Saccharicrinis marinus sp. nov., isolated from marine sediment

Qian-Qian Liu,1 Juan Li,2 Di Xiao,2 Jin-Xing Lu,2 Guan-Jun Chen1 and Zong-Jun Du1

1College of Marine Science, Shandong University at Weihai, Weihai 264209, PR China
2State Key Laboratory for Infectious Disease Prevention and Control, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Disease, National Institute for Communicable Disease Control and Prevention, China CDC, PO Box 5, Changping, Beijing 102206, PR China

A novel bacterial strain, designated Y11T, was isolated from marine sediment at Weihai in China. Comparative analysis of 16S rRNA gene sequences demonstrated that the novel isolate showed highest similarity to Saccharicrinis fermentans DSM 9555T (94.0 %) and Saccharicrinis carchari SS12T (92.7 %). Strain Y11T was a Gram-stain-negative, rod-shaped, non-endospore-forming, yellow-pigmented bacterium and was able to hydrolyse agar weakly. It was catalase-negative, oxidase-positive, facultatively anaerobic and motile by gliding. Optimal growth occurred at 28–30 °C, at pH 7.0–7.5 and in the presence of 2–3 % (w/v) NaCl. The DNA G+C content was 34.4 mol%. The strain contained MK-7 as the prevalent menaquinone. The major cellular fatty acids were iso-C15 : 0, anteiso-C15 : 0 and C15 : 1 ω6c. The predominant polar lipids were phosphatidylethanolamine and two unknown lipids. Data from the present polyphasic taxonomic study clearly place the strain as representing a novel species within the genus Saccharicrinis, for which the name Saccharicrinis marinus sp. nov. is proposed. The type strain is Y11T (=CICC10837T=KCTC42400T).

The family Marinilabiliaceae initially included three genera, namely Marinilabilia, Alkaliflexus and Anaerophaga, when proposed by Ludwig et al. (2011), and was composed of Gram-stain-negative, slender, flexible rods. Members of the family are saccharolytic, motile by gliding and require NaCl for growth. Subsequently, a further seven genera, Geofilum, Mangroviflexus, Natronoflexus, Alkalitalea, Thermophilus, Carboxylicivirga and Saccharicrinis, have been classified as members of the family Marinilabiliaceae. These recognized members have been isolated from different habitats such as marine environments (Veldkamp, 1961; Miyazaki et al., 2012; Zhao et al., 2012; Shalley et al., 2013; Liu et al., 2014; Yang et al., 2014), while some were obtained from soda lakes (Zhilina et al., 2004; Sorokin et al., 2011; Zhao & Chen, 2012), oily sludge (Denger et al., 2002) and hot spring sediment (Gao et al., 2013).

During an investigation focused on marine microbial diversity, strain Y11T was isolated from a marine sediment sample taken at Weihai, China. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain Y11T was affiliated with the family Marinilabiliaceae and appeared to represent a novel species within the genus Saccharicrinis.

The sediment sample was collected from a coastal area at Weihai, China (122° 03′ 44.01″ E 37° 32′ 01.93″ N) and treated with an enrichment culture technique as described by Du et al. (2014), except that the enrichment incubation was performed for 5 days. A bright yellow-pigmented colony that was able to hydrolyse agar weakly, designated strain Y11T, was obtained and purified on fresh marine agar 2216 (MA; Difco) plates at 28 °C and stored at −80 °C in 20 % (v/v) glycerol or maintained as lyophilized pellets in vacuum-sealed glass vials stored at 4 °C.

The taxonomic status of the novel strain was determined using a polyphasic approach. Unless otherwise stated, the morphological, physiological and biochemical properties were investigated with cells cultivated on MA at 28 °C and experiments were conducted according to the methods described by Liu et al. (2014).

Morphologically, cells of strain Y11T were slender, straight or slightly curved rods, 0.3–0.5 μm in width and 2–17 μm in length when observed using light microscopy (E600; Nikon). Cells were Gram-stain-negative and lacked spores under all cultivation conditions and growth phases. Gliding motility was observed by using oil-immersion phase-contrast...
microscopy (AX70; Olympus). The novel strain was negative for catalase activity and positive for oxidase activity. Strain Y11T showed no growth under anaerobic conditions after being incubated in an anaerobic chamber on MA with or without 0.25 % (w/v) NaNO₃ for 7 days at 28 °C. Nitrate reduction was positive when tested using the procedure described by Liu et al. (2014), with the medium amended using filter-sterilized seawater. The oxidation-fermentation test was positive and Tween 80 was not hydrolysed when tested according to Dong & Cai (2001). The biochemical and physiological characterization of strain Y11T was performed with API 20E, API ZYM and API 50 CHB fermentation kits (bioMérieux) and Biolog Gen III microplates as recommended by the manufacturers, except that the suspension was prepared in 3 % (w/v) sterile sea-salt solution (Sigma-Aldrich) as poor growth was observed with NaCl only. All of the above tests, including the determination of temperature, pH and salinity growth ranges and susceptibility to antimicrobial agents, were run in triplicate. The results are presented in the species description.

For the determination of G+C content and for phylogenetic analyses, DNA was extracted and purified using a genomic DNA extraction kit (Tiangen) following the manufacturer’s protocol. The DNA G+C content was determined directly by HPLC according to Mesbah et al. (1989) and was 34.4 mol%. The almost-complete 16S rRNA gene sequence of strain Y11T (1448 nt) was determined as described previously (Liu et al., 2014). Comparison of this sequence with the 16S rRNA gene sequences of established species, using version 2.1 of the EzTaxon server (Kim et al., 2012), indicated that the novel organism’s closest relatives were members of the family Marinilabilaceae in the phylum Bacteroidetes. In pairwise comparisons, strain Y11T was related most closely to the genus Saccharicrinis (approximately 92.7–94.0 % gene sequence similarity). More distantly related organisms included species of the genera Carboxylicivirga (91.2 %) and Marinifilum (89.4–89.7 %) and the type strains of other established species in the family Marinilabilaceae (<90.0 %). The 16S rRNA gene sequences of the novel strain and related strains were aligned using CLUSTAL X (Thompson et al., 1997) and alignment gaps and ambiguous bases were manually omitted. A neighbour-joining tree and a maximum-likelihood tree were reconstructed with bootstrapping of 1000 replications based on Kimura’s two-parameter model (Kimura, 1980) using MEGA version 6 (Tamura et al., 2013). A tree was also reconstructed with the maximum-parsimony algorithm available in PAUP (Swofford, 2002). As high congruence was noted between the tree-making methods, so only the neighbour-joining tree is shown. In this tree, the novel strain shared an affinity with members of the genus Saccharicrinis, and clustered in a separate clade within the genus with a high bootstrap value of 99 % (Fig. 1). The unique phylogenetic position of the novel isolate and low level of sequence similarity with respect to other recognized bacterial species studied indicated that strain Y11T could represent a novel species in the genus Saccharicrinis.

In addition to its unique 16S rRNA gene sequence, several features could be used to clearly distinguish the novel organism from its phylogenetically related neighbours, as shown in Table 1. Strain Y11T exhibited gliding motility, which is as expected for members of the genus Saccharicrinis. However, the fact that strain Y11T was positive for oxidase, negative for catalase and unable to grow at 40 °C notably distinguishes it from the other members of genus Saccharicrinis, and also distinguishes it from members of the type genus Marinilabila. The gliding motility of the novel strain contrasted with data for members of the genera Carboxylicivirga and Marinifilum. Unlike strain Y11T, members of the genera Alkaliflexus, Alkalitalea and Natronoflexus are slightly alkaliphilic and mainly grow under anaerobic conditions. The complete morphological, physiological and biochemical characteristics of strain Y11T are given in the species description.

For the analysis of cellular fatty acid methyl esters, cells of strain Y11T were harvested from a culture grown at 28 °C for 72 h in marine broth 2216 (MB; Difco), in the early stationary phase of growth. The analyses, performed according to the methods of Sasser (1990), revealed that strain Y11T was characterized by the fatty acids iso-C₁₅:₀ (25.4 %), anteiso-C₁₅:₀ (20.1 %) and C₁₅:₀(10.3 %) as the major components (>10 % of the total fatty acids). The fatty acid composition is similar to those found in phylogenetically closely related members of the genus Saccharicrinis under the same conditions and methodology. Additional differences in fatty acid patterns between strain Y11T and related members of the genus Saccharicrinis are detailed in Table S1 (available in the online Supplementary Material). Menaquinones were isolated by using the methods of Minnikin et al. (1984) and then separated by HPLC (Kroppenstedt, 1982). The predominant respiratory quinone detected in strain Y11T was menaquinone 7 (MK-7), in line with related members of the genus Saccharicrinis. Polar lipid analysis was carried out by the Identification Service of the Leibniz-Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany. The polar lipid profile of strain Y11T was dominated by phosphatidylethanolamine (PE) and two unidentified lipids (L4, L6), with moderate to minor amounts of aminophospholipid (PN), two unknown phospholipids (PL1, PL2), three unknown aminolipids (AL1, AL2, AL3) and another five unknown lipids (L1, L2, L3, L5, L7) also detected (Fig. S1). Although the novel bacterium shared similar main polar lipids with its closest relatives, the proportions and kinds of less abundant polar lipids were quite different. For instance, the novel isolate differed from other members of the genus Saccharicrinis in the presence of the aminophospholipid and the unknown aminolipids, and the absence of glycolipid.
In addition, analysis by matrix-assisted laser desorption ionization-time of flight MS was performed with a Microflex LT mass spectrometer (Bruker Daltonics) using the software package FlexControl (version 3.0; Bruker Daltonics) as previously reported (Xiao et al., 2012). The intact-cell profile (Fig. S2) clearly distinguished strain Y11T from other members of the genus *Saccharicrinis* when the mass data were compared.

Results of these phenotypic, biochemical and physiological analyses, together with the phylogenetic and chemotaxonomic differences, clearly indicate that strain Y11T can be assigned to the genus *Saccharicrinis* of the family *Marinilabiliaceae* as representing a novel species, for which the name *Saccharicrinis marinus* sp. nov. is proposed.

**Description of *Saccharicrinis marinus* sp. nov.**

*Saccharicrinis marinus* (ma.ri’nus. L. masc. adj. marinus of the sea).

Cells are slender, straight or slightly curved rods, approximately 0.3–0.5 μm wide and 2–17 μm long. Gram-stain-negative, non-spore-forming and motile by gliding. Colonies on MA are yellow-pigmented, circular, convex, entire and about 1–2 mm in diameter after 3 days of growth at 28 °C. Facultatively anaerobic and chemo-heterotrophic. Catalase-negative and oxidase-positive. Able to grow with 1–5 % (w/v) NaCl, with optimal growth with 2–3 % (w/v) NaCl. No growth occurs in the absence of NaCl. Able to grow at 4–33 °C, but not at 40 °C; the optimal temperature for growth is 28–30 °C. The pH range is 6.5–8.0; optimal pH is between 7.0 and 7.5. Hydrolyses agar and aesculin, but not urea, gelatin or Tween 80. Nitrate is reduced and oxidation-fermentation test is positive. In API 20E tests, positive results are obtained for production of ONPG and indole, and utilization of Simmons’s citrate, but negative results for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophane deaminase, H₂S production and

---

**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain Y11T. Bootstrap values (>50 %) based on 1000 resamplings are shown at branch nodes. GenBank accession numbers of 16S rRNA gene sequences are given in parentheses. *Streptomyces albus* DSM 40313T was used as an outgroup. Bar, 0.05 substitutions per nucleotide position.
Table 1. Comparison of the major features of strain Y11<sup>T</sup> with its phylogenetically related neighbours

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Colony colour</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Bright yellow</td>
<td>Yellow</td>
<td>Yellow</td>
<td>Ivory/brownish</td>
<td>ivory</td>
</tr>
<tr>
<td>Catalase</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Oxidase</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gliding motility</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>O₂ metabolism</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Optimal pH</td>
<td>7.0–7.5</td>
<td>7.0–7.5</td>
<td>8.0</td>
<td>7.0</td>
<td>7.5</td>
<td>7.0</td>
<td>ND</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agar</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Gelatin</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>34.4</td>
<td>40</td>
<td>37.56</td>
<td>44.2</td>
<td>44.1</td>
<td>35.8</td>
<td>35.7</td>
</tr>
</tbody>
</table>
Voges–Proskauer reaction. Glucuronamide, mucic acid, Tween 40 and acetoacetic acid are oxidized in Biolog Gen III microplates. Acid is produced from D-galactose, D-mannose, amygdalin, ascinulin, cellobiose, maltose, D-lactose, amidon, glycogen, gentiobiose and potassium 5-ketogluconate, but not from glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, L-xyllose, D-adonitol, methyl β-D-xylopyranoside, D-fructose, L-sorbose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-glucopyranoside, N-acetylgalactosamine, arbutin, salicin, melibiose, sucrose, trehalose, inulin, melezitose, raffinose, xylitol, turanose, D-lyxose, D-fucose, L-fucose, D-arabinitol or potassium gluconate. Acid is produced weakly from D-xyllose, D-glucose, L-rhamnose, D-tagatose and potassium 2-ketogluconate in API 50CH strips. API ZYM tests show positive results for the production of alkaline phosphatase, esterase (C4), acid phosphatase and naphthol-AS-Bl-phosphohydrolase, but negative results for esterase lipase (C8), trypsin, β-galactosidase, β-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, β-fucosidase, lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, chymotrypsin, Z-galactosidase, β-glucuronidase and β-mannosidase. Resistant to the following antimicrobial agents: tobramycin, norfloxacin, streptomycin, kanamycin, gentamycin and neomycin, but sensitive to penicillin G, clindamycin, rifampicin, cefotaxime sodium, lincomycin, ceftriaxone sodium, ampicillin and acetylsalicylamycin. The main respiratory quinine is MK-7. The predominant cellular fatty acids are iso-C_{15:0} anteiso-C_{15:0} and C_{15:1}ω6c. The major polar lipids are phosphatidylethanolamine and two unidentified lipids; in addition, aminophospholipid, two unknown phospholipids, three unknown aminolipids and five other unknown lipids are present in moderate to minor amounts in the polar lipid profile.

The type strain, Y11^T (＝CICC 10837^T＝KCTC 42400^T), was isolated from marine sediment at Weihai in China. The DNA G+C content of the type strain is 34.4 mol%.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (31370057, 31290231), National Science and Technology Major Project of China (2013ZX10004217), and the China Ocean Mineral Resources R & D Association (COMRA) Special Foundation (DY125-15-T-05).

References


