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# *Rhodohalobacter halophilus* gen. nov., sp. nov., a moderately halophilic member of the family *Balneolaceae*

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**Abstract**

A novel Gram-stain-negative, rod-shaped, facultatively anaerobic, oxidase-negative and catalase-positive bacterium, designated JZ3C29$^T$, was isolated from a saltern located in Feicheng, PR China. JZ3C29$^T$ was tolerant of moderately saline conditions. Optimal growth occurred at 40 °C (range 20–50 °C) and pH 7.5–8.5 (range pH 7.0–9.0) with 8–10 % (w/v) NaCl (range 2–16 %). Phylogenetic analysis based on 16S rRNA gene sequences revealed that JZ3C29$^T$ shared highest similarity with *Gracilimonas tropica* CL-CB462$^T$ (90.5 %), *Gracilimonas mengyeensis* YIM J14$^T$ (90.5 %) and *Gracilimonas rosea* CL-KR2$^T$ (90.4 %) and less than 90.0 % similarity with other species of the phylum *Bacteroidetes*. The isolate formed a novel genus-level clade in the recently described family *Balneolaceae*. The polar lipid profile of the novel isolate consisted of diphosphatidylglycerol, phosphatidylethanolamine, three unidentified glycolipids, four unidentified phospholipids and two unidentified lipids. The dominant cellular fatty acids (>10 %) were summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH) and iso-$C_{15:0}$ and the sole respiratory quinone was menaquinone 7 (MK-7). The DNA G+C content of JZ3C29$^T$ was 44.4 mol%. On the basis of these phenotypic and phylogenetic data, JZ3C29$^T$ should be classified as representing a novel genus and species within the family *Balneolaceae*, for which the name *Rhodohalobacter halophilus* gen. nov., sp. nov. is proposed. The type strain is JZ3C29$^T$ (=MCCC 1H00131$^T$=KCTC 52046$^T$=JCM 31413$^T$).

The recently recognized family *Balneolaceae* currently consists of four genera: *Fodinibius, Aliifodinibius, Gracilimonas* and *Balneola* [1]. The family was identified by phylogenetic analysis based on 16S rRNA gene sequences obtained from nine cultured strains, which originated from salt mines, marine solar salterns or seawater. Members of the family are Gram-stain-negative, rod-shaped, non-spore-forming, pigmented pink/orange, grow aerobically (some can be facultative anaerobes) at temperatures from 10 to 45 °C, need NaCl (5–10 % =0.9–1.8 M), and have DNA G+C contents of 39–49 mol%. In this study, a novel moderately halophilic bacterium with characteristics similar to those of members of the family *Balneolaceae* is reported and the novel genus *Rhodohalobacter* is proposed to accommodate it, on the basis of 16S rRNA gene sequence-based phylogenetic clustering and physiological and chemotaxonomic characteristics.

During a study into the diversity of halophilic bacteria in saltern environments, a novel facultatively anaerobic, red, non-motile, Gram-stain-negative bacterial strain, designated JZ3C29$^T$, was isolated on modified marine agar 2216 (MA), which consisted of (all g $l^{-1}$ in distilled water): sea salts (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 modified MA (excluding agar) was used when necessary; this medium was used for all studies with the aforementioned modifications. Samples of brine were collected from the saltern (36° 8′ 24.45″ N, 116° 49′ 22.46″ E) in early October 2015. The pH was 8.0–9.0, the total salinity was 301 ‰ (w/v), 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. For isolation of bacterial strains, 1 ml brine was added to 9 ml sterilized seawater with glass beads and shaken vigorously. The suspension was serially diluted to $1\times10^{-3}$ with sterilized seawater and 0.1 ml aliquots of each dilution were spread on modified MA. Plates were incubated at 37 °C for 5–7 days, and JZ3C29$^T$ was isolated and stored at −80 °C in sterile 1 % (w/

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**Abbreviation:** POCP, percentage of conserved proteins.
The GenBank accession number for the 16S rRNA gene sequence of *Rhodohalobacter halophilus* JZ3C29$^T$ is KU862657. The whole-genome shotgun project sequence of *Rhodohalobacter halophilus* JZ3C29$^T$ has been deposited into DDBJ/EMBL/GenBank under the accession number MDVE00000000. The version described in this paper is version MDWE01000000.
Four supplementary figures are available with the online Supplementary Material.

v) saline supplemented with 15 % (v/v) glycerol. Modified MA was used for routine growth at 40 °C (optimum growth could be obtained after 3–5 days). *Balneola vulgaris* DSM 17893$^T$, *Gracilimonas tropica* DSM 19535$^T$ and *Fodinibius salinus* DSM 21935$^T$, which were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; Germany), and another strain, *Aliifodinibius roseus* KCTC 23442$^T$, obtained from the Korean Collection for Type Cultures (KCTC; South Korea), were used as reference strains in this study and cultured under the same conditions as JZ3C29$^T$ (unless otherwise specified).

Genomic DNA of JZ3C29$^T$ was extracted and purified using a bacteria genomic DNA Mini kit (TaKaRa Bio), following the manufacturer's protocol. The gene encoding 16S rRNA was amplified by PCR with two universal primers, 27f and 1492r [2]. The purified PCR product was ligated into the PMD18-T vector (TaKaRa) and cloned according to the manufacturer's instructions. Sequencing was performed by Life Biotechnology (Shanghai, China) using the universal primers M13r and M13f. The nearly complete 16S rRNA gene sequence (1456 bp) of JZ3C29$^T$was submitted to GenBank/EMBL/DDBJ databases; similar sequences were searched for using the BLAST algorithm. The EzTaxon server (http://eztaxone.ezbiocloud.net/; [3]) was used to determine the similarity values among the sequences. The 16S rRNA gene sequence of JZ3C29$^T$ was aligned using SINA online [4], with removal of bases remaining unaligned at the ends. The aligned sequence was imported into the database of the Living Tree Project [5], release 123, using the ARB software package [6]. Close phylogenetic relatives of JZ3C29$^T$ were found and marked using ARB, and then a positional filter of 50 % conservation-based marked sequences was calculated and applied. The phylogenetic tree was reconstructed with the neighbour-joining (NJ) method [7], with Jukes–Cantor correction [8]. The arithmetic average distance between any two folded groups (sequences which clustered in a phyletic clade were folded as a group) visible in the ARB NJ tree shown was calculated using the 'Calculate Compressed Matrix' function in ARB. 16S rRNA gene sequences of several closely related species were first aligned by using CLUSTAL X (version 1.81) [9], and alignments were then manually adjusted. 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 a Kimura two-parameter nucleotide substitution model [13]. The stability of groupings was estimated by bootstrap analysis with 1000 replications [14]. Phylogenetic trees were also reconstructed using MP and ML (Figs S1 and S2, available in the online Supplementary Material).

The almost-complete 16S rRNA gene sequence was obtained from JZ3C29$^T$. Searches via the BLAST program revealed that the novel isolate had the highest similarity to *Gracilimonas tropica* CL-CB462$^T$ (90.5 %) [15] and *Gracilimonas mengyeensis* YIM J14$^T$ (90.5 %) [16], followed by *Gracilimonas rosea* CL-KR2$^T$ (90.4 %) [17], *Balneola alkaliphila* CM41_14b$^T$ (89.0 %) [18], *Aliifodinibius roseus* KCTC 23442$^T$ (88.9 %) [19], *Aliifodinibius sediminis* DSM 21194$^T$ (88.2 %) [19], *Balneola vulgaris* 13IX/A01/164$^T$ (88.1 %) [20] and *Fodinibius salinus* DSM 21935$^T$ (86.6 %) [21]. Except for the above-mentioned strains, published sequences from other strains showed less than 82.0 % 16S rRNA gene sequence similarity with that of JZ3C29$^T$. On the basis of the results of 16S rRNA gene sequence similarity comparison and phylogenetic analysis, JZ3C29$^T$ represents a distinct species of a novel genus, as the level of similarity is well below the traditional 97 % threshold cut-off. In the NJ phylogenetic tree based on 16S rRNA gene sequences, the novel strain was distinct from the members of the genera *Fodinibius*, *Aliifodinibius*, *Balneola* and *Gracilimonas* within the family *Balneolaceae* (Fig. 1), indicating that the relationship of the novel strain to other genera of the family *Balneolaceae* is consistent at the genus level. The MP (Fig. S1) and ML (Fig. S2) phylogenies displayed similar topologies, which further supported our conclusion that JZ3C29$^T$ represents a novel genus within the family *Balneolaceae*.

The genome of JZ3C29$^T$was sequenced by Shanghai Personal Biotechnology (Shanghai, 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 (ANI) values (ANIb) in JSpecies was implemented as described by Goris *et al.* [22] and the percentage of conserved proteins (POCP) between paired genomes was calculated according to the method of Qin *et al.* [23]. Draft genome sequencing of JZ3C29$^T$ yielded a genome of 3 122 722 bp after assembly, and the assembly produced 92 contigs, with a coverage of 137.9-fold. All contigs were larger than 664 bp, and the largest was 173 911 bp. The DNA G+C nucleotide content of the bacterium calculated from the draft genome sequence was 44.4 %, which correlated well with the estimated DNA G+C content (44.9 mol%) obtained by HPLC [24] and is similar to the values for related genera (Table 1). The average opening reading frame (ORF) length was 967.72 bp, and 2870 ORFs were detected. The ANIb values between JZ3C29$^T$ and *Gracilimonas tropica* DSM 19535$^T$, *Balneola vulgaris* DSM 17893$^T$, and *Salisaeta longa* DSM 21114$^T$ were 66.0, 65.9 and 62.2 %, respectively. These values were lower than the proposed species cut-off of 95–96 % [25]. In addition, The POCP values between the genome of JZ3C29$^T$ and those of closely related strains are as follows: *G. tropica* DSM 19535$^T$ (54.1 %), *B. vulgaris* DSM 17893$^T$ (51.7 %) and *S. longa* DSM 21114$^T$ (28.2 %), which were on the edge of or lower than the proposed 50 % cut-off for the genus boundary of prokaryotic lineages [23].

The morphology of colonies was examined after 4 days of incubation at 40 °C on modified MA. Transmission electron microscopy (TEM; Jem-1200; JEOL) was used to assess cell size and morphology. Light microscopic examinations (using an E600 light microscope; Nikon) were also performed to supplement TEM observations. Motility was examined according to the hanging-drop method. Gram staining was carried out as described by Smibert and Krieg

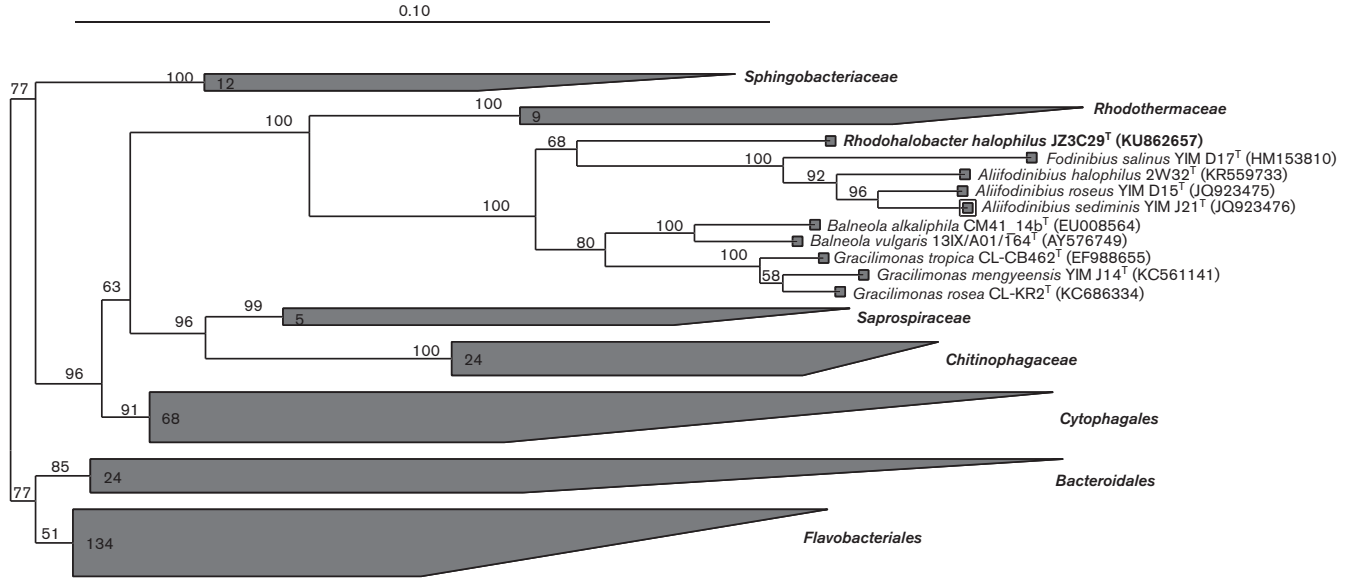


**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of JZ3C29$^T$ and members of the phylum *Bacteroidetes*. The database of LTP release 123 was used. Bootstrap values (>50 %) of neighbour-joining (1000 replications) methods are shown at the nodes. Bar, 0.1 substitutions per nucleotide position.

[26]. Susceptibility to antibiotics was tested on cultures incubated at 40 °C for up to 7 days using filter-paper discs containing various antibiotics. The effects of different growth temperatures were tested after incubations at 15, 20, 25, 28, 30, 33, 37, 42, 45 and 50 °C for approximately 7 days on modified MA until growth was indicated by visible colonies (growth conditions were recorded every 6 h). To test the effects of pH on growth, the pH of modified MB was adjusted to different levels with the addition of buffers [MES (pH5.5 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) (Sangon)] at concentrations of 20 mM, and $OD_{600}$ values of the cultures were measured after 72 h incubation at 40 °C. The pH of the medium was adjusted by adding 1M HCl or NaOH before autoclaving. The effects of different salt concentrations on growth (recorded every 6 h) were assessed by using a medium consisting of (g $l^{-1}$): yeast extract, 1; peptone, 5; ferric citrate, 0.1 and agar, 18, prepared with artificial seawater [0.32 % $MgSO_4$, 0.12 % $CaCl_2$, 0.07 % KCl and 0.02 % $NaHCO_3$ (all w/v)] and containing different concentrations of NaCl (0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 %, w/v). The hydrolysis of the following substrates was tested in modified MA as adapted from that described by Cowan and Steel [27]: 0.2 % (w/v) soluble starch, 1 % (v/v) Tween 80, 1 % (v/v) Tween 40, 1 % (v/v) Tween 20, 0.5 % (w/v) carboxymethyl cellulose or 2 % (w/v) sodium alginate, respectively. Hydrogen sulfide ($H_2S$) production was assayed according to the procedures of Smibert and Krieg [26].

Tests for other physiological or biochemical characteristics were performed using the API 20E and API 50CHB identification systems (bioMérieux) and the Biolog GEN III identification system, according to the manufacturer's instructions (except for salinity, which was adjusted to 8 %). The API 50CHB strips and Biolog were read every 12 h and for up to 7 days of culture at 40 °C. As the Biolog system may give inconsistent results for strains that require high salt concentrations for growth, we repeated the carbon source utilization tests using classical methodology. Carbon source utilization patterns of JZ3C29$^T$ were determined according to procedures described by Williams *et al.* [28]. All tests were performed in duplicate on experimental and reference strains under identical conditions, with appropriate positive and negative controls. 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 40 °C. For the nitrate reduction test, modified MB in test tubes supplemented with 0.1 % (v/v) nitrate was used. The inoculated test tubes were placed in aerobic or anaerobic conditions at 40 °C for 4 days. Uninoculated test tubes served as controls. Oxidase activity was tested using the bioMérieux oxidase reagent kit, according to the manufacturer's instructions. Catalase activity was tested by measuring the production of oxygen bubbles in 3 % (v/v) aqueous hydrogen peroxide solution. Production of indole and liquefaction of gelatin were determined using the API 20E system, as well as by conventional methods, as described by Holdeman *et al.* [29].

JZ3C29$^T$ formed circular, mucoid, reddish colonies, 1 mm in diameter, with entire and transparent edges after incubation for 3 days at 40 °C. The colour of the colonies deepened to red when the culture time was extended to 5 days. Red, reddish or orange colonies are common in members of the family *Balneolaceae* (Table 1). Cells of JZ3C29$^T$ were non-motile and no

**Table 1.** Characteristics that distinguish the proposed novel genus *Rhodohalobacter* from closely related genera in the family *Balneolaceae*

Genera: 1, *Rhodohalobacter* (data from this study); 2, *Gracilimonas* [15–17]; 3, *Balneola* [18, 20]; 4, *Aliifodinibius* [1, 19]; 5, *Fodinibius* [21]. +, Positive; −, negative. V, variable. ND, no data available.

| Characteristic | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Colony colour | Reddish | Orange, pink, pale red | Orange, pale orange | Red, rose red, salmon pink | Pink |
| Cell shape | Rods | Long irregular rods | Rods, straight rods | Rods | Rods/slightly curved |
| Motility | − | − | +/− | − | − |
| Spore formation | − | V | − | − | − |
| Salinity (% NaCl) | | | | | |
| Range | 2–16 | 1–20 | 0–8 | 2–20 | 4–23 |
| Optimum | 8–10 | 3–8 | 2–3 | 6–10 | 10–15 |
| Temperature (°C) | | | | | |
| Range | 20–50 | 15–40 | 10–40 | 20–45 | 25–45 |
| Optimum | 40 | 28–35 | 25–30 | 28–37 | 37 |
| Oxidase reaction | − | + | − | V | + |
| Nitrate reduction | − | V | + | V | + |
| Urease | − | V | − | V | + |
| Hydrolysis of: | | | | | |
| Aesculin | − | V | − | V | + |
| Gelatin | + | V | − | + | + |
| Starch | + | V | − | − | − |
| Tween 40 | − | V | − | V | + |
| Tween 80 | − | V | − | V | + |
| Major quinone | MK-7 | MK-7 | ND | MK-7 | MK-7 |
| Polar lipids* | DPG, PE, GL, PL, L | DPG, PE, PG, PL | DPG, PE, PG, GL, AL, L | DPG, PC, PE, GL, PL, AL, L | DPG, PE, PC, PG, GL, AL, PL, L |
| DNA G+C content (mol%) | 44.4 | 42.7–47.2 | 39.0–42.0 | 47.5–49.0 | 43.0 |

*DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; GL, glycolipid; PL, unknown phospholipids; AL, unkown aminolipids; L, unknown lipids.

flagella were observed by TEM, similar to results obtained for other closely related species. Electron micrographs (Fig. S3) showed that the cells of JZ3C29$^T$ were rod-shaped, ranging in size from 0.3 to 0.6 µm wide and 2.5 to 16.5 µm long. There were no obvious differences in morphological characteristics between JZ3C29$^T$ and its close relatives, which is consistent with the results of the phylogenetic analysis and 16S comparisons indicating that JZ3C29$^T$ represents a member of the family *Balneolaceae*.

Optimal growth of JZ3C29$^T$ occurred at 40 °C (range 20–50 °C) and pH 7.5–8.5 (range pH 7.0–9.0) with 8–10 % (w/v) NaCl (range 2–16 %). However, members of the genus *Aliifodinibius*, grew optimally at 28 °C. In addition, members of the family *Balneolaceae* showed no growth at 50 °C except for JZ3C29$^T$. With regard to optimal salinity, there were also obvious differences for the novel strain that distinguished it from its close relatives; the optimal salinity for the genus *Fodinibius* was greater than 10 % while for the genera *Gracilimonas* and *Balneola*, optimal salinity was 3–8 % and 2–3 % respectively (Table 1). JZ3C29$^T$ formed visible colonies on modified MA with or without 0.1 % (v/v) $KNO_3$ under micro-aerobic conditions, but not in an anaerobic jar. The isolate could not reduce nitrate under aerobic or anaerobic conditions. Among the related species, *Aliifodinibius sediminis* DSM 21194$^T$ and *Fodinibius salinus* DSM 21935$^T$ showed facultatively anaerobic growth and could reduce nitrate to nitrite. In addition, the differences in carbon utilization and acid production from carbohydrates between JZ3C29$^T$ and its close relatives were also significant (details shown in Table 2). In addition to the characteristics mentioned above, JZ3C29$^T$ was positive for catalase activity and starch hydrolysis but negative for oxidase activity and did not hydrolyse algin, Tweens 20, 40, 80 or carboxymethyl cellulose. Moreover, $H_2S$ and indole were not produced. Tests for gelatinase (performed using API 20E test strips) were positive. A total of 39 substrates gave positive reactions in the Biolog tests and 26 in the 50CH tests (details are given in the species description).

JZ3C29$^T$ was resistant to tobramycin (10 µg), neomycin (30 µg), tetracycline (30 µg), nalidixic acid (30 µg), gentamycin (30 µg), streptomycin (10 µg) and kanamycin (30 µg) but sensitive to ampicillin (10 µg), penicillin G (10 µg), ceftriaxone (30 µg), erythromycin (15 µg), rifampicin (5 µg), chloromycetin (30 µg), acetylspiramycin (30 µg), lincomycin (2 µg), clindamycin (2 µg) and cefotaxime (30 µg). Other cultural, physiological and biochemical characteristics of the

**Table 2.** Characteristics that distinguish JZ3C29$^T$ from closely related species with validly published names

Strains: 1, JZ3C29$^T$; 2, *Gracilimonas tropica* DSM 19535$^T$; 3, *Balneola vulgaris* DSM 17893$^T$; 4, *Aliifodinibius roseus* KCTC 23442$^T$; 5, *Fodinibius salinus* DSM 21935$^T$. +, Positive; −, negative; w, weak. All data are from this study.

| Characteristic | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| Enzymatic activities (API ZYM) | | | | | |
| Acid phosphatase | + | + | + | + | − |
| α-Chymotrypsin | + | + | + | − | − |
| Cystine arylamidase | − | + | w | + | + |
| Esterase (C4) | w | + | − | + | − |
| Lipase (C14) | − | w | − | + | + |
| Naphthol-AS-BI-phosphohydrolase | − | + | − | + | + |
| *N*-Acetyl-β-glucosaminidase | − | + | − | − | + |
| Trypsin | + | − | + | − | − |
| Utilization of: | | | | | |
| Aspartate | − | + | − | + | − |
| Citrate | + | − | − | − | + |
| Lactose | + | − | w | − | − |
| Maltose | − | + | + | + | − |
| Mannitol | + | − | w | − | − |
| *myo*-Inositol | + | + | + | − | − |
| Sorbitol | + | − | + | + | − |
| Sucrose | + | − | − | + | − |
| Acid production from: | | | | | |
| D-Mannose | w | + | + | + | − |
| D-Melibiose | + | − | − | − | − |
| D-Raffinose | + | w | w | − | − |
| D-Tagatose | − | + | + | + | + |
| Aesculin | + | + | − | + | − |
| Glycerol | + | + | + | − | − |
| Glycogen | + | − | − | − | − |
| Starch | + | − | − | − | − |

novel strain are given in the species description. Differential characteristics between JZ3C29$^T$ and its closest phylogenetic relatives are listed in Table 1, 2 and 3.

To determine fatty acid composition, cells were cultured on modified MA at 40 °C for 72 h to allow the bacterial communities to reach the late-exponential stage of growth (according to the four-quadrant streak method) [30]. Fatty acids were saponified, methylated and extracted using the standard protocol of the Sherlock microbial identification system (MIDI) version 6.1, equipped with a model 6890N gas chromatograph (Agilent). Peaks were automatically integrated and fatty acids were identified and percentages were calculated using the MIS standard software with the database TSBA40. The fatty acids (with amounts comprising >1.0 % of the total) in JZ3C29$^T$ were summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH; 46.5 %), iso-$C_{15:0}$ (27.3 %), iso-$C_{17:1}\omega9c$ (8.8 %), anteiso-$C_{15:0}$ (6.9 %), $C_{16:0}$ (6.5 %), $C_{14:0}$ (2.4 %) and $C_{16:1}\omega5c$ (1.7 %). The dominant fatty acids of the novel strain were similar to those of its close relatives, although there were differences in percentage composition and presence of certain major fatty acids. The fatty acids profiles enabled the four strains of species of the genera *Gracilimonas*, *Balneola*, *Aliifodinibius* and *Fodinibius* to be distinguished from JZ3C29$^T$ (Table 3).

Polar lipids were separated by two-dimensional silica gel TLC using the method of Minnikin *et al.* [31]. Four separate TLC plates (EMD Millipore, 1.16487.0001) were prepared for each sample and individually stained using phosphomolybdic acid solution (total lipids), molybdenum blue solution (phosphates), α-naphthol sulfuric solution (carbohydrates) and ninhydrin (amines) (all reagents were from Sigma-Aldrich). Respiratory quinones were extracted from 300 mg freeze dried cell material using methods described previously [32, 33], separated into different classes by TLC on silica gel, removed from the plate and analysed further by HPLC. The major polar lipids found in JZ3C29$^T$ were diphosphatidylglycerol, phosphatidylethanolamine, three unidentified glycolipids, four unidentified phospholipids and two unidentified lipids (Fig. S4). The sole respiratory quinone of JZ3C29$^T$ was MK-7, which is consistent with those of its closest relatives of the family *Balneolaceae* (Table 1).

In summary, the results of polyphasic analysis indicate that JZ3C29$^T$ represents a novel genus and species of the family *Balneolaceae*. This conclusion is supported based

**Table 3.** Fatty acid compositions of JZ3C29$^T$ and type strains of closely related species

Strains: 1, JZ3C29$^T$; 2, *Gracilimonas tropica* DSM 19535$^T$; 3, *Balneola vulgaris* DSM 17893$^T$; 4, *Aliifodinibius roseus* KCTC 23442$^T$; 5, *Fodinibius salinus* DSM 21935$^T$. All data are from this study. TR, <1.0 %. –, not detected. Fatty acids amounting to <1.0 % are not shown. Values are percentages of the total fatty acids. In this study, cells cultured at 37 °C for 4 days (end of the exponential phase) were used to determine fatty acids.

| Fatty acid | 1 | 2 | 3 | 4 | 5 |
|---|---|---|---|---|---|
| iso-$C_{13:0}$ | – | 8.9 | 14.7 | TR | TR |
| iso-$C_{14:0}$ | – | TR | 1.6 | – | – |
| $C_{14:0}$ | 2.4 | 1.2 | TR | TR | TR |
| $C_{15:1}\omega6c$ | – | 5.0 | 9.9 | TR | tr |
| iso-$C_{15:1}$ | – | 2.0 | TR | TR | 1.7 |
| iso-$C_{15:0}$ | 27.3 | 33.5 | 28.4 | 22.6 | 24.0 |
| $C_{15:0}$ | – | 2.9 | 7.0 | TR | TR |
| Anteiso-$C_{15:0}$ | 6.9 | 8.2 | 1.4 | 5.5 | 5.8 |
| $C_{16:1}\omega5c$ | 1.7 | 2.5 | 1.1 | 6.2 | 3.9 |
| $C_{16:0}$ | 6.5 | 1.8 | 1.2 | 4.0 | 1.4 |
| iso-$C_{17:1}\omega9c$ | 8.8 | 9.6 | 7.8 | 14.0 | 32.7 |
| $C_{17:1}\omega6c$ | – | 1.1 | 1.7 | TR | TR |
| $C_{17:1}\omega8c$ | – | 2.7 | 6.7 | TR | TR |
| iso-$C_{17:0}$ | – | TR | TR | 4.3 | TR |
| Anteiso-$C_{17:1}$ | – | – | – | 2.6 | TR |
| Summed feature* | | | | | |
| 3 | 46.5 | 14.3 | 13.3 | 32.3 | 22.9 |

*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.

on the results of (1) our phylogenetic and 16S gene sequence analyses, (2) comparisons of major fatty acids between species (Table 3) and (3) phenotypic, physiological and biochemical analyses. Regarding the latter, of particular note are the ability of JZ3C29$^T$ to hydrolyse starch, to utilize citrate, lactose, mannitol and sucrose and its inability to hydrolyse Tweens 40 and 80. JZ3C29$^T$ can also be readily distinguished from closely related members of the family *Balneolaceae* by its ability to produce acid from melibiose, glycogen and starch but not from D-tagatose. Therefore, on the basis of the result of these phylogenetic and chemotaxonomic analyses, we propose to recognize strain JZ3C29$^T$ as the type strain of a novel taxon *Rhodohalobacter halophilus* sp. nov.

## DESCRIPTION OF *RHODOHALOBACTER* GEN. NOV.

*Rhodohalobacter* (Rho.do.ha.lo.bac′ter. Gr. n. *rhodon*, the rose; Gr. n. *hals halos*, salt; N.L. n. *bacter*, rod; N.L. masc. n. *Rhodohalobacter*, a red halophilic rod.)

Cells are Gram-stain-negative, rod-shaped, facultatively anaerobic, heterotrophic and without flagella. Catalase-positive and oxidase-negative. Halophilic; NaCl is required for growth. The sole methyl naphthoquinone is MK-7. The major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, unidentified glycolipids, unidentified phospholipids and unidentified lipids. The type species is *Rhodohalobacter halophilus*.

## DESCRIPTION OF *RHODOHALOBACTER HALOPHILUS* SP. NOV.

*Rhodohalobacter halophilus* (ha.lo′phi.lus. Gr. n. *hals*, *halos* salt; Gr. adj. *philos* loving; N.L. masc. adj. *ha.lo'phi.lus* salt-loving).

Displays the following properties in addition to those listed for the genus: cells are 0.3–0.6 µm in width and 2.5–16.5 µm in length; colonies are circular, viscid, reddish, 1 mm in diameter, with entire and transparent edges after incubation for 3 days at 40 °C on modified marine agar 2216. The colour of the colonies deepens to red when the culture time is extended to 5 days. Optimal growth occurs at 40 °C (range 20–50 °C) and pH 7.5–8.5 (range pH 7.0–9.0) with 8–10 % (w/v) NaCl (range 2–16 %). Cells are positive for catalase activity and starch hydrolysis but negative for oxidase activity and do not hydrolyse algin, Tweens 20, 40 and 80 or carboxymethyl cellulose. Gelatin hydrolase is positive. Indole, acetoin (Voges–Proskauer reaction) and $H_2S$ are not produced. Dextrin, trehalose, gentiobiose, sucrose, turanose, stachyose, raffinose, lactose, melibiose, *N*-acetyl-$\beta$-D-mannosamine, *N*-acetyl-D-galactosamine, *N*-acetyl neuraminic acid, $\alpha$-D-glucose, D-mannose, D-fructose, 3-methyl glucose, L-rhamnose, inosine, D-sorbitol, D-mannitol, D-arabitol, *myo*-inositol, glycerol, D-glucose-6-phosphate, L-alanine, L-arginine, L-histidine, L-pyroglutamic acid, D-galacturonic acid, D-gluconic acid, D-glucuronic acid, glucuronamide, D-saccharic acid, D-lactic acid methyl ester, L-lactic acid, citric acid, L-malic acid and acetoacetic acid are oxidized as sole carbon and energy sources. Acids can be produced from

glycerol, L-arabinose, D-ribose, D-xylose, D-glucose, D-fructose, L-rhamnose, D-mannitol, arbutin, aesculin, salicin, maltose, melibiose, sucrose, trehalose, raffinose, starch and glycogen, and weakly produced from D-adonitol, D-galactose, D-mannose, inositol, methyl-$\alpha$-D-glucopyranoside, *N*-acetyl glucosamine, amygdalin and potassium-5-ketogluconate. The dominant fatty acids are summed feature 3 ($C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH) and iso-$C_{15:0}$.

The type strain, JZ3C29$^T$ (=KCTC 52046$^T$=MCCC 1H00131$^T$=JCM 31413$^T$), was isolated from a saltern near the city of Feicheng, China. The DNA G+C content of the type strain is 44.4 mol%.

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

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