Modicisalibacter tunisiensis gen. nov., sp. nov., an aerobic, moderately halophilic bacterium isolated from an oilfield-water injection sample, and emended description of the family *Halomonadaceae* Franzmann *et al.* 1989 emend Dobson and Franzmann 1996 emend. Ntougias *et al.* 2007

Zouhaier Ben Ali Gam,^{1,2} Slim Abdelkafi,^{1,3} Laurence Casalot,¹ Jean Luc Tholozan,¹ Ridha Oueslati² and Marc Labat¹

Correspondence

Marc Labat
marc.labat@esil.univmed.fr

¹Laboratoire de Microbiologie IRD, Biotechnologie Microbienne des Environnements Chauds, UMR D180, IRD ESIL, Universités de Provence et de la Méditerranée, 163 avenue de Luminy, 13288 Marseille cedex 9, France

²Laboratoire d'Immuno-Microbiologie Environnementale et Cancérogénèse, Faculté des Sciences de Bizerte, 70021 Zarzouna, Tunisia

³Laboratoire des Bio-Procédés, Centre de Biotechnologie de Sfax, Route de Sidi Mansour Km 6, BP 'K', Sfax 3038, Tunisia

An aerobic, moderately halophilic, Gram-negative, motile, non-sporulating bacterium, strain LIT2^T, was isolated from an oilfield-water injection after enrichment on crude oil. Strain LIT2^T grew between 15 and 45 °C and optimally at 37 °C. It grew in the presence of 1–25 % (w/v) NaCl, with an optimum at 10 % (w/v) NaCl. Predominant fatty acids were C_{16:0} (26.9 %), C_{18:1ω7c} (22.6 %), C_{16:1ω7c} (20.4 %) C_{19:0} cyclo ω8c (10.9 %) and C_{17:0} (8 %). Interestingly, the relative percentages of these last two fatty acids were intermediate compared with most species among the family *Halomonadaceae* for which fatty acid composition has been determined. The DNA G + C content was 53.7 mol%, which is very low among the family *Halomonadaceae*. Strain LIT2^T exhibited 16S rRNA gene sequence similarity values of 94.06–95.15 % to members of the genus *Chromohalobacter*, 94.21–94.65 % to members of the genus *Halomonas* and 93.57 % with the single species representative of the genus *Cobetia*. Based on the phylogenetic and phenotypic evidence presented in this paper, we propose the name *Modicisalibacter tunisiensis* gen. nov., sp. nov. to accommodate strain LIT2^T. The type strain of *Modicisalibacter tunisiensis* is LIT2^T (=CCUG 52917^T =CIP 109206^T). A reassignment of the descriptive 16S rRNA signature characteristics of the family *Halomonadaceae* permitted placement of the new genus *Modicisalibacter* into the family.

The main groups of micro-organisms that are adapted to life in hypersaline environments are distributed across the domains *Archaea*, *Eucarya* and *Bacteria* (Oren, 2002). The halophilic micro-organisms that belong to the *Bacteria* comprise several representatives that are part of larger phylogenetic groups. The different branches of the phylum *Proteobacteria* have various halophilic representatives with close relatives that are non-halophilic (Kushner, 1978). Amongst the bacterial families that are part of the class

Gammaproteobacteria, the family *Halomonadaceae* is characterized by halophilic, halotolerant and non-halophilic species that belong to different genera (Franzmann *et al.*, 1988; Prado *et al.*, 2006).

Halomonas, the largest genus in this family, contains 38 species, followed by the genus *Chromohalobacter*, with seven species. The genus *Cobetia* was created with the single species *Cobetia marina* (Arahal *et al.*, 2002). To our knowledge, all the species belonging to these three genera are moderately halophilic, aerobic, Gram-negative, heterotrophic rods. Furthermore, almost all species are motile and capable of reducing nitrate. They have been isolated

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain LIT2^T is DQ641495.

from different terrestrial and aquatic environments, mainly with moderately to high salt concentrations and/or alkaline pH (Ventosa *et al.*, 1989; Arahal *et al.*, 2001a, b; Quillaguamán *et al.*, 2004a).

This report describes the phylogenetic and phenotypic characterization of isolate LIT2^T, obtained with two phylogenetically closely related strains (LIT3 and LIT4) from oilfield-water injection samples collected in the south of Tunisia. Based on the physiological and phylogenetic characteristics presented, we propose a new genus and species to accommodate strain LIT2^T.

Samples were collected in sterile glass bottles from different water injections of oilfields located in the Sidi Litayem area near Sfax (Tunisia) and stored in the dark at 4 °C until use. Enrichments were performed with a basal medium containing (l⁻¹ distilled water) 100 g NaCl, 0.3 g KH₂PO₄, 0.3 g K₂HPO₄, 1 g NH₄Cl, 0.33 g MgCl₂·6H₂O, 0.1 g CaCl₂, 1 g yeast extract and 1 ml trace-element solution (Widdel & Pfennig, 1981). The pH was adjusted to 7.2 with a 10 M KOH solution. Aliquots of 25 ml were dispensed into flasks and sterilized by autoclaving at 121 °C for 20 min. Substrates were injected from concentrated sterile stock solutions to obtain the expected final concentration to check the need of the strains for other organic substrates.

Samples (2.5 ml) from oilfield-water injections were used to inoculate 25 ml basal medium supplemented with 1 % crude oil as a carbon source. The cultures were then incubated at 37 °C under agitation at 150 r.p.m. Each enrichment culture was subcultured several times under the same conditions prior to isolation. For isolation, aliquots (100 µl) of 10⁻¹ to 10⁻¹⁰ dilutions were plated onto 1 % crude-oil agar basal medium and incubated overnight at 37 °C. Single colonies, originating from different water-injection exhausts and belonging to the same oilfield biotope, were picked and used for screening.

For all experiments, basal medium containing 1 g yeast extract l⁻¹ was used. The pH of the medium was adjusted with 5 M HCl or 10 M KOH to obtain a range between pH 4 and 11. Different amounts of NaCl were weighed directly in flasks prior to dispensing 25 ml medium to obtain the expected NaCl concentration (range 0–250 g l⁻¹); the pH was then readjusted after having added salt in each flask. The temperature range for growth was analysed between 15 and 55 °C (5 °C intervals). Nine strains were isolated from the different enrichment cultures initiated with crude oil. All strains were routinely cultured and maintained in the basal medium supplemented with 1 % crude oil, as described below. Three strains, LIT2^T, LIT3 and LIT4, were chosen, and strain LIT2^T was characterized further. In addition to the isolate, *Chromohalobacter canadensis* DSM 6769^T, *Chromohalobacter israelensis* DSM 6768^T, *Chromohalobacter salexigens* DSM 3043^T, *Chromohalobacter marismortui* DSM 6770^T, *Chromohalobacter sarencensis* CCUG 47987^T, *Chromohalobacter nigrandesensis*

DSM 14323^T, *Halomonas ventosae* DSM 15911^T, *Halomonas desiderata* DSM 9502^T, *Halomonas campisalis* ATCC 700597^T, *Halomonas alimentaria* DSM 15356^T and *Cobetia marina* DSM 4741^T were used as references for phenotypic characteristics. These micro-organisms were cultivated under the same conditions.

Optical and electron microscopy were performed as described by Abdelkafi *et al.* (2005). For heat resistance, cells grown in basal medium containing yeast extract were exposed to temperatures of 80, 90 and 100 °C for 10 min (Abdelkafi *et al.*, 2006a). The cells were cooled quickly to room temperature and then inoculated into fresh glucose-containing medium and growth was recorded after 24 h of incubation at 37 °C under agitation (150 r.p.m.).

The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Catalase activity was determined by bubble production in 3 % (v/v) hydrogen peroxide solution. Oxidase activity was determined by oxidation of 1 % *p*-aminodimethylaniline oxalate. Other phenotypic characters, including morphological, physiological and biochemical tests, were determined as described previously (Yoon *et al.*, 2001; Romanenko *et al.*, 2003; Quillaguamán *et al.*, 2004a; Abdelkafi *et al.*, 2005). Enzyme activities were examined by using the API ZYM system (bioMérieux). Resistance to antibiotics was determined on Mueller–Hinton agar (Difco 0252; Becton Dickinson) with standard antibiotic discs (bioMérieux). Inhibition diameters were recorded after 24 h of incubation at 37 °C under aerobic conditions. Classification of the strain as sensitive, not sensitive or intermediately sensitive to the antibiotics was proposed according to the disc-manufacturer's instructions (bioMérieux). Fatty acid methyl esters were analysed using the standard procedure of the Microbial Identification System (Microbial ID) and compared to the fatty-acid database. For fatty acid methyl ester analysis, bacteria were grown on blood agar at 30 °C. Bacterial growth was followed by measuring turbidity at 600 nm by using a Shimadzu model UV 160A spectrophotometer as described by Abdelkafi *et al.* (2006b).

The G+C content of the DNA was determined by the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany) using HPLC as described by Mesbah *et al.* (1989).

DNA was extracted from nine different isolates, including strain LIT2^T, according to the protocol described for the Wizard Genomic DNA purification kit (Promega). The 16S rRNA genes of these isolates were amplified by using primers Fd1 (5'-AGAGTTTGATCCTGGCTCAG-3') and 1525R (5'-AAGGAGGTGATCCAGCC-3') under the following reaction conditions: 1 min at 96 °C, 30 cycles of 20 s at 96 °C, 30 s at 55 °C and 2 min at 72 °C and a final elongation step for 5 min at 72 °C. PCR products were then cloned into the pGEM-T Easy vector (Promega) according to the manufacturer's protocol. Recombinant clones with inserts of the expected length were sequenced

by using the vector-specific primers SP6 (5'-ATTTAGG-TGACACTATAGAA-3') and T7 (5'-TAATACGACTCAC-TATAGGG-3') (Genome Express). Plasmids containing inserts of the expected length were isolated using the Wizard Plus SV Minipreps DNA purification system (Promega) according to the manufacturer's protocol and purified plasmids were sent for sequencing to GATC (Konstanz, Germany). Sequence data were imported into the sequence editor BioEdit version 5.0.9 (Hall, 1999); base calling was examined and a contiguous sequence was obtained. The full sequence was aligned by using the RDP (version 8) Sequence Aligner program (Maidak *et al.*, 2001) and adjusted manually to conform to the 16S rRNA secondary-structure model (Lane *et al.*, 1985). A non-redundant BLAST search (Altschul *et al.*, 1997) identified its closest relatives. Sequences used in the phylogenetic analysis were obtained from the RDP (version 8) (Maidak *et al.*, 2001) and GenBank (Benson *et al.*, 1999) databases. Positions of sequence and alignment ambiguity were omitted and pairwise evolutionary distances were calculated with the method of Jukes & Cantor (1969). A dendrogram was constructed using the neighbour-joining method (Saitou & Nei, 1987). Confidence in the tree topology was determined by using 100 bootstrapped trees (Felsenstein, 1985).

The enrichment cultures were positive for crude-oil tolerance after 1 week of incubation at 37 °C under agitation (150 r.p.m.). After several dilutions and sub-cultures in the same liquid medium, different stable microbial consortia developed. The morphologically dominant bacterial population was a motile and non-spore-forming bacterium. Subsequently, these enrichment cultures were serially diluted and used to inoculate Petri dishes. Numerous isolates were obtained from these different oilfield-water injection exhausts, and nine different isolates were chosen. The 16S rRNA genes of these nine strains were compared by amplified rDNA restriction analysis (ARDRA) profiles. Three of the pure colonies (LIT2, LIT3 and LIT4) showed similar ARDRA profiles (data not shown). Strain LIT2^T was selected for further characterization.

Characterization and morphology

Table 1 provides a comparison of the taxonomic features of strain LIT2^T with those of some species from the genera *Chromohalobacter* and *Halomonas* and the type strain of *Cobetia marina*. The isolate is a mesophilic, Gram-negative, motile and strictly aerobic bacterium. The catalase reaction is positive. Cells are rod-shaped (1.0–4.0 µm long and 0.6–1.0 µm wide). Colonies are smooth, circular, low-convex, cream and 2–3 mm in diameter after 48 h at 37 °C. The temperature range supporting growth is 15–45 °C, the optimal temperature being 37 °C. The pH range for growth is 5–10 with an optimum at pH 7.2. Moderately halophilic, with a NaCl range for growth of 10–250 g l⁻¹ and an optimum at 100 g l⁻¹. In API ZYM analysis, positive reactions occurred for alkaline phosphatase, esterase, leucine arylamidase, acid phosphatase and esterase lipase.

Negative reactions were observed for naphthol-AS-BI-phosphohydrolase, α-glucosidase, α-fucosidase, cystine arylamidase, lipase, valine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase and α-mannosidase. The same biochemical tests were performed with strains LIT3 and LIT4, giving similar results (data not shown). The oxidase reaction distinguished strain LIT2^T clearly from all species of the genus *Halomonas* (Yoon *et al.*, 2002). Strain LIT2^T could be differentiated from *Cobetia marina* by ribose utilization, nitrate reduction, urease activity and by its mobility (Arahal *et al.*, 2002). Strain LIT2^T could also be distinguished from species of the genus *Chromohalobacter* by several representative biochemical tests. For example, strain LIT2^T can be clearly distinguished from its closest phylogenetic relative, *Chromohalobacter canadensis* DSM 6769^T (Arahal *et al.*, 2001a), by its citrate reaction, mannose and ribose utilization and by its aesculin hydrolysis. Similarly, strain LIT2^T differs from *Chromohalobacter israelensis* DSM 6768^T (Quillaguamán *et al.*, 2004b) by its utilization of maltose, mannose and aesculin. LIT2^T can also be distinguished from both *Chromohalobacter salexigens* DSM 3043^T and *Chromohalobacter marismortui* DSM 6770^T by its utilization of mannose, maltose and ribose. Urease activity and H₂S production confirmed the differentiation of strain LIT2 from *Chromohalobacter salexigens* and nitrate reduction and fructose utilization confirmed its differentiation from *Chromohalobacter marismortui*.

According to this combination of physiological characteristics, strain LIT2^T can be distinguished by at least three traits from all the species listed in Table 1 belonging to the genera *Chromohalobacter*, *Halomonas* and *Cobetia*. Other useful characteristics to differentiate strain LIT2^T from other phylogenetically related species belonging to the family *Halomonadaceae* are listed in Table 1.

The growth behaviour of strain LIT2^T was studied in the presence of different antibiotics. The strain was susceptible to polymyxin (25 µg) and colistin (50 µg) and was weakly susceptible to penicillin (10 µg), but the strain was resistant to tetracycline (30 µg), streptomycin (10 µg), kanamycin (30 µg), neomycin (30 µg) and chloramphenicol (30 µg).

Strain LIT2^T has a cellular fatty acid profile containing large amounts of saturated and unsaturated fatty acids (Table 2). The major fatty acids detected in strain LIT2^T were C_{16:0} (26.9%), C_{18:1ω7c} (22.6%), C_{16:1ω7c} (20.4%), C_{19:0} (10.9%) and C_{17:0} (8%). This profile is similar in many points to those of the type strains of previously described species belonging to the genera *Chromohalobacter*, *Halomonas* and *Cobetia*, confirming its position in the family *Halomonadaceae* (Yoon *et al.*, 2002; Peçonek *et al.*, 2006). Moreover, similar to other members of the family *Halomonadaceae*, strain LIT2^T possessed low levels of the saturated cellular fatty acids C_{10:0} (2.1%), C_{12:0} (2.8%), C_{12:0} 3-OH (5.6%) and C_{14:0} (0.7%). However, important differences were detected in the fatty acid profile of strain LIT2^T when compared with the profiles of representative

Table 1. Differential characteristics of strain LIT2^T and other strains

Strains: 1, strain LIT2^T; 2, *Chromohalobacter canadensis* DSM 6769^T (Arahal *et al.*, 2001b); 3, *Chromohalobacter israelensis* DSM 6768^T (Quillaguamán *et al.*, 2004b); 4, *Chromohalobacter salexigens* DSM 3043^T (Vargas *et al.*, 2005); 5, *Chromohalobacter marismortui* DSM 6770^T (Ventosa *et al.*, 1989); 6, *Halomonas ventosae* DSM 15911^T (Martinez-Canovas *et al.*, 2004); 7, *Halomonas desiderata* DSM 9502^T (Yoon *et al.*, 2002); 8, *Halomonas campisalis* ATCC 700597^T (Martinez-Canovas *et al.*, 2004); 9, *Halomonas alimentaria* DSM 15356^T (Yoon *et al.*, 2002); 10, *Cobetia marina* CCUG 49558^T (Arahal *et al.*, 2002). +, Positive; –, negative; ND, not determined; NR, not reported.

Characteristic	1	2	3	4	5	6	7	8	9	10
Straight rods	+	+	+	+	+	+	+	+	–	+
Motility	+	+	+	+	+	+	+	+	–	–
Cell size (µm)	1.0–4.0 × 0.6–1.0	2.0–3.8 × 0.6–1.2	1.5–4 × 0.6–1.0	2–3.0 × 0.7–1.0	1.5–4.0 × 0.6–1	1.2–1.4 × 0.7–0.8	1–2.6 × 0.4–0.6	3–5 × 1	1.3–1.9 × 0.8–1.2	1.6–4 × 0.8–1.2
Colony form	Convex	Convex	Flat	NR	Convex	Convex	NR	NR	Low-convex	NR
Pigmentation	Cream	White	Cream	White–cream	Brown–yellow	Cream	Cream	White	Cream–yellow	NR
NaCl concentration for growth (%)										
Range	1–25	3–25	3.5–20	0.9–25	2–30	3–15	0–20	1–26	0.5–23	0.5–20
Optimum	10	8	8	7.5–10	10	8	1–5	NR	1–13	5
Temperature for growth (°C)										
Range	4–45	15–45	15–45	4–45	5–45	15–50	10–45	4–50	4–45	10–42
Optimum	37	30	30	37	37	NR	30	NR	30	37
pH for growth										
Range	5–10	5–9	5–9	5–9	5–10	6–10	7–11	6–12	6–12	5–10
Optimum	7–7.5	NR	NR	7.5	7.5	NR	NR	NR	6.5–7.5	7.5
Nitrate reduction	+	+	+	+	–	+	+	+	+	–
Indole production	–	–	–	–	–	–	–	NR	–	–
Urease activity	–	–	–	+	–	–	+	–	+	+
H ₂ S production	–	–	–	+	–	+	+	–	NR	–
Oxidase	–	–*	–*	–*	–*	+	+	+	+	–
Growth on:										
Citrate	–	+	ND	+	–	ND	NR	NR	NR	NR
Fructose	+	+	ND	+	–	ND	NR	NR	NR	NR
Glucose	+	+	ND	+	+	+	+	+	NR	+
Mannose	–	+	+	+	+	–	+	–	NR	–
Maltose	–	–	+	+	+	+	+	+	NR	–
Ribose	–	+	NR	+	+	ND	–	–	NR	+
Xylitol	–	–	NR	NR	NR	ND	NR	NR	NR	NR
Hydrolysis of:										
Gelatin	–	–	–	ND	–	–	–	–	–	–
Aesculin	–	+	+	ND	–	–	–	–	–	–
DNA G + C content (mol%)	53.7	62	65	62.4–66	62–65	72.6–74.6	66	63	66	63

*Performed according to Kovacs (1956).

type strains of the genera *Chromohalobacter*, *Halomonas* and *Cobetia*. Indeed, the proportion of C_{19:0} cyclo ω8c found in strain LIT2^T (10.9%) is rather different from that reported for *Chromohalobacter sarecensis* (28%) and *Cobetia marina* (2.7%). Quantitative differences for C_{17:0} might be used to confirm the differentiation of strains LIT2^T and LIT3 (ranging from 5.3–8%) from *Halomonas* species (ranging from 0.2–1.8%). In summary, the fatty acid profiles of strains LIT2^T and LIT3 differed significantly from those of other members of the family *Halomonadaceae* (Table 2).

The G + C content of genomic DNA of strain LIT2^T was 53.7 mol% as determined by HPLC, which is very low amongst the family *Halomonadaceae*. Nevertheless, this value falls within the range described for species of the family *Halomonadaceae* (52–74.6 mol%). Only two species, which are phylogenetically far from strain LIT2^T, show a lower G + C content (*Halomonas venusta*, 52–55 mol%; *Halomonas aquamarina*, 57–58 mol%). The DNA G + C content supported the assignment of strain LIT2^T to a new genus.

Table 2. Cellular fatty acid profiles of strains LIT2^T and LIT3 compared with some species of *Chromohalobacter* and *Halomonas* and *Cobetia marina*

Species/strains: 1, strain LIT2^T; 2, strain LIT3; 3, *Cobetia marina* CCUG 49558^T; 4, *Cobetia marina* CCUG 16090; 5, *Chromohalobacter sarencensis* CCUG 47987^T; 6, *Chromohalobacter beijerinckii* CCUG 49679^T; 7, *Chromohalobacter marismortui* DSM 6770^T (Peçonek *et al.*, 2006); 8, *Halomonas alimentaria* (Yoon *et al.*, 2002); 9, *Halomonas pacifica* (Yoon *et al.*, 2002); 10, *Halomonas cupida* (Yoon *et al.*, 2002); 11, *Halomonas venusta* CCUG 16063^T. Data in columns 3–6 and 11 were obtained from the CCUG; data in columns 7–10 were taken from the publications indicated. Cellular fatty acids were identified with the MIDI system in all cases. Values are percentages of total fatty acids. ND, Not detected.

Fatty acid	1	2	3	4	5	6	7	8	9	10	11
C _{10:0}	2.1	2.3	1.8	2.3	1.5	0.9	2.2	2.4	2.4	3.1	2.5
C _{12:0}	2.8	2.8	3.1	3.6	ND	3.2	3.6	ND	1.5	4.0	1.0
C _{12:0} 3-OH	5.6	6.0	6.6	4.4	7.5	6.7	8.2	7.0	5.6	8.5	6.5
C _{14:0}	0.7	0.8	ND	ND	1.1	1.0	ND	0.7	0.3	0.3	2.6
C _{16:0}	26.9	27.5	29.6	29.8	31.6	36.1	18.0	18.7	25.3	22.0	10.0
C _{17:0}	8.0	5.3	13.5	10.4	20.5	23.3	3.6	0.3	0.2	0.5	ND
C _{19:0} cyclo ω8c	10.9	7.9	2.7	1.0	28.0	24.9	25.5	7.8	18.5	15.5	ND
C _{16:1} ω7c	20.4	22.2	23.2	32.3	2.4	1.7	ND	ND	ND	ND	15.6
C _{18:1} ω7c	22.6	25.3	19.5	13.3	2.0	2.2	29.8	41.4	29.8	30.7	59.0

Almost-complete 16S rRNA gene sequences were determined for strains LIT3, LIT4 and LIT2^T. These sequences were aligned and compared with all bacterial sequences available in the GenBank database. A phylogenetic analysis based on 16S rRNA gene sequences showed that strains LIT2^T, LIT3 and LIT4 were members of the family *Halomonadaceae*. A neighbour-joining tree, showing the phylogenetic relationships between strains LIT2^T, LIT3 and LIT4 and selected representatives of the *Halomonadaceae*, is presented in Fig. 1. The phylogenetic analyses showed that strains LIT2^T, LIT3 and LIT4 formed a new cluster which was adequate for genus assignment. This new proposed genus is phylogenetically as far from the genus *Halomonas* as from the genus *Chromohalobacter*. Thus, strains LIT2^T, LIT3 and LIT4 formed a phylogenetically very cohesive group, where strain LIT2^T exhibited similarity levels of 95.15–94.06 % with species of the genus *Chromohalobacter*, 94.65–94.21 % with species of the genus *Halomonas* and 93.57 % with the type strain of *Cobetia marina*. Moreover, no other bacterial species shared more than 95.15 % sequence similarity with strain LIT2^T. This low level of sequence similarity indicated that strain LIT2^T could be assigned to a new genus. The sequence of the type strain contained 12 signatures associated with the family *Halomonadaceae* (data not shown). By extension, it is also proposed that the related strains LIT3 and LIT4 be placed inside the same species of this new genus.

On the basis of these findings, it is proposed that strains LIT2^T, LIT3 and LIT4 represent a novel species in a new genus, *Modicisalibacter tunisiensis* gen. nov., sp. nov.

Description of *Modicisalibacter* gen. nov.

Modicisalibacter (Mo'di.ci.sa'li.bac'ter. L. adj. *modicus* moderate, limited; L. n. *sal*, *salis* salt; N.L. masc. n. *bacter* a rod; N.L. masc. n. *Modicisalibacter* a moderately halophilic rod).

Cells are Gram-negative, non-spore-forming, motile rods. Moderately halophilic. Strictly aerobic and require Na⁺ for growth. Mesophilic, growing well at 15–45 °C, oxidase-negative and reduce nitrate. Predominant fatty acids are C_{16:0}, C_{18:1} ω7c, C_{16:1} ω7c, C_{19:0} cyclo ω8c and C_{17:0}. Contents of C_{19:0} cyclo ω8c and C_{17:0} differ significantly from those of other members of the *Halomonadaceae*. The DNA G + C content of the type strain of the type species is

Fig. 1. Phylogenetic dendrogram based on 1322 unambiguous positions of the 16S rRNA gene sequence indicating the positions of strains LIT2^T, LIT3 and LIT4 (*Modicisalibacter tunisiensis* gen. nov., sp. nov.) and their closest relatives with validly published names belonging to the genera *Chromohalobacter* and *Halomonas* and other relatives belonging to the genera *Cobetia*, *Carnimonas*, *Zymobacter* and *Halotalea*. The tree was constructed using the neighbour-joining algorithm. Accession numbers are included. Bar, 0.02 substitutions per nucleotide position.

53.7 mol% (HPLC). The genus belongs to the family *Halomonadaceae*. The type species is *Modicisalibacter tunisiensis*.

Description of *Modicisalibacter tunisiensis* sp. nov.

Modicisalibacter tunisiensis (tu.ni.si.en'sis. N.L. masc. adj. *tunisiensis* of Tunisia, where the first strains were isolated).

Displays the following properties in addition to those in the genus description. Cells are approximately 1.0–4.0 µm long and 0.6–1.0 µm wide. Colonies on marine agar are circular, smooth, convex and 2–3 mm in diameter after 48 h of incubation at 37 °C. Cells grow at 4–45 °C, with optimum growth at 37 °C. The pH range for growth is 5–10, with an optimum at pH 7.2. Optimal growth occurs in the presence of 100 g NaCl l⁻¹. Growth occurs in the range 1–250 g NaCl l⁻¹. Catalase reaction is positive. ONPG hydrolysis is negative. Citrate is not utilized. Urease and arginine dihydrolase are not produced. Gelatin, alginate and aesculin are not hydrolysed. Hydrogen sulfide and indole are not produced. Utilizes D-glucose, D-fructose, tryptone, peptone and Casamino acids. Other characteristics in comparison with closely related species of the genera *Chromohalobacter*, *Halomonas* and *Cobetia* are shown in Table 1. Tests for alkaline phosphatase, esterase, leucine arylamidase, acid phosphatase and esterase lipase are positive. Tests for naphthol-AS-BI-phosphohydrolase, α-glucosidase, α-fucosidase, cystine arylamidase, lipase, valine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-glucuronidase, β-glucosidase, N-acetyl-β-glucosaminidase and α-mannosidase are negative. Susceptible to polymyxin (25 µg) and colistin (50 µg), weakly susceptible to penicillin (10 µg) and resistant to tetracycline (30 µg), streptomycin (10 µg), kanamycin (30 µg), neomycin (30 µg) and chloramphenicol (30 µg). Predominant fatty acids are C_{16:0} (26.9 %), C_{18:1ω7c} (22.6 %), C_{16:1ω7c} (20.4 %), C_{19:0} cyclo ω8c (10.9 %) and C_{17:0} (8 %). C_{10:0}, C_{12:0}, C_{12:0} 3-OH and C_{14:0} are present in smaller amounts.

The type strain is LIT2^T (=CCUG 52917^T =CIP 109206^T), which was isolated from a sample of oilfield-water injection collected in the Sidi Litayem area near Sfax, Tunisia.

Emended description of the family *Halomonadaceae* Franzmann *et al.* 1989

The description of the family *Halomonadaceae* is as given by Franzmann *et al.* (1988) and emended previously by Dobson & Franzmann (1996) and Ntougias *et al.* (2007), with a single alteration in the 16S rRNA signature nucleotide characteristics. Alignment of sequences was carried out using the CLUSTAL W submission form according to Ntougias *et al.* (2007). All previous positions of these 16S rRNA genes are retrieved with the inclusion of the new genus *Modicisalibacter* except position 640 (A instead of G). The 16S rRNA signature nucleotides characteristic of the family *Halomonadaceae* can be redefined as follows:

position 484 (A or G), position 486 (C or U), position 640 (A or G), position 660 (A), position 668 (A), position 669 (A), position 737 (U), position 738 (U), position 745 (U), position 776 (U), position 1124 (U or G), position 1297 (U), position 1298 (C), position 1423 (A), position 1424 (C or U), position 1439 (U or C), position 1462 (A or G), position 1464 (C or U). The family comprises the genera *Carnimonas*, *Chromohalobacter*, *Cobetia*, *Halomonas*, *Halotalea*, *Modicisalibacter* and *Zymobacter*.

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