:0}$ (3.9 %) and iso-$C_{15:1}$ (2.8 %). Strain SOL3f37^T grew between 20 and 48 °C (optimum 40 °C) and at pH 6.2–8.1 (optimum pH 6.9). Strain SOL3f37^T required at least 0.5 g NaCl l^{-1} and grew in the presence of NaCl concentrations up to 150 g l^{-1} (optimum 40 g l^{-1}). Yeast extract (2 g l^{-1}) was required for degradation of pyruvate, fumarate, fructose, glucose and mannitol. Also, strain SOL3f37^T grew heterotrophically on yeast extract, peptone and bio-Trypticase, but was unable to grow on Casamino acids. Sulfate, thiosulfate, sulfite, elemental sulfur, fumarate, nitrate and nitrite were not reduced. The DNA G+C content was 30.7 mol%. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain SOL3f37^T was a member of the family *Clostridiaceae* in the order *Clostridiales*; strain SOL3f37^T was related to members of various genera of the family *Clostridiaceae*. It exhibited highest 16S rRNA gene sequence similarity (93.4 %) with *Clostridiisalibacter paucivorans* 37HS60^T, 91.8 % with *Thermohalobacter berrensis* CTT3^T and 91.7 % with *Caloranaerobacter azorensis* MV1087^T. On the basis of genotypic, phenotypic and phylogenetic data, it is suggested that strain SOL3f37^T represents a novel species in a new genus. The name *Sporosalibacterium faouarense* gen. nov., sp. nov. is proposed, with SOL3f37^T (=DSM 21485^T =JCM 15487^T) as the type strain of *Sporosalibacterium faouarense*.

The *Clostridiales* constitutes one of the largest of the eubacterial orders. It encompasses a complex range of bacteria that may be Gram-negative or Gram-positive, psychrophilic, mesophilic or thermophilic, spore-forming or non-spore-forming, chemo-organoheterotrophic or chemolithotrophic and have been found in a variety of habitats, and it includes a large number of eukaryotic pathogens (Cato *et al.*, 1986). The family *Clostridiaceae* was the first of the 19 families within the order *Clostridiales* to be described, and it contains the type genus of the order. It contains 13 recognized genera, of which the type genus, *Clostridium*, contains about 190 recognized species at the time of writing. Recently, the systematics of the *Clostridiaceae* have been found to differ significantly from previous descriptions (Wiegel, 2009). Several of the species that were not in the radiation of the genus *Clostridium sensu stricto* have been transferred to new genera and other families, in some cases even in different orders. The taxa presently placed into the *Clostridiaceae* are generally obligately anaerobic rods. Although most of the species are neutrophiles, several alkaliphilic, alkalithermophilic, moderately halophilic, haloalkaliphilic and slightly acidophilic species have been described (Wiegel, 2009).

Abbreviation: DMA, dimethyl acetal.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SOL3f37^T is EU567322.

Here, the isolation and characterization are reported of a novel strictly anaerobic, moderately halophilic, heterotrophic, spore-forming bacterium, belonging to the family *Clostridiaceae*, which originated from a hydrocarbon-polluted soil surrounding a deep petroleum environment located in the El Faouar area in south Tunisia. It is suggested that this strain, SOL3f37^T, represents a novel species in a new genus of the order *Clostridiales*.

Samples were collected in sterile tubes from a hydrocarbon-polluted soil surrounding a deep petroleum environment located in the El Faouar area in south Tunisia, transported to our laboratory and stored at 4 °C until analysis. The NaCl concentration of the samples was 36 g l^{-1}. The temperature of the soil at the sampling site was 35–39 °C during the day. Samples were collected at the surface of the soil (0–2 cm deep) and the pH of the soil was 6.8.

Enrichment and isolation were performed using an anaerobic enrichment medium, which contained (per litre distilled water) 0.3 g KH_2PO_4, 0.3 g K_2HPO_4, 1.0 g NH_4Cl, 40 g NaCl, 0.5 g KCl, 0.2 g $CaCl_2$, 0.5 g cysteine hydrochloride, 2 g yeast extract (Difco), 5 g bio-Trypticase, 3 g glucose, 1 ml mineral element solution (Widdel & Pfennig, 1981) and 1 ml 0.1 % (w/v) resazurin. The medium was adjusted to pH 7.1 with 10 M KOH. This enrichment medium was boiled under a stream of O_2-free N_2 gas and cooled to room temperature and 5 ml aliquots were then distributed into Hungate tubes under a stream of O_2-free N_2 gas. The N_2 gas phase was replaced with N_2/CO_2 (80 : 20) and the tubes were autoclaved. To initiate enrichment cultures, a small portion of the soil sample was inoculated into the growth medium and incubated at 37 °C without agitation. The Hungate technique was then used throughout isolation and study for physiological and metabolic characterization.

Enrichment cultures showed growth after 24 h incubation at 37 °C and microscopic examination revealed the presence of motile rod-shaped bacteria. Growth was determined by inserting culture tubes directly into a model Cary 50 Scan spectrophotometer (Varian) and measuring the OD_{580}. Cultures were purified by repeating the Hungate roll-tube method (Hungate, 1969) and using enrichment medium solidified with 2 % (w/v) agar (Difco). Several colonies were picked and cultured in the corresponding culture medium. The isolation process was repeated several times until isolates were deemed to be axenic. Several axenic cultures were obtained using this process and one strain, named SOL3f37^T, was used for further characterization.

Cells of strain SOL3f37^T were straight, thin and long rods (0.5 × 5.0–10.0 μm) that grew singly or in pairs. Cells were motile by means of a monotrichous laterally inserted flagellum (Fig. 1). Strain SOL3f37^T stained Gram-positive. Spherical and terminal spores were observed, mainly in old cultures. Strain SOL3f37^T showed a typical Gram-positive-type cell-wall structure, unlike the thick, multilayered cell wall of its closest relative *Clostridiisalibacter paucivorans*.

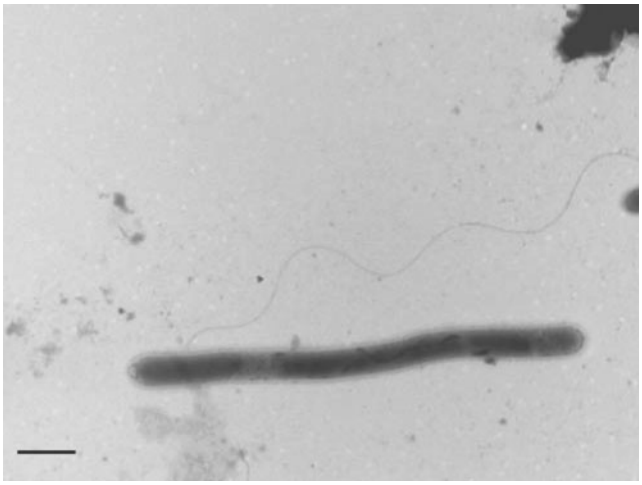

Fig. 1. Electron micrograph of a negatively stained cell of strain SOL3f37^T showing a monotrichous laterally inserted flagellum. Bar, 1 μm.

Fatty acid methyl esters were extracted from fresh biomass by the CCUG using the standard Microbial Identification System procedure (Microbial ID). The Microbial ID system was used to compare the fatty acid methyl esters of strain SOL3f37^T with fatty acid patterns stored in the fatty acid database. Six fatty acid components were found at levels greater than 2 % (Table 1), with an exceptionally high percentage of the fatty acid component iso-$C_{15:0}$ (41 %). The other major components were identified as iso-$C_{14:0}$ 3-OH and/or iso-$C_{15:0}$ dimethyl acetal (DMA) (21.6 %), iso-$C_{13:0}$ (4.4 %), anteiso-$C_{15:0}$ (3.9 %), iso-$C_{15:1}$ (2.8 %) and one major component that remained unidentified (12.5 %).

Strain SOL3f37^T did not grow in anaerobic enrichment medium containing traces of oxygen (as indicated by the pink-coloured resazurin in the growth medium) and was therefore described as a strict anaerobe. It grew anaerobically at 20–48 °C, with an optimum at 40 °C.

Substrate utilization was studied using anaerobic basal medium containing (per litre distilled water) 0.3 g KH_2PO_4, 0.3 g K_2HPO_4, 1.0 g NH_4Cl, 40.0 g NaCl, 0.5 g KCl, 0.2 g $CaCl_2$ and 0.5 g cysteine hydrochloride. Yeast extract (0.2 g or 2.0 g l^{-1}) was added to this basal medium. For determination of NaCl requirements, NaCl was weighed directly into tubes at concentrations up to 200 g l^{-1} before dispensing basal medium without NaCl. The strain did not grow when NaCl was omitted from the basal medium. It grew in NaCl concentrations ranging from 0.5 to 150 g l^{-1} (optimum 40 g l^{-1}). For pH studies, the same medium was adjusted to the desired pH using anaerobically prepared stock solutions of $NaHCO_3$ (10 %) or Na_2CO_3 (8 %). Growth occurred between pH 6.2 and 8.1; the optimum pH was 6.9. Yeast extract (2 g l^{-1}) was required for degradation of the carbon substrates pyruvate,

Table 1. Cellular fatty acid contents of strain SOL3f37^T and its closest relative, *Clostridiisalibacter paucivorans* 37HS60^T

Strains: 1, *Sporosalibacterium faouarense* gen. nov., sp. nov. SOL3f37^T; 2, *Clostridiisalibacter paucivorans* 37HS60^T (data from Liebgott *et al.*, 2008). Values are percentages (w/v) of total fatty acids. FAME, fatty acid methyl ester; DMA, dimethyl acetal; *c*, *cis*; cyc, cyclopropyl fatty acid; ND, no detected.

Fatty acid	1	2
iso-$C_{11:0}$ FAME	0.3	ND
iso-$C_{13:0}$ FAME	4.4	ND
anteiso-$C_{13:0}$ FAME	0.4	ND
iso-$C_{13:0}$ 2-OH FAME	ND	3.2
iso-$C_{13:0}$ 3-OH FAME and/or iso-$C_{15:1}$ I/H FAME	0.9	1.3
$C_{14:0}$ FAME	0.9	14.3
iso-$C_{14:0}$ 2-OH FAME	1.5	ND
iso-$C_{14:0}$ 3-OH FAME and/or iso-$C_{15:0}$ DMA	21.6	ND
iso-$C_{14:1}$ FAME	0.6	ND
iso-$C_{15:1}\omega7c$ FAME	ND	2.2
iso-$C_{15:1}$ F FAME	2.8	ND
iso-$C_{15:0}$ FAME	41.0	6.6
anteiso-$C_{15:0}$ FAME	3.9	1.5
$C_{15:1}\omega5c$ FAME	0.8	ND
$C_{16:0}$ FAME	1.2	7.6
$C_{16:1}\omega7c$ alcohol	0.3	ND
iso-$C_{16:1}$ I and/or $C_{14:0}$ 3-OH FAME	0.3	ND
$C_{16:1}\omega9c$ FAME	ND	2.5
$C_{16:1}\omega7c$ FAME	ND	9.9
$C_{16:1}\omega9c$ DMA	ND	1.8
$C_{16:1}\omega7c$ DMA	ND	8.5
iso-$C_{17:0}$ FAME	0.4	ND
$C_{17:0}$ cyc FAME	0.6	2.2
$C_{17:0}$ cyc DMA	ND	1.6
anteiso-$C_{17:1}\omega9c$ FAME	ND	2.2
anteiso-$C_{17:1}$ B FAME and/or iso-$C_{16:1}$	1.5	ND
$C_{17:1}\omega10c$ and/or anteiso-$C_{17:1}\omega3c$ FAME	ND	19.3
$C_{18:0}$ FAME	1.3	ND
iso-$C_{19:1}$ FAME	1.0	ND

fumarate, fructose, glucose and mannitol (all at 20 mM). Furthermore, strain SOL3f37^T grew heterotrophically on yeast extract, peptone and bio-Trypticase, but was unable to grow on Casamino acids. Fumarate was reduced to succinate. End products from pyruvate utilization were acetate, H_2 and CO_2. Strain SOL3f37^T did not use cellobiose, cellulose, galactose, lactose, maltose, mannose, ribose, sucrose, xylose, melibiose, arabinose, glycerol, ethanol, methanol, acetate, propionate, butyrate, lactate, citrate, xylan or starch. Acetate was produced after growth on glucose, fructose and mannitol. None of the amino acids tested (at 20 mM), including threonine, glycine, proline, tyrosine, phenylalanine, leucine, isoleucine, cysteine, lysine, serine, valine, alanine, arginine, tryptophan, asparagine, aspartic acid, glutamine, glutamic acid, methionine and histidine, supported growth.

Sulfate, thiosulfate and elemental sulfur were added separately to the anaerobic basal medium at final concentrations of 20 mM, 20 mM and 0.1 % (w/v), respectively, for potential use as an electron sink. Nitrate and nitrite utilization tests were carried out in triplicate in butyl-capped Hungate tubes filled with 5 ml pre-reduced medium (without resazurin and Na_2S) distributed under a nitrogen atmosphere. $NaNO_3$ (10 mM) or $NaNO_2$ (10 mM) was added to the tubes immediately before inoculation (10 %, v/v). These tests were carried out under similar conditions without pre-reducing the medium and without a nitrogen atmosphere for oxygen utilization.

Sulfate (20 mM), thiosulfate (20 mM), elemental sulfur (0.1 %), nitrate (20 mM) and nitrite (2 mM) were not used as electron acceptors. The sulfur test was carried out photometrically as colloidal CuS (Fardeau *et al.*, 1997) and nitrate/nitrite reduction was assayed using specific sticks (Quantofix; Macherey-Nagel).

The DNA G+C content, determined by the Identification Service of the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) using the method of Mesbah *et al.* (1989), was 30.7 mol%.

Genomic DNA of strain SOL3f37^T was extracted using the Wizard Genomic DNA Purification kit following the manufacturer's protocol (Promega). The universal primers Fd1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and Rd1 (5′-AAGGAGGTGATCCAGCC-3′) were used to amplify the 16S rRNA gene. The nucleotide sequence (1497 bases) was aligned manually using the sequence alignment editor BioEdit (Hall, 1999). Reference sequences were obtained from the Ribosomal Database Project II (Maidak *et al.*, 2001) and GenBank (Benson *et al.*, 1999). Pairwise evolutionary distances based on 1246 unambiguous nucleotides were computed by the method of Jukes & Cantor (1969). The phylogenetic tree obtained by the neighbour-joining method (Saitou & Nei, 1987) is shown in Fig. 2; its topology was supported by the maximum-parsimony and maximum-likelihood algorithms (not shown).

16S rRNA gene sequence analysis of strain SOL3f37^T revealed that it was a member of the family *Clostridiaceae* of the order *Clostridiales*, as defined in the currently proposed taxonomy in *Bergey's Manual of Systematic Bacteriology* (Wiegel, 2009). The three most closely related strains with validly published names were *Clostridiisalibacter paucivorans* 37HS60^T, *Caloranaerobacter azorensis* MV1087^T and *Thermohalobacter berrensis* CTT3^T, with sequence similarity of 93.4, 91.7 and 91.8 %, respectively. These three type strains belong to three different genera. Other closely related type strains with validly published names include *Clostridium puriniliticum* ATCC 33906^T, *Clostridium acidurici* ATCC 7906^T and *Eubacterium angustum* ATCC 43737^T, which showed sequence similarities with strain SOL3f37^T below 92 % (91.9, 91.6 and 88.8 %, respectively; Fig. 2).

Numerous phenotypic characteristics of strain SOL3f37^T enabled it to be distinguished unambiguously from

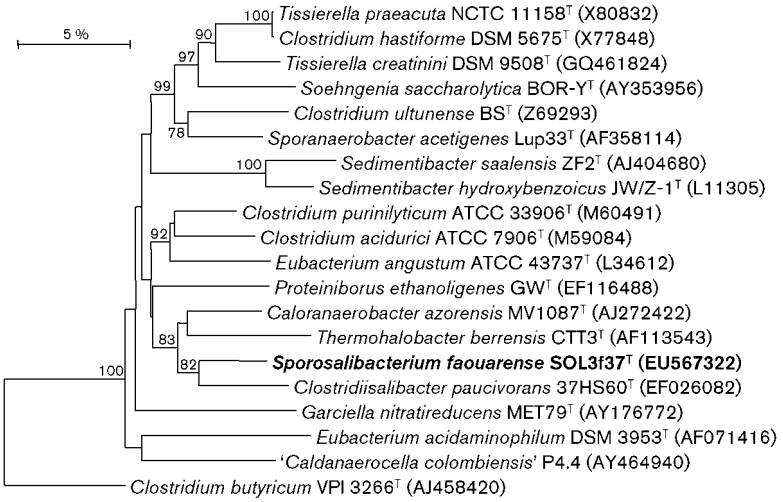

Fig. 2. Phylogenetic dendrogram based on 16S rRNA gene sequence data (1315 unambiguous alignment positions) showing the position of strain SOL3f37^T. Bootstrap values (expressed as percentages of 1000 replications) greater than 70 % are shown at branch points. Sequence accession numbers are given in parentheses. Bar, 5 % sequence divergence.

phylogenetically closely related type strains, including *Clostridium purinilyticum* ATCC 33906^T (Dürre *et al.*, 1981) and *Clostridium acidurici* ATCC 7906^T (Liebert, 1909). Isolate SOL3f37^T was metabolically distinct from these species, which grew on purines such as uric acid and adenine and on amino acids such as glycine. However, none of them grew on sugars (Wery *et al.*, 2001), whereas strain SOL3f37^T grew on two sugars (glucose and fructose), but not on amino acids, including glycine.

Strain SOL3f37^T differed markedly from *Thermohalobacter berrensis* CTT3^T. Strain SOL3f37^T stained Gram-positive and was spore-forming, whereas *Thermohalobacter berrensis* CTT3^T stained Gram-negative and was a non-sporulating rod (Cayol *et al.*, 2000). Strains SOL3f37^T and *Thermohalobacter berrensis* CTT3^T also differed in their growth conditions; strain SOL3f37^T, a slightly thermotolerant bacterium, grew between 20 and 48 °C (optimum 40 °C), whereas *Thermohalobacter berrensis* CTT3^T, a strict thermophile, grew at up to 70 °C (optimum 65 °C), but not below 45 °C.

Strain SOL3f37^T also differed unambiguously from *Caloranaerobacter azorensis* MV1087^T (Wery *et al.*, 2001). Strain SOL3f37^T stained Gram-positive, whereas *Caloranaerobacter azorensis* MV1087^T was Gram-negative and had a characteristic Gram-negative cell wall. In addition, the DNA G+C content of *Caloranaerobacter azorensis* MV1087^T (27.0 mol%) was significantly lower than the value calculated for strain SOL3f37^T (30.7 mol%). Furthermore, *Caloranaerobacter azorensis* MV1087^T and strain SOL3f37^T did not use the same carbohydrates; in contrast to *Caloranaerobacter azorensis* MV1087^T, strain SOL3f37^T was able to use glucose, fructose and mannitol but not arabinose, ribose, xylose, galactose or sorbose. Strain SOL3f37^T also differed from *Caloranaerobacter azorensis* MV1087^T in terms of physiological growth conditions; the optimum growth temperature of strain SOL3f37^T was 40 °C, whereas *Caloranaerobacter azorensis* MV1087^T was able to grow at up to 70 °C, with optimum growth at 65 °C. Hence, unlike *Caloranaerobacter azorensis* MV1087^T, which is thermophilic, strain SOL3f37^T was only slightly thermotolerant.

Finally, strain SOL3f37^T could be distinguished unambiguously from *Clostridiisalibacter paucivorans* 37HS60^T in terms of marked differences in their morphological, genetic and physiological traits. *Clostridiisalibacter paucivorans* 37HS60^T (Liebgott *et al.*, 2008) was able to utilize the organic acids succinate, fumarate and pyruvate (all at 20 mM), whereas strain SOL3f37^T was unable to use succinate, but could use fumarate and pyruvate, as well as glucose, fructose and mannitol (Table 2). Strain SOL3f37^T and *Clostridiisalibacter paucivorans* 37HS60^T both reduced fumarate slowly to succinate. End products from pyruvate fermentation were acetate, H_2 and CO_2. Unlike *Clostridiisalibacter paucivorans* 37HS60^T, which was unable to use sugars as carbon sources, strain SOL3f37^T produced acetate after growth on glucose, fructose and mannitol. Like *Clostridiisalibacter paucivorans* 37HS60^T, strain SOL3f37^T was an anaerobic, spore-forming, rod-shaped bacterium. However, the 16S rRNA gene sequence similarity between strain SOL3f37^T and *Clostridiisalibacter paucivorans* 37HS60^T was less than 93.4 %, and numerous phenotypic characteristics of isolate SOL3f37^T were completely distinct. Strain SOL3f37^T differed from *Clostridiisalibacter paucivorans* 37HS60^T in its inability to use Casamino acids and amino acids. *Clostridiisalibacter paucivorans* 37HS60^T was able to use cysteine, lysine, serine and valine for growth, whereas strain SOL3f37^T was unable to grow on any of the 20 amino acids tested. Strain SOL3f37^T and *Clostridiisalibacter paucivorans* 37HS60^T were both mesophilic and slightly thermotolerant, with a similar temperature range for growth, but *Clostridiisalibacter paucivorans* 37HS60^T grew at up to 50 °C (optimum 42 °C), whereas SOL3f37^T only grew at up to 48 °C (optimum 40 °C). In addition, some physiological characteristics enabled SOL3f37^T to be distinguished from its closest relative. Thus, in contrast to *Clostridiisalibacter paucivorans* 37HS60^T, which was able to grow at pH 5.5, strain SOL3f37^T did not grow

Table 2. Differential phenotypic characteristics of strain SOL3f37^T and type strains of phylogenetically related species

Strains: 1, *Sporosalibacterium faouarense* gen. nov., sp. nov. SOL3f37^T (data from the present study); 2, *Clostridiisalibacter paucivorans* 37HS60^T (Liebgott *et al.*, 2008); 3, *Caloranaerobacter azorensis* MV1087^T (Wery *et al.*, 2001); 4, *Thermohalobacter berrensis* CTT3^T (Cayol *et al.*, 2000). ND, No data available. None of the four strains was able to reduce thiosulfate or sulfate, but they all used pyruvate as a substrate.

Characteristic	1	2	3	4
Cell width (μm)	0.5	0.5	0.3–0.5	0.5
Cell length (μm)	5.0–10.0	3.0–8.0	0.5–2.0	3.0–8.0
Gram type	Positive	Atypical positive	Negative	Negative
Type of flagella*	ML	P	P	ML
Temperature for growth (°C)				
Range	20–48	20–50	45–65	45–70
Optimum	40	42	65	65
pH for growth				
Range	6.2–8.1	5.5–8.5	5.5–9.0	5.2–8.8
Optimum	6.9	6.8	7.0	7.0
NaCl concentration for growth (g l^{-1})				
Range	0.5–150	10–100	6.5–65	20–150
Optimum	40	50	20	50
DNA G+C content (mol%)	30.7	33.0	27.0	33.0
Growth on Casamino acids	−	+	+	−
S^0 reduction	−	−	+	−
Utilization of:				
Arginine	−	−	+	−
Cysteine	−	+	ND	−
Serine	−	+	ND	−
Lysine	−	+	ND	−
Valine	−	+	ND	−
Fructose	+	−	+	+
Galactose	−	−	+	−
Glucose	+	−	+	+
Mannose	−	−	ND	+
Ribose	−	−	+	−
Xylose	−	−	+	−
Glycerol	−	−	ND	+
Starch	−	−	+	+
Mannitol	+	−	+	+
Fumarate	+	+	−	−
Succinate	−	+	−	ND

*ML, Monotrichous, laterally inserted; P, peritrichous.

below pH 6.2. Also, unlike SOL3f37^T, *Clostridiisalibacter paucivorans* 37HS60^T was unable to grow in media containing less than 10 g NaCl l^{-1}. The maximum NaCl concentration for growth of *Clostridiisalibacter paucivorans* 37HS60^T was 100 g l^{-1}, whereas strain SOL3f37^T was able to grow in NaCl concentrations up to 150 g l^{-1}. Also, the DNA G+C content of *Clostridiisalibacter paucivorans* 37HS60^T (33.0 mol%) was significantly higher than the value calculated for strain SOL3f37^T (30.7 mol%). *Clostridiisalibacter paucivorans* 37HS60^T was motile by means of peritrichous flagella, whereas strain SOL3f37^T was motile with a monotrichous laterally inserted flagellum (Fig. 1). Finally, *Clostridiisalibacter paucivorans* 37HS60^T showed an atypical thick Gram-positive cell-wall structure composed of dense and stratified multiple layers, which is restricted to date within these closely related genera to the genus *Clostridiisalibacter*, whereas strain SOL3f37^T showed a typical Gram-positive-type cell-wall structure.

Taking into account the important differences in phylogenetic distances, strain SOL3f37^T represents a novel species in a new genus in the family *Clostridiaceae* in the order *Clostridiales*, for which the name *Sporosalibacterium faouarense* gen. nov., sp. nov. is proposed. This proposal is strongly supported by significant differences in genetic, metabolic and physiological traits and cell-wall structure.

Description of *Sporosalibacterium* gen. nov.

Sporosalibacterium (Spo.ro.sa.li.bac.te′ri.um. Gr. n. *spora* a seed and, in bacteriology, a spore; L. n. *sal*, *salis* salt; L. neut. n. *bacterium* a rod; N.L. neut. n. *Sporosalibacterium* a moderately halophilic sporulated rod).

Rod-shaped, motile, Gram-positive bacteria, forming ovoid to spherical and subterminal to terminal spores. Spores appear mainly in old cultures. Exhibit a typical Gram-positive-type cell-wall ultrastructure, unlike the thick, multilayered cell wall of the closest relative *Clostridiisalibacter paucivorans*. Moderately halophilic and slightly thermotolerant. Strictly anaerobic, chemo-organotrophic. Able to grow on yeast extract, peptone, bio-Trypticase, pyruvate, fumarate, glucose, fructose and mannitol, but unable to grow on Casamino acids. Yeast extract is required for growth. Sulfur, sulfate, thiosulfate, nitrate and nitrite are not necessary for growth. 16S rRNA gene sequence comparisons place *Sporosalibacterium* in the lineage of the low-G+C-content Gram-positive bacteria, in the family *Clostridiaceae* of the order *Clostridiales*. The type species is *Sporosalibacterium faouarense*.

Description of *Sporosalibacterium faouarense* sp. nov.

Sporosalibacterium faouarense (fa.ou.a.ren′se. N.L. neut. adj. *faouarense* from the El Faouar area in south Tunisia, where the type strain was isolated).

Displays the following properties in addition to those described for the genus. Cells are monotrichously flagellated, 0.5×5.0–10.0 μm, occurring singly or in pairs. The major fatty acids detected are iso-$C_{15:0}$, iso-$C_{14:0}$ 3-OH and/or iso-$C_{15:0}$ DMA, iso-$C_{13:0}$, anteiso-$C_{15:0}$ and iso-$C_{15:1}$ and one major component that remains unidentified, with small proportions of various other fatty acids. Growth occurs at 20–48 °C (optimum 40 °C). The optimum pH for growth is 6.9; growth occurs at pH 6.2–8.1. Optimum NaCl concentration for growth is 40 g l^{-1}; grows in the presence of NaCl concentrations up to 150 g l^{-1}. Heterotrophic. Requires yeast extract in order to degrade pyruvate, fumarate, fructose, glucose and mannitol. Does not use elemental sulfur, sulfate, thiosulfate, sulfite, nitrate, nitrite or oxygen as electron acceptors.

The type strain is SOL3f37^T (=DSM 21485^T =JCM 15487^T), isolated from a hydrocarbon-polluted soil surrounding a deep petroleum environment located in the El Faouar area in south Tunisia. The G+C content of DNA of the type strain is 30.7 mol% (HPLC).

Acknowledgements

We gratefully acknowledge Olfa Houari for valuable advice and Pierre-Pol Liebgott for technical assistance. Many thanks to Pierre Thomas and Manon Joseph for electronic microscopy and Faten Trakhna for assistance with drafting.

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