Salibacter halophilus gen. nov., sp. nov., isolated from a saltern

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Abstract

A Gram-stain-negative and facultatively anaerobic bacterium, JZ3C34ᵀ, was isolated from a saltern in Feicheng, China (36° 8′ 24.45″ E 116° 49′ 22.46″ N). Cells of strain JZ3C34ᵀ were 0.3–0.4 µm wide and 1.5–2.0 µm long, catalase-positive and oxidase-negative. Colonies on modified marine agar 2216 were orange, circular, convex, translucent and approximately 1 mm in diameter after incubation for 96 h at 33°C. Growth occurred at 20–50°C (optimally at 33°C), at pH 6.5–8.5 (optimally at 7.0–8.0) and in the presence of 2–18 % (w/v) NaCl (optimally in 6 % NaCl). Phylogenetic analysis of the 16S rRNA gene indicated that strain JZ3C34ᵀ was a member of the family Cryomorphaceae within the order Flavobacteriales and the most closely related species was Owenweeksia hongkongensis DSM 17368ᵀ (89.2 % 16S rRNA gene sequence similarity). The major respiratory quinone of strain JZ3C34ᵀ was menaquinone MK-7, and the dominant fatty acids were iso-C₁5:0 and iso-C₁5;1 G. The major polar lipids were two unidentified lipids and phosphatidylethanolamine, and the genomic DNA G+C content was 39.6 mol%. Polyphasic taxonomy clearly places the new strain as a novel species within a new genus of the family Cryomorphaceae, for which the name Salibacter halophilus gen. nov., sp. nov. The type strain of Salibacter halophilus is JZ3C34ᵀ (=KCTC 52047ᵀ=MCCC 1K02288ᵀ).
motility was examined according to the method described by Bowman [9]. The Gram-reaction was carried out as described by Smibert and Krieg [10]. Anaerobic growth was tested for 7 days at 33 °C on modified MA with or without 0.1 % (w/v) NaNO₃ in an anaerobic jar with an atmosphere of 60 % CO₂, 30 % N₂ and 10 % H₂. Susceptibility to antibiotics was determined using filter-paper discs containing various antibiotics as described by Du et al. [11] and according to the Clinical and Laboratory Standards Institute (2012). Growth was evaluated at various temperatures (4, 15, 20, 25, 28, 30, 33, 37, 42, 45, 50, 55 °C) on modified MA until growth was indicated by visible colonies. The effects of different salt concentrations on growth were detected by using a medium comprising 0.1 % (w/v) yeast extract, 0.5 % (w/v) peptone and 1.8–2.0 % (w/v) agar, artificial seawater (0.32 % MgSO₄, 0.12 % CaCl₂, 0.07 % KCl and 0.02 % NaHCO₃, all w/v) and containing different concentrations of NaCl [0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20 and 25 % (w/v)]. The effect of pH on growth was investigated in modified MB from pH 5.5 to 10.0 adding the following buffers during pH adjustment: MES (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, 9.5 and 10.0), at concentrations of 20 mM. OD₆₀₀ values of the culture were measured after 96 h of incubation at 33 °C. The pH of the medium was adjusted by adding 1 M HCl or NaOH before autoclaving. The requirement for Mg²⁺ and Ca²⁺ was examined according to Zhou et al. [1]. Hydrolysis of starch, lipids, cellulose, alginate, and weens 20, 40 and 80 was determined on modified MA as described previously [10].

Tests for other physiological or biochemical characteristics were performed using the API 20E, API ZYM and API 50CHB kits (bioMérieux) and the Biolog GEN III system, according to the manufacturer’s instructions (except for salinity, which was adjusted to 6 % with sea salts). Oxidation-fermentation and nitrate reduction were tested as described by Dong and Cai [12]. Oxidase activity was tested using an oxidase reagent kit (bioMérieux) according to the manufacturers instructions. Catalase activity was tested by measuring the production of oxygen bubbles in 3 % (v/v) aqueous hydrogen peroxide solution.

The 16S rRNA gene was amplified by PCR using two universal primers as described by Liu et al. [13]. The PCR product was ligated into the pGM-T vector (Tiangen) for cloning, as described by Liu et al. [13]. Sequencing reactions were carried out using an ABI BigDye 3.1 Sequencing Kit (Applied Biosystems) and an automated DNA sequencer (model ABI 3730; Applied Biosystems). A nearly complete sequence (1448 bp) was submitted to GenBank. The EzTaxon server (eztaxone.ezbiocloud.net; [14]) was used to obtain sequences of reference type strains and to determine which taxa possessed 16S rRNA gene sequences that shared a high level of nucleotide similarity with strain JZ3C34ᵀ. Alignment of sequences was carried out using the alignment program CLUSTAL X (version 1.81) [15]. Phylogenetic analysis based on almost-complete 16S rRNA gene sequences of strain JZ3C34ᵀ and members of the family Cryomorphaceae was used to reconstruct the tree using the neighbour-joining method [16] implemented in the software package MEGA (version 6.0) [17]. The maximum-likelihood [18] and minimum-evolution [19] methods were also used to estimate and verify the taxonomic position of the novel isolate. Genomic DNA was extracted using a commercial genomic DNA extraction kit (TaKaRa MiniBEST Bacteria Genomic DNA Extraction Kit Ver. 3.0). The DNA G+C content was determined using HPLC according to the methods described by Tamaoka and Komagata [20] and Mesbah et al. [21].

To determine the major menaquinone, whole-cell fatty acids and polar lipid profiles, cells shaken (120 r.p.m.) in modified MB at 33 °C for 96 h were harvested and subjected to freeze-drying. The major menaquinone was detected according to the method described by Minnikin et al. [22]. Fatty acid methyl esters were extracted and prepared according to the

<table>
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<th>Characteristic</th>
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<th>8</th>
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<td>Y-O</td>
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<td>Growth at 4 °C/50 °C</td>
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<td>Gliding motility</td>
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<td>−</td>
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<td>v</td>
<td>−</td>
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<td>+</td>
<td>v</td>
<td>+</td>
<td>−</td>
<td>v</td>
<td>−</td>
<td>+</td>
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**Table 1.** Characteristics that distinguish strain JZ3C34ᵀ from closely related genera of the family Cryomorphaceae

Taxa: 1, strain JZ3C34ᵀ (this study); 2, Owenweeksia (data from this study except DNA G+C content) [7]; 3, Phaeocystidibacter (data from this study except DNA G+C content, [24]); 4, Wandonia [3]; 5, Fluvicola [8, 25]; 6, Lishizhenia [4, 26]; 7, Cryomorpha [5]; 8, Salinirepens [2]; 9, Brumimicrobium [5, 27]; 10, Crocinitomix [5]; 11, Luteibaculum [6]. +, Positive; −, negative; w, weakly positive; v, variable. O, orange; Y-O, yellow-orange; Y, yellow.
standard protocol of the MIDI/Hewlett Packard Microbial Identification System [25]. Polar lipids were determined using two-dimensional TLC using the method of Minnikin et al. [22]. 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-naphthyl sulfuric solution (carbohydrates) and ninhydrin (amines) all reagents were from Sigma-Aldrich.

Cells of strain JZ3C34T rods, 0.3–0.4 µm in width and 1.5–2.0 µm in length (Fig. S1, available in the online Supplementary Material). Like all members of the Cryomorphaceae, strain JZ3C34T Gram-stain-negative and hydrolyse starch. However, strain JZ3C34T be readily distinguished from other members of the family Cryomorphaceae by certain physiological features, such as tolerance to high levels of salinity (as much as 15 %, w/v, NaCl) and temperature (as high as 50 °C). Other characteristics that differentiate this strain from other representative members of the family Cryomorphaceae are shown in Table 1.

A number of other traits and characteristics of strain JZ3C34T, including antibiotic susceptibility, enzymatic activities and substrate oxidation, were also assessed. With respect to antibiotic susceptibility, strain JZ3C34T was sensitive to penicillin (10 µg), streptomycin (10 µg), cefotaxime sodium (30 µg), rifampin (5 µg), chloramphenicol (30 µg), erythromycin (15 µg), lincomycin (2 µg) and acetylspiramycin (30 µg). The novel isolate was positive for activities of alkaline phosphatase, leucine arylamidase, acid phosphatase, esterase (C4) and esterase lipase (C8), but negative for valine arylamidase, naphthol-AS-BI-phosphohydrolase, cystine arylamidase, lipase (C14), trypsin, chymotrypsin, β-galactosidase, α-glucosidase, β-glucosidase, α-mannosidase, β-fucosidase, α-galactosidase, β-glucuronidase and N-acetyl-β-glucosaminidase activities. Acid was produced from D-ribose, D-fructose, L-sorbose, trehalose, D-tagatose and 5-keto-potassium gluconate. Turanose, sodium lactate, D-serine, D-fructose 6-phosphate, L-histidine, D-glucuronic acid, glucuronamide, α-ketoglutaric acid, acetoacetic acid and gentiobiose were oxidized by strain JZ3C34T. A detailed comparison of the major features of strain JZ3C34T with its phylogenetically related neighbours is presented in Table 2.

The major menaquinone was MK-7, which differed significantly from most members of the family Cryomorphaceae except Wandonia haliotis KCTC 22610T [3]. The predominant fatty acids (>10 %) were iso-C15:0 (59.0 %) and iso-C17:0 anteiso (14.9 %), which accounted for 73.9 % of the total fatty acids (Table S1). Similar to most members of the family Cryomorphaceae, strain JZ3C34T possessed high levels of C14:0 and C15:0 branched-chain fatty acids (83.3 %). Overall, the chemotaxonomic characteristics of strain JZ3C34T are typical for bacteria of this family, although there were some differences in the proportions of major fatty acids and the type of minor fatty acids. Detailed cellular fatty acid compositions of strain JZ3C34T, Phaeocystidibacter luteus MCCC 1F01079T and Owenweeksia hongkongensis JCM 12287T are shown in Table S1. The major polar lipids of strain JZ3C34T were two unidentified lipids and phosphatidylethanolamine. Diphosphatidylglycerol, glycolipid, two unidentified phospholipids and two unidentified lipids were present in moderate to minor amounts in the polar lipid profile (Fig. 1).

The DNA G+C content of strain JZ3C34T was 39.6 mol%, which is within the range of values for members of the family Cryomorphaceae (35–45 mol%). The results of the phylogenetic analysis of the 16S rRNA gene sequences showed that strain JZ3C34T formed a distinct lineage within the family Cryomorphaceae and was most closely related to Owenweeksia hongkongensis [7] (Fig. 2), which was also confirmed by the maximum-likelihood and minimum-

### Table 2. Characteristics that distinguish strain JZ3C34T from the type strains of the two genera in the family Cryomorphaceae to which it is most closely related

<table>
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<th>Characteristic</th>
<th>1</th>
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<tr>
<td>Isolation source</td>
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<td>Marine</td>
<td>Marine</td>
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<td>Salinity (%, w/v, NaCl)</td>
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<td>1.0–7.5</td>
<td>0.25–7.5</td>
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<tr>
<td>Optimum</td>
<td>6</td>
<td>3–5</td>
<td>2–5</td>
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<tr>
<td>Temperature for growth (°C)</td>
<td>20–50</td>
<td>4–37</td>
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<tr>
<td>Oxidase reaction</td>
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<tr>
<td>Hydrolysis of</td>
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<tr>
<td>Tween 20</td>
<td>+</td>
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<td>–</td>
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<td>Tween 80</td>
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<td>+</td>
<td>–</td>
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<tr>
<td>Gelatin</td>
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<td>Utilization of</td>
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<tr>
<td>Gentiobiose</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>D-Fructose</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>L-Galactonic acid lactone</td>
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<td>+</td>
<td>–</td>
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<tr>
<td>D-Glucuronic acid</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>L-Rhamnose</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Bromosuccinic acid</td>
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<td>–</td>
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<tr>
<td>D-Fructose 6-phosphate</td>
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<td>–</td>
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<tr>
<td>Acid production from</td>
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<tr>
<td>Mannose</td>
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<tr>
<td>D-Ribose</td>
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<tr>
<td>D-Fructose</td>
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<tr>
<td>L-Sorbose</td>
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<tr>
<td>Ascin</td>
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<td>+</td>
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<tr>
<td>Trehalose</td>
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<td>–</td>
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<tr>
<td>D-Tagatose</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Major quinone</td>
<td>MK-7</td>
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<tr>
<td>DNA G+C content (mol %)</td>
<td>39.6</td>
<td>39–40</td>
<td>44.6</td>
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</table>
analyses, we propose that strain JZ3C34T is recognized as the type strain of a new taxon within the family Cryomorphaeae, for which the name Salibacter halophilus gen. nov., sp. nov. is proposed.

**DESCRIPTION OF SALIBACTER GEN. NOV.**

Salibacter (Sa.li.bac’ter. L. masc. n. sal, salis salt; N.L. masc. n. bacter a rod; N.L. masc. n. Salibacter a salted rod).

Cells are Gram-stain-negative, halophilic, facultatively anaerobic, oxidase-negative, catalase-positive, non-flagellated rods that do not form endospores. The main respiratory quinone is MK-7. The major polar lipids include phosphatidylethanolamine and two unidentified lipids. The major cellular fatty acids are iso-C15 : 0 and iso-C15 : 1 G.

The type species is Salibacter halophilus.

**DESCRIPTION OF SALIBACTER HALOPHILUS SP. NOV.**

Salibacter halophilus [ha.lo’phi.lus. Gr. n. hals, halos salt; N.L. adj. halophilus -a -um (from Gr. adj. philos -e -on) friend, loving; N.L. masc. adj. halophilus salt-loving].

Has the following properties in addition to those given in the genus description. Cells are non-motile rods, approximately 0.3–0.4 µm in width and 1.5–2.0 µm in length. Colonies on modified MA are orange, circular, convex, translucent and approximately 1 mm in diameter after incubation for 96 h at 33°C. Growth occurs at 20–50°C (optimum 33°C), at pH 6.5–8.5 (optimum pH 7.0–8.0) and in the presence of 2.0–
18.0 % (w/v) NaCl (optimum 6.0 %). Growth occurs on modified MA and requires Na⁺, Mg²⁺, and Ca²⁺. Gelatin, and Tweens 20, 40 and 80 are hydrolysed, but starch, agar and alginate are not hydrolysed. Nitrate is not reduced to nitrite. Tweens 20, 40 and 80 are hydrolysed, but starch, agar and DNA G+C content of the type strain is 39.6 mol%.

The type strain is JZ3C34T (=KCTC 52047T=MCCC 1K02288T), isolated from a saltern in Feicheng, China. The DNA G+C content of the type strain is 39.6 mol%.

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Conflicts of interest

Ethical statement
This article does not contain any studies with animals performed by any of the authors.

References