Longimonas halophila gen. nov., sp. nov., isolated from a marine solar saltern

Jun Xia,¹ Yan-Xia Zhou,¹ Li-Hua Zhao,¹ Guan-Jun Chen¹,² and Zong-Jun Du¹,²

¹College of Marine Science, Shandong University at Weihai, Weihai 264209, PR China
²State Key Laboratory of Microbial Technology, Shandong University, Jinan 250100, PR China

A bacterial strain, designated SYD6ᵀ, was isolated from a marine solar saltern on the coast of Weihai, Shandong Province, PR China. Cells of strain SYD6ᵀ were rod-shaped, red, and approximately 5.0–9.0 μm in length and 0.4–0.6 μm in width. This strain was Gram-stain-negative, facultatively anaerobic, heterotrophic, catalase-positive and oxidase-negative. Growth occurred in 4–25 % (w/v) NaCl [with 2–15 % (w/v) MgCl₂·6H₂O also present], at 20–50 °C and pH 6.5–8.5. Optimal growth was observed at 37–42 °C, pH 7.5–8.0, with 6–8 % (w/v) NaCl [with 2–4 % (w/v) MgCl₂·6H₂O]. Nitrate was not reduced. Glucose, sucrose, maltose, fructose and ribose stimulated growth, but not glycerol, xylitol or mannitol. The G+C content of the genomic DNA was 61.5 mol% (HPLC). The sole methyl naphthoquinone was MK-7 and the predominant polar lipids were phosphatidylethanolamine, phosphatidylethanolamine, diphosphatidylglycerol and an unknown lipid. Phylogenetic analysis, based on 16S rRNA gene sequences, demonstrated that strain SYD6ᵀ was affiliated with the phylum Bacteroidetes. The most closely related neighbours were species of the genus Salisaeta and strain SYD6ᵀ had a 16S rRNA gene sequence similarity of 91.97 % with Salisaeta longa DSM 21114ᵀ. On the basis of these phenotypic and phylogenetic data, strain SYD6ᵀ represents a novel species of a new genus of the family Rhodothermaceae, for which the name Longimonas halophila gen. nov., sp. nov. is proposed. The type strain of the type species is SYD6ᵀ (=CICC 10838ᵀ = KCTC 42399ᵀ).

At the time of writing, the family Rhodothermaceae of the class Cytophagia in the phylum Bacteroidetes (Ludwig et al., 2010) includes five genera with validly published names: Rhodothermus (Alfredsson et al., 1988), Salinibacter (Antón et al., 2002), Salisaeta (Vaisman & Oren, 2009), Rubricoccus (Park et al., 2011), and the most recently proposed genus Rubrivirga (Park et al., 2013), according to the List of Prokaryotic Names with Standing in Nomenclature (Parte, 2014). Members of these genera are Gram-stain-negative, require NaCl for growth and are mostly orange–reddish. All genera have, so far, been isolated from saline environments. This paper reports on the characterization of the halophilic bacterial strain, SYD6ᵀ, isolated from a marine solar saltern, and the classification of this strain in a novel genus and species within the family Rhodothermaceae.

Abbreviations: DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SYD6ᵀ is KF955302.

A supplementary figure and a supplementary table are available with the online Supplementary Material.

Salisaeta longa DSM 21114ᵀ was used as a reference strain in this study.

During a study into the diversity of halophilic bacteria, a sediment sample was collected from a marine solar saltern on the Weihai coast, Shandong Province, PR China (37° 25' 07.91" N 121° 59' 21.48" E). The sample was serially diluted to 10⁻⁴ with sterilized seawater and 0.1 ml aliquots of each dilution were spread onto solid medium. Plates were incubated at 37 °C for 5–7 days, and strain SYD6ᵀ was isolated. Routine growth was optimum after 3–5 days at 37 °C in medium consisting of seawater with the following additions (all g l⁻¹): NaCl, 50; MgCl₂·6H₂O, 30; K₂SO₄, 5; CaCl₂·2H₂O, 0.1; yeast extract, 1; peptone, 1; and soluble starch, 2. The pH was adjusted to 7.5 and the medium autoclaved. This medium was used for all studies, with modifications as described. For solid medium, 1.8 % (w/v) agar was added. Cell morphology was observed using light microscopy (E600; Nikon) after staining with Ryu staining solution (West et al. 1977). The morphology of colonies was observed after incubation for 4 days at 37 °C. Gram staining was carried out as described by Smibert & Krieg (1994). Susceptibility to antibiotics was...
tested on cultures incubated at 37 °C for up to 7 days using filter-paper discs containing various antibiotics. The effects of different temperatures on growth were tested on incubations at 5, 10, 18, 28, 33, 37, 42, 45 and 50 °C for approximately 7 days in solid medium. To test the effects of pH on growth, standard medium was modified with the addition of buffers [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) (Sangon)] at concentrations of 20 mM and then the OD_{600} was measured. The pH of the medium was adjusted by adding 1 M HCl or NaOH before autoclaving. The effects of different salt concentrations were assessed by using the standard solid medium made with artificial seawater (0.32 % MgSO_{4}, 0.12 % CaCl_{2}, 0.07 % KCl and 0.02 % NaHCO_{3}, all w/v), with NaCl at 0–25 % (w/v), and the concentration of MgCl_{2}.6H_{2}O kept at 3 % (w/v). MgCl_{2}.6H_{2}O concentrations ranging from 0–20 % (w/v), were also tested in the same media with an NaCl concentration of 8 % (w/v). Hydrolysis of agar, starch, CM-cellulose, sodium alginate and Tween 80 were determined according to the methods of Dong & Cai (2001). The genomic DNA G+C content was determined according to Gonzalez & Saiz-Jimenez (2002).

Physiological and biochemical characteristics were tested with API 20E and API ZYM kits (bioMérieux) according to the manufacturer’s instructions, except that the suspension medium was supplemented with 8 % (w/v) NaCl and 3 % (w/v) MgCl_{2}.6H_{2}O. Tests of acid production from carbohydrates were performed using the API 50CHB fermentation kit (bioMérieux), according to the manufacturer’s instructions. NaCl and MgCl_{2}.6H_{2}O concentrations in 50CHB medium were adjusted to 8 % and 3 % (w/v), respectively. Stimulation of growth by sugars (5 g l^{-1}) was tested in soluble starch-free medium with the concentrations of yeast extract and peptone both lowered to 0.1 g l^{-1}, and 20 mM HEPES buffer (pH 7.0) added (Vaisman & Oren, 2009). All tests were performed in duplicate, with appropriate positive and negative controls and also on the reference strain Salisaeta longa DSM 21114 \textsuperscript{T} under identical conditions. Reduction of nitrate and the oxidation-fermentation test were performed as described by Dong & Cai (2001). Growth under anaerobic conditions was determined after cultivation in an anaerobic chamber on solid medium 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 according to the manufacturer’s instructions, and catalase activity was determined from the production of oxygen bubbles in 3 % (w/v) aqueous hydrogen peroxide solution.

Cells cultured at 37 °C for 3 days (end of the exponential phase) were used to determine fatty acid and respiratory quinone compositions. Fatty acids were saponified, methylated and extracted using the standard protocol of the Sherlock Microbial Identification System (MIS; MIDI) version 6.1 with an Agilent model 6890N gas chromatograph. Peaks were automatically integrated and fatty acids names and percentages calculated using the MIS standard software with the database TSBA40. Respiratory quinones were extracted and purified according to Collins (1985) and analysed by HPLC (Kroppenstedt, 1982). Polar lipids were separated by two-dimensional silica gel TLC. Total lipids were detected using molybdophosphoric acid, and defined functional groups were detected using spray reagents specific for the groups. Full details are given by Tindall et al. (2007). Cellular fatty acid and polar lipid analyses were carried out by the Identification Service of the Leibniz-Institut Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany.

DNA was extracted and purified using a bacteria genomic DNA Mini kit (TaKaRa Bio) following the manufacturer’s protocol. The 16S rRNA gene was amplified by PCR with two universal primers, 27f and 1492r (Li et al., 2014). The purified PCR product was ligated into the pGM-T vector (Tiangen) and cloned according to the manufacturer’s instructions. Sequencing was performed by Shanghai Sunny Biotechnology, China. The nearly complete 16S rRNA gene sequence of strain SYD6 \textsuperscript{T} was submitted to the GenBank database and similar sequences searched for using the BLAST and EzBioCloud algorithm. 16S rRNA gene sequences of several closely related species were first aligned by using CLUSTAL X (version 1.81) (Thompson et al. 1997), and alignments were then manually adjusted. Phylogeny was inferred by using the neighbour-joining method with Kimura’s two-parameter model (Kimura, 1980) implemented in the computer program MEGA version 6 (Tamura et al., 2013), and statistical reliability was assessed from 1000 bootstrap replicates.

Colonies of strain SYD6 \textsuperscript{T} were circular with entire edges, red, opaque and slightly sticky. Cells were rod-shaped with no flagella. Growth occurred in 4–25 % (w/v) NaCl (with MgCl_{2}.6H_{2}O at 2–15 %, w/v), at 20–50 °C and at pH 6.5–8.5. Optimal growth was observed at 37–42 °C, pH 7.5–8.0 and with 6–8 % (w/v) NaCl (with MgCl_{2}.6H_{2}O at 2–4 %, w/v). Growth was not observed without NaCl or MgCl_{2}.6H_{2}O or under anaerobic conditions. The complete morphological, physiological and biochemical characteristics of strain SYD6 \textsuperscript{T} are given in the species description.

Strain SYD6 \textsuperscript{T} contained MK-7 as the sole menaquinone. The major polar lipids of strain SYD6 \textsuperscript{T} were phosphatidyethanolamine (PE), phosphatidylcholine (PC), diphosphatidylglycerol (DPG) and an unknown lipid (L4). In addition, three lipids (L1–L3), an aminolipid (AL) and two glycolipids (GL1–GL2) were detected (Fig. S1, available in the online Supplementary Material). The predominant cellular fatty acids (with amounts comprising >10 % of the total) of strain SYD6 \textsuperscript{T} were iso-C_{15}:0, 2-OH/C_{16}:1, 10t7c (23.9 %), iso-C_{16}:0 (13.1 %), C_{18}:1,10t7c/C_{18}:1,10t6c (11.8 %) and C_{18}:1,10t7c (11.5 %).

The DNA G+C content of strain SYD6 \textsuperscript{T} was determined to be 61.5 mol\%, which is close to that determined for Salisaeta longa DSM 21114 \textsuperscript{T}, but much lower than that of other related genera in the family Rhodothermaceae. Strain SYD6 \textsuperscript{T} and Salisaeta longa DSM 21114 \textsuperscript{T}, like most
members of the other related genera, were catalase-positive and negative for nitrate reduction. The predominant cellular fatty acid of strain SYD6\textsuperscript{T} and *Salisaeta longa* DSM 21114\textsuperscript{T} was C\textsubscript{16}:\textit{i}07c and or/iso-C\textsubscript{15}:0 2-OH (Summed feature 3). However, there were some differences in the proportions and types of the less abundant fatty acids present in these two strains. Indeed, the relative proportions of C\textsubscript{16}:0 and C\textsubscript{18}:1 17c distinguished strain SYD6\textsuperscript{T} from *Salisaeta longa* DSM 21114\textsuperscript{T} (Table S1).

Despite the common chemotaxonomic traits of strain SYD6\textsuperscript{T} and the closely related *Salisaeta longa* DSM 21114\textsuperscript{T}, strain SYD6\textsuperscript{T} differed sufficiently from *Salisaeta longa* DSM 21114\textsuperscript{T}, as demonstrated by its ability to hydrolyze Tween 80, but not to produce indole, and to produce acid from arabinose, inulin and glycogen, but not from cellobiose, melibiose, trehalose or melezitose. Strain SYD6\textsuperscript{T} could also be readily distinguished from *Salisaeta longa* DSM 21114\textsuperscript{T} in that its growth could be stimulated by fructose and ribose, but not by glycerol, and also *Salisaeta longa* DSM 21114\textsuperscript{T} did not grow when MgCl\textsubscript{2}.6H\textsubscript{2}O was replaced with MgSO\textsubscript{4}.7H\textsubscript{2}O, while strain SYD6\textsuperscript{T} grew well in both. In addition, many more characteristics of strain SYD6\textsuperscript{T} are different from those of members of other related genera in the family *Rhodothermaceae*. For example, cells of strain SYD6\textsuperscript{T} are much longer than *Rubricoccus marinus* NBRC 107124\textsuperscript{T} (Park et al., 2011), *Rhodothermus marinus* DSM 4252\textsuperscript{T} (Alfredsson et al., 1988), *Salinibacter ruber* DSM 13855\textsuperscript{T} (Antón et al., 2002) and *Rubrivirga marina* KCTC 23867\textsuperscript{T} (Park et al., 2013). Strain SYD6\textsuperscript{T} can grow at 45 °C, but *Rubricoccus marinus* NBRC 107124\textsuperscript{T} and *Rubrivirga marina* KCTC 23867\textsuperscript{T} cannot. The NaCl concentration required for growth of strain SYD6\textsuperscript{T} is much higher than that of *Rubricoccus marinus* NBRC 107124\textsuperscript{T}, *Rhodothermus marinus* DSM 4252\textsuperscript{T} and *Rubrivirga marina* KCTC 23867\textsuperscript{T}. Detailed information of characteristics that distinguished strain SYD6\textsuperscript{T} from closely related genera in the family *Rhodothermaceae* are presented in Table 1.

The 16S rRNA gene analysis placed strain SYD6\textsuperscript{T} within the class *Cytophagia*. The sequences obtained were aligned with sequences deposited in the GenBank database using BLAST software (Altschul et al., 1997). The nearly complete 16S rRNA gene sequence of strain SYD6\textsuperscript{T} had a similarity of 91.97 % with that of *Salisaeta longa* DSM 21114\textsuperscript{T}. 16S rRNA gene sequence similarities to other close relatives were less than 90.0 %, including *Salinibacter ruber* DSM 13855\textsuperscript{T} (88.23 %), *Salinibacter luteus* CGMCC 1.11002\textsuperscript{T} (87.55 %) and *Salinibacter iranicus* CGMCC 1.11003\textsuperscript{T} (87.36 %). Phylogenetic trees obtained using the neighbour-joining (Fig. 1) method revealed a clear affiliation of the novel isolate to a separate branch within the family *Rhodothermaceae*. This topology was also supported by the minimum-evolution and maximum-likelihood algorithms (data not shown). The clade accommodating strain SYD6\textsuperscript{T} was also shown to be a lineage that is well separated from other recognized genera within the family *Rhodothermaceae*. These phylogenetic data, in conjunction with other phenotypic and chemotaxonomic characteristics, strongly suggest that isolate SYD6\textsuperscript{T} represents a distinct species of a novel genus in the family *Rhodothermaceae*, for which we propose the name *Longimonas halophila* gen. nov., sp. nov.

### Table 1. Differential characteristics of strain SYD6\textsuperscript{T} and representatives of closely related genera in the family *Rhodothermaceae*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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</thead>
<tbody>
<tr>
<td>Cell length (μm)</td>
<td>5–9</td>
<td>15–30</td>
<td>0.3–0.5</td>
<td>2–2.5</td>
<td>2–6</td>
<td>0.7–5.0</td>
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<tr>
<td>Temperature for growth (°C)</td>
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<tr>
<td>NaCl for growth (% w/v)</td>
<td>4–25</td>
<td>5–20</td>
<td>1–5</td>
<td>2–6</td>
<td>15–30</td>
<td>1–5</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>ND</td>
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<tr>
<td>Oxidase reaction</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Catalase reaction</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Sensitivity to:</td>
<td></td>
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<tr>
<td>Penicillin G</td>
<td>–</td>
<td>–</td>
<td>ND</td>
<td>+</td>
<td>+</td>
<td>ND</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>+</td>
<td>–</td>
<td>ND</td>
<td>–</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>Polar lipids*</td>
<td>PE, PC, DPG, L</td>
<td>DPG, PE, PG, 2L</td>
<td>DPG, PE</td>
<td>DPG, PC 3GL, L</td>
<td>DPG, PE, PG, PL, 2L</td>
<td></td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>61.5</td>
<td>62.9</td>
<td>68.9</td>
<td>64.4</td>
<td>66.5</td>
<td>64.8–65.8</td>
</tr>
</tbody>
</table>

*DPG, diphostatidglycerol; GL, unknown glycolipid; L, unknown lipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol.

†Data from Vaisman & Oren (2009).
Longimonas halophila gen. nov., sp. nov.

Longimonas (Lon.gi.mo’nas. L. adj. longus long; Gr. fem. n. monas a unit, monad; N.L. fem. n. Longimonas, a long monad).

Cells are Gram-stain-negative, rod-shaped, facultatively anaerobic, heterotrophic and without flagella. Catalase-positive and oxidase-negative. Halophilic; both NaCl and anaerobic, heterotrophic and without flagella. Catalase-

Description of Longimonas gen. nov.

Longimonas halophila (ha.lo’phi.la. Gr. n. hals, halos salt; Gr. adj. philos loving; N.L. fem. adj. halophilia salt-loving, referring to the ability of the type strain to grow at high NaCl concentrations).

Displays the following properties in addition to those given in the genus description. Cells are red, approximately 5.0–9.0 μm × 0.4–0.6 μm and mesophilic. Nitrate is not reduced. Glucose is slightly fermented under anaerobic conditions and no gas is produced. Tween 80 and starch are hydrolysed, but CM-cellulose, sodium alginate and agar are not. Grows in glucose, sucrose, maltose, fructose and ribose, but not in glycerol, xylose or mannitol. API 20E tests are positive for gelatinase. Acid is produced from glycerol, D-arabinose, L-arabinose, D-ribose, L-xylene, D-adonitol, D-glucose, D-fructose, D-mannose, L-sorbitose, aesculine, maltose, sucrose, inulin, raffinose, starch, glycojen, turanose, D-tagatose, potassium gluconate, potassium 2-ketogluconate and potassium 5-ketogluconate (API 50CHB strips). In API ZYM tests, alkaline phosphatase, leucine arylamidase, valine arylamidase, trypsin, chymotrypsin and acid phosphatase activities are positive; esterase (C4), cystine arylamidase are weakly active. Resistant to tobramycin, trimethoprim, kanamycin, penicillin G and cefotaxime, but sensitive to tetracycline, nalidixic acid, polymyxin B, vancomycin, cefamandole and streptomycin. The sole methyl naphthoquinone is MK-7 and the predominant fatty acids are iso-C15 : 0 2-OH/C16 : 1ω7c, iso-C16 : 0 2-OH/C18 : 1ω7c, iso-C18 : 1ω6c and C18 : 1ω7c. The major polar lipids are PE, PC, DPG and an unknown lipid.

The type strain, SYD6T (=CICC 10838T=KCTC 42399T), 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 61.5 mol% (HPLC).

Acknowledgements

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References


