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NOTE

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Halofilum ochraceum gen. nov., sp. nov., a gammaproteobacterium isolated from a marine solar saltern

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Abstract

A Gram-stain-negative, oxidase-negative, catalase-positive, facultative anaerobe, designated XJ16^T, was isolated from a marine solar saltern on the coast of Weihai, China. Cells of strain XJ16^T were long and rod-shaped. The colonies were ochre in colour and were able to reduce nitrate to nitrite. Optimal growth occurred at 33–37 °C (range, 20–45 °C) and in the presence of 8–10 % (w/v) NaCl (range, 2–20 %). The pH range for growth was found to be 6.5–9.5, with optimum growth at pH 7.5–8.0. Phylogenetic analysis based on the 16S rRNA gene sequence demonstrated that strain XJ16^T was related to the phylum *Proteobacteria*. The most closely related neighbours were species of the genus *Thioalkalivibrio*, and the 16S rRNA gene sequence of strain XJ16^T shared 93.1 % similarity with that of *Thioalkalivibrio sulfidiphilus* HL-EbGr7^T and 93.0 % similarity with that of *Thioalkalivibrio denitrificans* ALJD^T. The G+C content of the genomic DNA was 65.9 mol% (HPLC). The sole respiratory quinone was Q-8, and the predominant cellular fatty acids (>10 %) were iso-$C_{15:0}$ 2-OH/$C_{16:1}\omega7c$, $C_{18:0}$ and $C_{16:0}$ 10-CH_3. The predominant polar lipids in strain XJ16^T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine. Based on these phylogenetic, physiological and biochemical characteristics, strain XJ16^T should be classified representing a novel species of a new genus within the family *Ectothiorhodospiraceae*, for which the name *Halofilum ochraceum* gen. nov., sp. nov. is proposed. The type strain of the type species is XJ16^T (=KCTC 42259^T=MCCC 1H00120^T=CICC 23817^T).

Members of the family *Ectothiorhodospiraceae*, described by Imhoff [1], are famous for their various types of metabolism, such as photoautotrophy, photoheterotrophy, chemoautotrophy and chemoheterotrophy. At the time of writing, the family *Ectothiorhodospiraceae* comprised 15 genera: *Acidiferrobacter*, *Alkalilimnicola*, *Alkalispirillum*, *Aquisalimonas*, *Arhodomonas*, *Ectothiorhodosinus*, *Ectothiorhodospira*, *Halorhodospira*, *Natronocella*, *Nitrococcus*, *Thioalbus*, *Thioalkalivibrio*, *Thiohalospira*, *Thiorhodospira* and *Spiribacter*. The type genus of the family is *Ectothiorhodospira* [2], with *Ectothiorhodospira mobilis* as the type species. Most members of the family *Ectothiorhodospiraceae* favour saline and alkaline growth conditions. Accordingly, in nature, members of the family *Ectothiorhodospiraceae* are found in marine to extremely saline environments that contain sulfide and have an alkaline pH, and these bacteria can be selectively enriched under such conditions. In this study, we report the characterization of a halophilic strain isolated from a marine solar saltern. The novel isolate showed the highest 16S rRNA gene sequence similarity to the established genus *Thioalkalivibrio* [3]. Based on distinct genotypic and phenotypic properties when compared to members of the most closely related taxa, the novel strain is proposed to represent a novel species of a new genus in the family *Ectothiorhodospiraceae*.

During a study of the diversity of halophilic bacteria in the marine solar saltern environment, a novel, facultatively anaerobic, ochre, Gram-stain-negative bacterial strain, XJ16^T, was isolated on modified marine agar 2216 (MA), which consisted of (all g l^{-1} in distilled water): sea salt (Sigma), 40; NaCl, 50; yeast extract, 1; peptone, 5; ferric citrate, 0.1; and agar, 18. The pH of the medium was adjusted to 7.5 before autoclaving. Modified marine broth 2216 (MB) with the same composition as the modified MA (excluding agar) was used when necessary. This medium was used for all studies with the described modifications. The sample was collected from a marine solar saltern (37° 25′ 21.7″ N 121° 59′ 23.11″ E) on the coast of Weihai, Shandong Province, China. Samples of blended water and sediment were collected from the marine solar saltern in early August 2014.

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Abbreviations: ML, maximum-likelihood ; MP, maximum-parsimony ; NJ, neighbour-joining .
The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and whole-genome shotgun project of *Halofilum ochraceum* XJ16^T are KP052777 and LVEG00000000, respectively. The version described in this paper is version LVEG02000000.
Two supplementary figures are available with the online Supplementary Material.

The pH was 8.0–9.0, the temperature on the soil surface was 40–45 °C and NaCl was the major salt. In the course of their formation, other salts, particularly Na_2CO_3, generally gave rise to samples that were both alkaline and saline to varying degrees. To isolate bacteria, 1 g wet sediment was blended with 99 ml sterilized seawater with glass beads and shaken vigorously. The suspension was serially diluted to 10^{-6} with sterilized seawater, and 0.1 ml aliquots of each dilution were spread onto modified MA plates. The plates were incubated at 37 °C for 5–7 days. Strain XJ16^T was isolated and then stored in sterile 1 % (w/v) NaCl supplemented with 15 % (v/v) glycerol at −80 °C. Cultures were routinely grown at 37 °C on modified MA, and optimum growth was obtained after 3–5 days. *Thioalkalivibrio versutus* DSM 13738^T was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Germany) and was used as the reference strain in this study.

Genomic DNA of strain XJ16^{T}was extracted and purified using a bacterial genomic DNA Mini kit (TaKaRa Bio) following the manufacturer's protocol, and the G+C content of the genomic DNA was determined by HPLC [4]. The 16S rRNA gene was amplified by PCR with the universal primers 27f and 1492r [5]. The purified PCR product was ligated into pGM-T (Tiangen) and cloned according to the manufacturer's instructions. Sequencing was performed by Shanghai Sunny Biotechnology (China) using the universal primers T7 and SP6. The nearly complete 16S rRNA gene sequence of strain XJ16^T was submitted to the GenBank database, and similar sequences were retrieved from the EzTaxon database [6]. These sequences were used to produce a phylogenetic tree according to the neighbour-joining (NJ) method [7], after multiple sequence alignment using CLUSTAL X [8] and manual editing to remove gaps at the 3' and 5' ends and ambiguous bases using BioEdit version 7.0 [9]. Maximum-parsimony (MP [10]) and maximum-likelihood (ML [11]) methods were used to confirm the phylogenetic placement of the aligned sequences. MP and ML analyses were performed using the software package MEGA version 6 [12], with Kimura's two-parameter nucleotide substitution model [13]. The stability of groupings was estimated by bootstrap analysis with 1000 replications [14].

A nearly full-length (1463 bp) 16S rRNA gene sequence of strain XJ16^T was obtained, and comparison revealed that strain XJ16^T shared the highest 16S rRNA gene sequence similarity with *Thioalkalivibrio sulfidiphilus* HL-EbGr7^T (93.1 %) and *Thioalkalivibrio denitrificans* ALJD^T (93.0 %), followed by *Thioalkalivibrio thiocyanodenitrificans* ARhD 1^T (92.9 %), *Natronocella acetinitrilica* ANL 6-2^T (92.5 %), *Ectothiorhodospira salini* JA430^T (92.0 %), *Thiorhodospira sibirica* A12^T (91.9 %), *Ectothiorhodosinus mongolicus* M9^T (91.7 %), *Thioalbus denitrificans* Su4^T (91.6 %), *Alkalilimnicola ehrlichii* MLHE-1^T (91.2 %) and *Aquisalimonas halophila* YIM 95345^T (91.1 %). Similarity values to the next closest relatives were less than 91.0 %. In the NJ phylogenetic tree based on the 16S rRNA gene sequence, strain XJ16^T formed a separate clade from the genera *Thioalkalivibrio*, *Ectothiorhodospira* and *Ectothiorhodosinus* within the family *Ectothiorhodospiraceae* (Fig. 1), which indicated that it is a genus-level taxon. The ML and MP phylogenetic trees were similar, and supported the conclusion that the novel strain represented a novel genus within the family *Ectothiorhodospiraceae*.

Based on 16S rRNA gene sequence similarity, strain XJ16^T was selected for whole genome sequencing. The genome was sequenced by Shanghai Personal Biotechnology (China) using the Illumina HiSeq platform. Assembly of the raw sequencing data was performed using Newbler (version 2.8, 20110517_1502) and GapCloser (http://soap.genomics.org.cn/soapdenovo.html). The BLAST calculation of average nucleotide identity values (called ANIb) in JSpecies was implemented as described by Goris *et al.* [15]. Average nucleotide identity values were also calculated by using the NUCmer program in the MUMmer software package [16] (called ANIm), and the percentage of conserved proteins between paired genomes was calculated according to the method of Qin *et al.* [17].

The draft genome sequencing of strain XJ16^T yielded a genome of 3 644 302 bp after assembly, and the assembly produced 35 contigs, with the coverage of 164.96. All the contigs were larger than 717 bp, and the largest was 735 968 bp. The G+C content of the bacterium, calculated from the draft genome sequence, was 65.8 %, which correlated well with the data obtained by HPLC (65.9 mol%). The average ORF length was 940.26 bp, and 3355 ORFs were detected. The ANIb (ANIm) values between strain XJ16^T and *Thioalkalivibrio sulfidiphilus* HL-EbGr7^T, *Thioalkalivibrio thiocyanodenitrificans* ARhD 1^T, *Alkalilimnicola ehrlichii* MLHE-1^T and *Thioalkalivibrio versutus* D301 (a representative of the genus *Thioalkalivibrio* that was used since no genomic sequence data were found for type strain *Thioalkalivibrio versutus* DSM 13738^T) were 68.0 (82.2), 67.5 (82.5), 67.8 (82.2) and 67.3 % (82.5 %), respectively. These values were lower than the proposed species cut-off of 95–96 % [18]. In addition, the percentage of conserved protein values between the genomes of strain XJ16^T and closely related strains are as follows: *Thioalkalivibrio sulfidophilus* HL-EbGr7^T (38.0 %), *Thioalkalivibrio thiocyanodenitrificans* ARhD 1^T (38.3 %), *Alkalilimnicola ehrlichii* MLHE-1^T (41.5 %) and *Thioalkalivibrio versutus* D301 (38.7 %), which were lower than the proposed 50 % cut-off for the genus boundary of prokaryotic lineages [17].

The colony morphology and cell size were observed by transmission electron microscopy (Jem-1200; JEOL) and light microscopy (E600; Nikon) after incubation for 4 days at 37 °C. Gram staining was performed as described by Smibert and Krieg [19]. Antibiotic susceptibility was tested in cultures incubated at 37 °C for up to 7 days using filter-paper discs containing various antibiotics. The effect of different temperatures (15, 20, 28, 33, 37, 45 and 50 °C) on growth was tested using cultures on modified MA, which were incubated until visible colonies formed (approximately 7 days). To test the

effect of pH on growth, the pH of modified MB was altered by adding the following different buffers (each 20 mM, all from Sangon): MES (for pH 5.5 and 6.0), PIPES (pH 6.5 and 7.0), HEPES (pH 7.5 and 8.0), Tricine (pH 8.5) and CAPSO (pH 9.0 and 9.5). The pH of the medium was adjusted by adding 1 M HCl or NaOH before autoclaving. The OD_{600} values of the culture were measured after 4 days of incubation at 37 °C. The effects of various salts were assessed on standard solid medium prepared with artificial seawater containing 0.32 % $MgSO_4$, 0.12 % $CaCl_2$, 0.07 % KCl and 0.02 % $NaHCO_3$ (all w/v), at different NaCl concentrations [0–25 % (w/v), in 2.0 % increments]. Hydrolysis of casein, starch, Tween 80, cellulose and sodium alginate were determined on standard solid medium supplemented with 1 % (v/v) skimmed milk, 0.2 % (w/v) soluble starch, 1 % (v/v) Tween 80, 0.5 % (w/v) CM-cellulose and 2 % (w/v) sodium alginate, respectively [20]. DNase agar (Qingdao Hope Bio-technology) prepared with distilled water and sea salt (final salinity 10 %) was used to detect DNase activity. H_2S production was assayed according to the method of Smibert and Krieg [19]. The methyl red and Voges-Proskauer (VP) tests were performed as described by Smibert and Krieg [21].

Tests for other physiological and biochemical characteristics were performed using API 20E, API 20NE, API ZYM and API 50CHB kits (bioMérieux) and Biolog system, according to the manufacturers' instructions, except that the salinity was adjusted to 10 %. The API 50CHB strips and Biolog system were incubated at 37 °C and were read every 12 h for 7 days. As the API and Biolog systems sometimes give inconsistent results when a high salt concentration is used, the carbon source utilization and sugar fermentation tests were repeated using classical methodologies. The procedures used for determining the carbon source utilization patterns of strain XJ16^T have been described by Williams *et al.* [22]. Sugar fermentation tests were performed in modified MA with 0.3 % agar according to the method of Wilde *et al.* [23]. All tests were performed in duplicate, with appropriate positive and negative controls. The nitrate reduction and oxidation-fermentation tests were performed as described by Dong and Cai [24]. Growth under anaerobic conditions was determined after cultivation in an anaerobic chamber on modified MA with or without 0.1 % (w/v) KNO_3 for at least 2 weeks at 37 °C. Oxidase activity was tested using the bioMérieux oxidase reagent kit

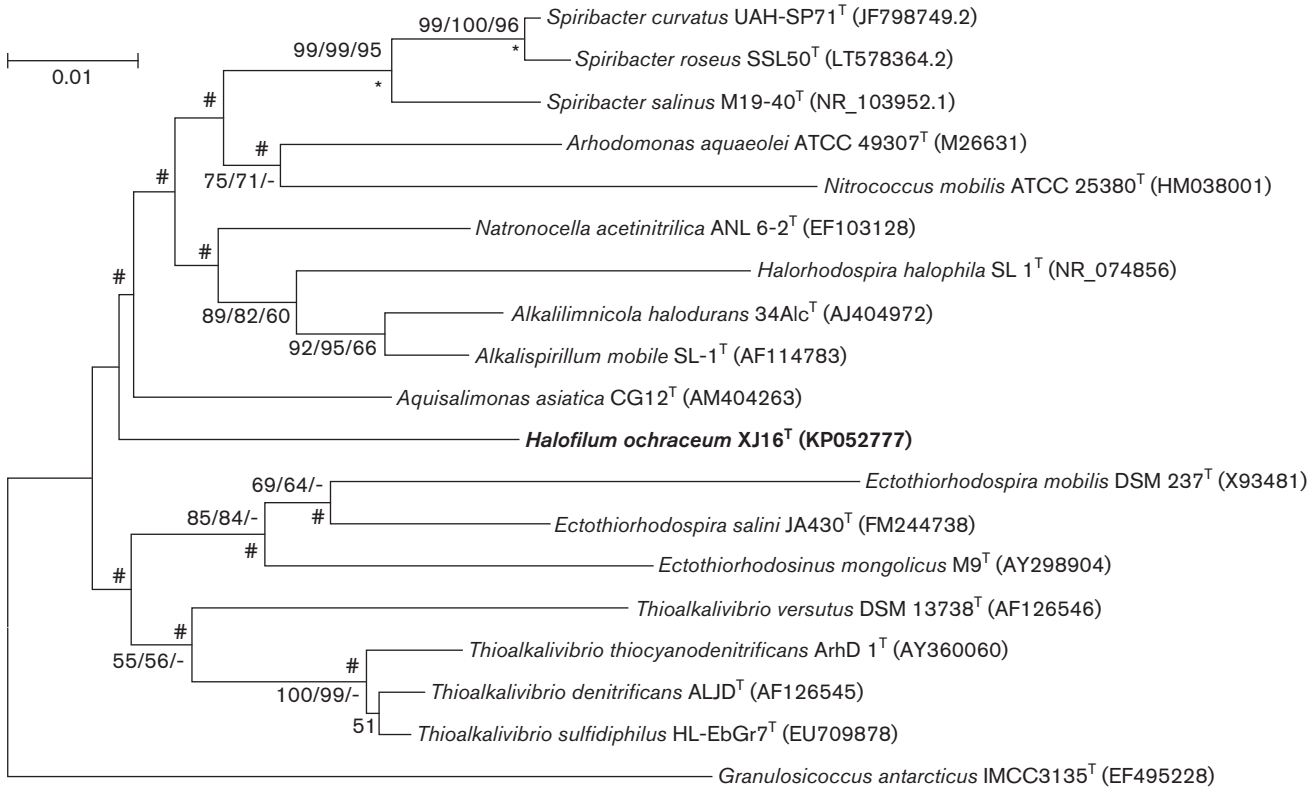

Fig. 1. Phylogenetic tree reconstructed from 16S rRNA gene sequences, showing the position of strain XJ16^T and the type strains of related taxa within the phylum *Proteobacteria*. The tree was reconstructed using the NJ algorithm. Bootstrap values (>50 %) of NJ (1000 replications)/ML (1000 replications)/MP (1000 replications) methods are shown at the nodes. Asterisks and pound signs indicate that the nodes were recovered reproducibly by all treeing methods or by two treeing methods, respectively. GenBank accession numbers for the 16S rRNA gene sequences are shown in parentheses. *Granulosicoccus antarcticus* IMCC3135^T was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

according to the manufacturer's instructions, and catalase activity was measured as the production of oxygen bubbles in a 3 % (v/v) aqueous hydrogen peroxide solution. Indole production (API 20E) and gelatin liquefaction (API 20E) were determined with the API system and by conventional methods as described by Holdeman *et al.* [25].

Strain XJ16^T formed ochre colonies of approximately 0.5 µm in diameter after incubation for 4 days on modified MA at 37 °C. Cells of strain XJ16^T were non-motile, and no flagella were observed in transmission electron micrographs (Fig. S1, available in the online Supplementary Material), which showed that the cells of strain XJ16^T were long, rod-shaped and 0.4–0.6 µm×2.0–10.0 µm in size. The morphological characteristics of XJ16^T are markedly different from those of the strains in related genera, as shown in Table 1.

Growth occurred in 2–20 % (w/v) NaCl, (optimum 8–10 %), at 20–45 °C (optimum 33–37 °C), and at pH 6.5–9.5 (optimum pH 7.5–8.0). Visible colonies formed under anaerobic conditions on modified MA with or without 0.1 % (w/v) KNO_3. Casein, starch, Tween 80, CM-cellulose, sodium alginate and DNA were not hydrolysed, H_2S and indole were not produced, while gelatin w hydrolysed according to not only the conventional method but also the API system. The methyl red test was negative, while the Voges-Proskauer test was positive. Strain XJ16^T and most of the strains in related genera were positive for nitrate reduction (except for *Halorhodospira*) and catalase. Strain XJ16^T could be readily distinguished from related members of the family *Ectothiorhodospiraceae* in that it was oxidase-negative. Strain XJ16^T was resistant to gentamicin (10 mg), vancomycin (30 mg), neomycin (30 mg), kanamycin (30 mg), tobramycin (10 mg), and norfloxacin (30 mg), but sensitive to methoxy pyrimidine (10 mg), erythromycin (15 mg), carbenicillin (10 mg), chloromycetin (30 mg), cefotaxime (30 mg), ceftriaxone (30 mg) and penicillin (10 mg). Other cultural, physiological and biochemical characteristics of this novel strain are reported in the species description. The characteristics that distinguish strain XJ16^T from its closest phylogenetic relatives are shown in Table 1.

The fatty acid and respiratory quinone composition were determined using cells cultured in modified MB at 37 °C for 4 days (i.e. at the end of the logarithmic growth phase). Fatty acids were saponified, methylated and extracted according to the standard protocol of the Sherlock Microbial Identification System (MIDI) version 6.1 using an Agilent model 6890N gas chromatograph. The peaks were automatically integrated, the fatty acid names were provided, and percentages were calculated using MIS standard software with the TSBA40 database. Respiratory quinones were extracted and purified according to Collins [26] and analysed by HPLC [27]. Polar lipids were separated by two-dimensional silica gel TLC. Total lipids were detected using molybdophosphoric acid, and specific functional groups were determined using spray reagents for specific functional groups. The complete details are given in Tindall *et al.* [28]. Cellular fatty acids and polar lipids were analysed by the Identification Service of the DSMZ (Braunschweig, Germany).

Table 1. Differential characteristics of strain XJ16^T and closely related genera in the family *Ectothiorhodospiraceae*

Taxa: 1, *Halofilum ochraceum* gen. nov., sp. nov. XJ16^T (data from this study); 2, *Thioalkalivibrio* ([3, 29–34] and this study*); 3, *Alkalilimnicola* [35, 36]; 4, *Aquisalimonas* [37, 38]; 5, *Arhodomonas* [39, 40]; 6, *Thioalbus* [41]. +, Positive; –, negative; ND, no data available; A, aerobic; F, facultatively anaerobic; CLH, chemolithoheterotrophic; CLA, chemolithoautotrophic; COH, chemo-organoheterotrophic; DPG diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidyliniositol; AL, aminolipid; PL, phospholipid.

Characteristic	1	2	3	4	5	6
Cell morphology	Long rod	Rods/barrel/short vibrioid	Oval rods/rods	Rods	Oval rods/rods	Rods
Mobility	None	Single, polar flagellum/ none	Single, polar/subpolar flagella	Motile	Single, polar flagellum	None
Pigment	Ochre	White/yellow/ brownish/reddish	Non-pigmented	Non-pigmented	Non-pigmented	Non-pigmented
Upper salt limit (%)	20	29	28	23	25	5
Oxidase reaction	–	+	+	+	+	+
Hydrolysis of:						
Gelatin	+	ND	–	–	–	ND
Tween 80	–	ND	+	+	+	ND
O_2 metabolism	F	A/F	A/F	A	A/F	F
Polar lipids	DPG, PG, PE, PC	ND	PG, PE, PC, DPG	DPG, PE, PG, PC, PI, AL, PL	PG, DPG, PGL, PE, PC	ND
Metabolism	Chemoheterotrophic	CLA	CLA/CLH	ND	COH/hydrogen autotrophic	CLA
DNA G+C content (mol%)	65.9	61.0–66.9	65.6–67.5	59.4–64.0	67.0–68.2	64.5

*Data for *Thioalkalivibrio versutus* DSM 13738^T are from this study.

The sole respiratory quinone detected in strain XJ16^T was Q-8. The fatty acids comprising >1.0 % of the total fatty acid content in strain XJ16^T were summed feature 3 ($C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH, 27.0 %), $C_{18:0}$ (24.4 %), $C_{16:0}$ 10-CH_3 (12.3 %), $C_{16:0}$ (9.8 %), $C_{18:1}\omega7c$ (7.9 %), $C_{12:0}$ (6.3 %), $C_{17:0}$ cyclo (4.6 %), $C_{14:0}$ (3.2 %) and $C_{17:0}$ (2.2 %). The predominant fatty acids (>10.0 %) in strain XJ16^T were very different from those in the reference strain *Thioalkalivibrio versutus* DSM 13738^T. $C_{18:0}$, $C_{16:0}$ 10-CH_3 and $C_{16:0}$ were the major fatty acids of XJ16^T, whereas *Thioalkalivibrio versutus* DSM 13738^T contained $C_{18:1}\omega7c$, $C_{16:1}$ and $C_{12:0}$ as the dominant fatty acids. Additional information is shown in Table 2. The polar lipids in strain XJ16^T were highly complex. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and phosphatidylcholine were the major polar lipids. An unknown phospholipid (PL4) and an unknown aminophospholipid were also present at moderate levels, and other unknown phospholipids (PL1, PL2 and PL3) were minor components. Detailed information is shown in Fig. S2.

Strain XJ16^T can be easily distinguished from members of the genera *Ectothiorhodosinus*, *Ectothiorhodospira*, *Halorhodospira*, *Thiohalospira* and *Thiorhodospira* based on O_2 metabolism, as species of these genera are either anaerobic or microaerobic, whereas strain XJ16^T is facultatively anaerobic. There is also a large difference between strain XJ16^T and other photo-autolithotrophic, photo-organoheterotrophic or photo-lithoheterotrophic members of the family *Ectothiorhodospiraceae*, for example, species of the genera *Acidiferrobacter*, *Arhodomonas*, *Ectothiorhodosinus*, *Ectothiorhodospira*, *Halorhodospira* and *Thiorhodospira*. Based on this discrepancy, five related genera of the family *Ectothiorhodospiraceae* were selected to compare with strain XJ16^T; detailed information from this analysis is shown in Table 1. The phylogenetic data, along with other phenotypic and chemotaxonomic characteristics, strongly suggest that isolate XJ16^T represents a distinct species of a novel genus in the family *Ectothiorhodospiraceae*, for which the name *Halofilum ochraceum* gen. nov., sp. nov. is proposed.

The DNA G+C content of the novel isolate was 65.9 mol%, which is much higher than that in the genera *Natronocella*, *Acidiferrobacter*, *Aquisalimonas*, *Ectothiorhodosinus*, *Nitrococcus* and *Thiorhodospira*, and this feature can also be used to differentiate XJ16^T from other related strains (Table 1).

Table 2. Fatty acid compositions of strain XJ16^T and the closely related species *Thioalkalivibrio versutus*

Strains: 1, *Halofilum ochraceum* gen. nov., sp. nov. XJ16^T; 2, *Thioalkalivibrio versutus* DSM 13738^T. All data are from this study; cells cultured at 37 °C for 4 days (end of the logarithmic phase) were used to determine fatty acids. Values are percentages of the total fatty acids. Fatty acids amounting to <1.0 % are not shown. Dominant fatty acids are highlighted in bold. –, Not detected.

Fatty acid	1	2
Straight-chain		
$C_{12:0}$	6.3	**10.9**
$C_{14:0}$	3.2	–
$C_{16:0}$	**9.8**	–
$C_{17:0}$	2.2	–
$C_{18:0}$	**24.4**	5.4
Unsaturated		
$C_{16:1}$	–	**24.6**
$C_{18:1}\omega7c$	8.0	**45.5**
Branched		
$C_{17:0}$ cyclo	4.6	–
$C_{19:0}$ cyclo $\omega8c$	–	1.6
10-methyl $C_{16:0}$	**12.3**	–
Summed feature 3*	**27.1**	**11.2**

*Summed features represent groups of two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 3 comprises $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH.

DESCRIPTION OF *HALOFILUM* GEN. NOV.

Halofilum (Ha.lo.fi′lum. Gr. n. *hals*, *halos* salt; L. neut. n. *filum* a thread; N.L. neut. n. *Halofilum* a salt-loving bacterium with a linear cell).

Cells are Gram-stain-negative, rod-shaped, facultatively anaerobic, chemoheterotrophic, catalase-positive, and oxidase-negative. Halophilic, i.e. NaCl is required for growth. Nitrate is reduced but nitrite is not. The major cellular fatty acids are $C_{16:1}\omega7c$/iso-$C_{15:0}$ 2-OH, $C_{18:0}$ and $C_{16:0}$ 10-CH_3, and the main ubiquinone system is Q-8. The major polar lipids comprise diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, and phosphatidylcholine.

The type species is *Halofilum ochraceum*, a member of the *Ectothiorhodospiraceae*.

DESCRIPTION OF *HALOFILUM OCHRACEUM* SP. NOV.

Halofilum ochraceum (o.chra′ce.um. L. n. *ochra* ochre, yellow ochre; N.L. neut. adj. *ochraceum* the colour of ochre).

Displays the following properties in addition to those listed in the genus description. Cells are long, ochre and approximately 2.0–10.0 µm in length and 0.4–0.6 µm in width. Growth occurs in 2–20 % (w/v) NaCl, at 20–45 °C, and at pH 6.5–9.5. Optimal growth is observed at 33–37 °C, pH 7.5–8.0, and with 8–10 % (w/v) NaCl. No growth occurs in the absence of NaCl. Visible colonies are formed under anaerobic conditions on standard solid medium with or without 0.1 % (w/v) KNO_3. Glucose is fermented under anaerobic conditions. Tween 80, casein, starch, CM-cellulose, sodium alginate and DNA are not hydrolysed. H_2S and indole are not produced. Voges-Proskauer and gelatin hydrolysis tests are positive, but the methyl red test is negative. Positive results for gelatinase, arginine dihydrolase, urease and β-glucosidase activities. Acid is produced from L-arabinose, D-ribose, D-xylose, D-mannose, lactose, trehalose, *N*-acetylglucosamine, arbutin, aesculin, maltose,

sucrose, gentiobiose, potassium gluconate and potassium 5-ketogluconate, and is weakly produced from D-galactose, D-glucose, D-fructose, L-sorbose, D-arabinose, L-rhamnose, D-sorbitol, cellobiose, salicin, melibiose, turanose, D-tagatose, L-fucose and potassium 2-ketogluconate. Esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase and cystine arylamidase activities are positive; activities of alkaline phosphatases and naphthol-AS-BI-phosphohydrolase are weakly positive. D-Glucuronic acid, glucuronamide and D-fructose 6-PO_4 are oxidized, and D-galacturonic acid, turanose, acetoacetic acid and L-fucose are weakly oxidized as the sole carbon and energy sources.

The type strain, XJ16^T (=KCTC 42259^T=MCCC 1H00120^T =CICC 23817^T), was isolated from a marine solar saltern on the coast of Weihai, Shandong Province, PR China. The DNA G+C content of the type strain is 65.9 mol% (HPLC).

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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