Lablibacter aurantiacus gen. nov., sp. nov., isolated from sea squirt (Styela clava) and reclassification of Saccharicrinis marinus as Lablibacter marinus comb. nov.

De-Chen Lu,1 Jin-Xin Zhao,1 Feng-Qing Wang,1 Zhi-Hong Xie2,* and Zong-Jun Du1,*

Abstract

A Gram-stain-negative, facultatively anaerobic, orange-pigmented bacterium, designated HQYD1T, was isolated from a sea squirt (Styela clava) and characterized using a polyphasic approach. Morphologically, strain HQYD1T exhibited rods with gliding motility. This novel isolate grew optimally at 28°C in the presence of 2–3% (w/v) NaCl. The 16S rRNA gene sequence was most similar to [Saccharicrinis] marinus Y11T (96.3%), followed by Saccharicinis fermentans DSM 9555T (93.8%). The dominant fatty acids of strain HQYD1T were identified as C16:0, C18:0 and iso-C15:0. Major polar lipids included an unidentified lipid and a phospholipid. The major respiratory quinone was found to be MK-7, and the genomic DNA G+C content was determined to be 35.1 mol%. Based on evidence from this taxonomic study, a novel genus, Lablibacter gen. nov., is proposed in the family Marinilabiliaceae with type species Lablibacter aurantiacus sp. nov. The type strain of the type species is HQYD1T (=MCCC 1K02304=KCTC 42583T). As [Saccharicrinis] marinus Y11T clustered phylogenetically with strain HQYD1T, we also propose [Saccharicrinis] marinus Y11T to be reclassified as Lablibacter marinus comb. nov. (type strain Y11T=CICC 10837T=KCTC 42400T).

The family Marinilabiliaceae suggested by Ludwig et al. [1] comprises 10 genera at the time of writing. Species in the genera Alkaliflexus, Alkalitalea, Anaerophaga, Mangroviflexus, Natronoflexus and Thermophagus are anaerobic, while species in the genera Geofilum, Marinilabilia, Carboxylicivirga and Saccharicrinis are capable of fermentative metabolism. Members of the family Marinilabiliaceae are Gram-stain-negative rods and contain MK-7 as the respiratory quinone [2–12].

In this study, an orange-pigmented bacterium, HQYD1T, was isolated from a sea squirt collected from the coast of Weihai, China. Phenotypic and genotypic characteristics, fatty acid and menaquinone compositions and phylogenetic findings support the establishment of a novel genus in the family Marinilabiliaceae. Based on our analyses, we also propose the reclassification of (Saccharicrinis) marinus Y11T to this novel genus.

Strain HQYD1T was isolated from a sea squirt (Styela clava) collected from the coastal area of Weihai, China (37°31′33″N 122°0′37″E). For isolation, sea squirt tissue was homogenized and then serially diluted in sterile water. Samples were then taken from each serial dilution, plated on marine agar 2216 (MA; Difco) and incubated at 28°C in aerobiosis for 7 days. Orange-pigmented colonies, designated HQYD1T, were picked and streaked several times on the same medium. Once isolated, strain HQYD1T was routinely grown on MA or MB-agarose (marine broth 2216 (MB; Difco) supplemented with 0.05% agarose) at 28°C. For long-term preservation, cells were stored at −80°C in sterile 1% (w/v) saline medium containing 15% (v/v) glycerol. [Saccharicinis] marinus Y11T (from our laboratory), Saccharicinis carcharii SS12T (from our lab) and Saccharicinis fermentans DSM 9555T [Leibniz-Institut Deutsches Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany] were used as reference strains. Unless stated otherwise, all organisms were grown on MA or MB-agarose under identical conditions for comparative purposes.

Cell size, morphology and the presence of flagella were investigated by electron microscopy (Cl-I; Nikon) and transmission electron microscopy (Jem-1200; Jeol), using cells grown on MA at 28°C for 48, 72, 120 and 168h. Gliding motility was examined according to the method described by Bowman [13]. Gram-reaction was performed as described by Snibert and Krieg [14]. Growth ranges and

**Keywords:** Lablibacter aurantiacus; polyphasic taxonomic; sea squirt; bacterium.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain HQYD1T is JF721990.

Two supplementary figures and one supplementary table are available with the online Supplementary Material.
optima of temperature were indicated by visible colonies on MA and in MB-agarose via turbidity change at 4, 8, 10, 12, 15, 20, 25, 28, 30, 33, 35, 37, 40, 42 and 45 °C. The tolerance range for NaCl was tested in MB-agarose prepared with artificial seawater (per litre: 3.2 g MgSO$_4$, 2.2 g MgCl$_2$, 1.2 g CaCl$_2$, 0.7 g KCl, 0.2 g NaHCO$_3$) containing NaCl at concentrations from 0 to 10 % (w/v, in 1% intervals). The effect of pH on growth was investigated in MB-agarose. The pH was adjusted by addition of 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). Bacterial growth was monitored by using a spectrophotometer at 660 nm. Oxidase activity was tested using the oxidase reagent kit (BioMérieux) according to manufacturer’s instructions (after incubation for 48 h). Catalase activity in bacterial colonies was detected by exposure to a 3% H$_2$O$_2$ solution. Anaerobic growth was tested for 7 days at 28 °C on MA with or without 0.1% (w/v) NaN$_3$ in an anaerobic jar (Whitley Jar Gassing System; Don Whitely Scientific). Degradation of agar, alginate and starch, susceptibility to antibiotics and hydrolysis of Tween 80 were tested as described previously [15]. Nitrate reduction was tested as described by Dong and Cai [16]. Oxidation of carbohydrates, alcohols, organic acids, amino acids and nucleosides as sole carbon sources was evaluated in Biolog GEN III MicroPlates. Other biochemical and physiological characterizations of strain HQYD$^T$ were performed using API 20E, API ZYM and API 50 CHB fermentation kits (bioMérieux) according to the manufacturer’s instructions, except that the suspension was prepared in 3% (w/v) sterile sea-salt solution (Sigma).

Cellular menaquinones and polar lipids were identified from a freeze-dried sample (200 mg) of cells grown under optimal culture conditions on MB-agarose to late-exponential growth phase. Menaquinones were analysed as described by Minnikin et al. [17] using reversed-phase HPLC. For fatty acids analysis, the cell mass of strain HQYD$^T$ was harvested from MA plates after cultivation for 72 h at 28 °C. Fatty acids were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System [18]. Polar lipid analysis was performed by the Identification Service of the DSMZ. Polar lipids were extracted from 100 mg freeze-dried cell material using a chloroform/methanol/0.3% (w/v) aqueous NaCl mixture (1 : 2 : 0.8, by vol.), modified from the Bligh and Dyer protocol [19]. Lipids were recovered in the chloroform phase by adjusting the chloroform/methanol/0.3% (w/v) aqueous NaCl mixture to a ratio of 1 : 1 : 0.9 (by vol.), and were separated by two-dimensional silica gel thin-layer chromatography (Macherey-Nagel Art. No. 818 135) [20].

Colonies of strain HQYD$^T$ were orange-pigmented, transparent, circular and approximately 0.5 mm in diameter on MA after incubation for 72 h at 28 °C under aerobic

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**Table 1. Differential characteristics of strain HQYD$^T$ and with its phylogenetically related neighbours**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1 (this study)</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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</thead>
<tbody>
<tr>
<td>Colony colour</td>
<td>Orange</td>
<td>Yellow</td>
<td>Bright yellow</td>
<td>Yellow</td>
<td>Yellow</td>
</tr>
<tr>
<td>Cell size (µm)</td>
<td>0.3–0.5×1.5–20.0</td>
<td>0.3–0.5×2–17</td>
<td>0.3–0.7×8–50</td>
<td>0.5–0.7×7–14</td>
<td>0.4–0.8×8.2–11.8</td>
</tr>
<tr>
<td>Temperature range (optimum) for growth (°C)</td>
<td>4–37 (28)</td>
<td>4–33 (28–30)</td>
<td>18.5–37.5 (30)</td>
<td>10–40 (28–30)</td>
<td>18.2–38.1 (30.3)</td>
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<tr>
<td>Gliding motility</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
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<tr>
<td>Catalase</td>
<td>w</td>
<td>–</td>
<td>+</td>
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<td>+</td>
<td>–</td>
<td>+</td>
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<td>Hydrolysis of:</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
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<td>Ag</td>
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<td>+</td>
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<tr>
<td>Urea</td>
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<td>–</td>
<td>+</td>
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<td>Enzyme activities</td>
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<tr>
<td>Esterase (C4)</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>w</td>
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<td>Esterase lipase (C8)</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Valine arylamidase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Major fatty acids</td>
<td>C$<em>{16:0}$, C$</em>{18:0}$, isoC$<em>{15:0}$, C$</em>{16:1}$, anteiso-C$<em>{15:0}$, iso-C$</em>{15:1}$, anteiso-C$<em>{15:0}$, iso-C$</em>{15:0}$, anteiso-C$_{15:0}$</td>
<td>35.1</td>
<td>36.1</td>
<td>37.6</td>
<td>40.0</td>
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<tr>
<td>DNA G+C content (mol%)</td>
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*a Data from: a, Yang et al. [11]; b, Liu et al. [12].
conditions, Cells were Gram-negative rods (0.3–0.5 μm × 1.5–20.0 μm) with gliding motility and had a single polar flagellum (Fig. S1, available in the online Supplementary Material). Strain HQYD1T and [Saccharicrinis] marinus Y11T were readily distinguished from S. fermentans DSM 9555T and S. carhari SS12T by physiological features, such as growth at 4° C and the presence of esterase, β-galactosidase and naphthol-AS-BI-phosphohydrolase. Detailed comparison of major features of strain HQYD1T with its phylogenetically related neighbours are shown in Table 1.

Genomic DNA of the isolate was extracted and purified using a genomic DNA extraction kit (Takara) and the DNA G+C content was determined by HPLC [21]. A restriction cut lambda standard was used as molecular ladder. The 16S rRNA gene was amplified by PCR using two universal primers as described by Liu et al. [12]. Sequencing of the 16S rRNA gene and phylogenetic analysis were performed as described by Wang et al. [15]. Comparison of this sequence with the 16S rRNA gene sequences of established species was performed using the EzTaxon server version 2.1 [22]. A phylogenetic tree was reconstructed with the neighbour-joining algorithm implemented in the software package MEGA (version 6.0) [23]. Phylogenetic trees were also generated with the maximum-likelihood [24] and maximum-parsimony algorithms [23] and showed the same phylogenetic trends as the neighbour-joining tree.

The 16S rRNA gene sequence of strain HQYD1T was composed of 1442 bp. Comparison of this sequence with the 16S rRNA gene sequences of established species indicated that the closest relatives of the novel organism were members of the family Marinilabiliaceae, order Bacteroidales, class Bacteroidia in the phylum Bacteroidetes. The sequence was 96.3 % identical to S. marinus Y11T, 93.8 % identical to S. fermentans DSM9555T, and 92.2 % identical to S. carhari SS12T. The sequence of [Saccharicrinis] marinus Y11T was 93.0 % identical to S. marinus Y11T and 93.4 % identical to S. fermentans DSM9555T. Strain HQYD1T formed a phylogenetic cluster with strain [Saccharicrinis] marinus Y11T at a bootstrap confidence level of 100 % and was distinctly separated from members of genus Saccharicrinis at a bootstrap confidence level of 98 % (Fig. 1). This can be estimated and verified by the maximum-likelihood tree and maximum-parsimony tree. Comparative 16S rRNA gene sequence analysis suggests that strain HQYD1T should be classified as a new genus and species in the family Marinilabiliaceae (Fig. 1).

In addition to its unique 16S rRNA gene sequence, there are a number of other features that may be used to clearly distinguish the proposed genus from other members of the Marinilabiliaceae. For example, members of the novel genus can grow under 10 °C, and cannot grow at 40 °C, unlike genera Saccharicrinis, Carboxylicivirga, Geofilum, Marinilabilia, Alkaliflexus, and Thermophagus. Additional differential characteristics are shown in Table 2. The DNA G+C content of strain HQYD1T is 35.1 mol%, which is closer to [Saccharicrinis] marinus Y11T (36.1 mol%). Similar to other species in the family Marinilabiliaceae, the predominant respiratory quinone present in strain HQYD1T was identified as MK-7. Strain HQYD1T and [Saccharicrinis] marinus Y11T have rich linear-saturated fatty acids which were quite different from those of S. fermentans DSM 9555T and S. carhari SS12T (Table S1). Furthermore, S. fermentans DSM 9555T and S. carhari SS12T have rich branched fatty acids (>50 %), which were quite different from those of strain HQYD1T and [Saccharicrinis] marinus Y11T (Table S1). These findings suggest that [Saccharicrinis] marinus Y11T is most closely related to strain HQYD1T, rather than S. fermentans DSM 9555T or S. carhari SS12T. The major polar lipids of strain HQYD1T included an unidentified lipid and phospholipid. Aminophospholipid was also detected (Fig. S2), which was similar to [Saccharicrinis] marinus Y11T [25] but unlike S. fermentans DSM 9555T and S. carhari SS12T [11, 12]. More differential characteristics are shown in Table 2.

Based on the results of this taxonomic study using a polyphasic approach, strain HQYD1T is considered to represent a novel species of a new genus of the family Marinilabiliaceae, for which the name Labilibacter aurantiacus gen. nov., sp. nov. is proposed. In addition, we propose that [Saccharicrinis] marinus Y11T be reclassified as Labilibacter marinus comb. nov.

**DESCRIPTION OF LABILIBACTER GEN. NOV.**

Labilibacter (Labi.li.bac’ter. L. adj. labilis, gliding; N.L. masc. n. bactér, rod; N.L. masc. n. Labilibacter, gliding rod).

Cells are Gram-negative, facultatively anaerobic rods with gliding motility. No growth is observed without NaCl. The main respiratory quinone is MK-7. The predominant cellular fatty acids are C_{16:0} C_{18:0} and iso-C_{15:0}. The range of DNA G+C contents is 35–37 mol%. Phylogenetically, the genus is a member of the family Marinilabiliaceae, order Marinilabiales, class Bacteroidia in the phylum Bacteroidetes.

The type species is Labilibacter aurantiacus.

**DESCRIPTION OF LABILIBACTER AURANTIACUS SP. NOV.**

Labilibacter aurantiacus (au.ran.ti’a.cus. N.L. masc. adj. aurantiacus orange-coloured).

In addition to the description of the genus, the following properties are exhibited. Cells are approximately 0.3–0.5 μm wide and 1.5–20.0 μm long. Colonies on MA are orange-pigmented, circular and about 0.5 mm in diameter after 72 h of growth at 28 °C. Growth was observed at 4–37 °C, pH 6.0–8.5 and in the presence of 1–5 % (w/v) NaCl [with optimum growth at 28 °C, pH 7.0–7.5 and with 2–3 % (w/v) NaCl]. Can hydrolyse agar gelatin and alginate, but not starch, urea or Tween 80. Cells are weakly positive for catalase and negative for oxidase activity and indole and H_{2}S production. Nitrate is not reduced to nitrite. When assayed
with the API ZYM system, alkaline phosphatase, esterase (C4), valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase are present, but esterase lipase (C8), lipase (C14), leucine arylamidase, cysteine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-

Table 2. Differential characteristics of the proposed novel genus Labilibacter sp. nov. and closely related genera in the family Marinilabiliaceae

<table>
<thead>
<tr>
<th>Taxa: 1, Labilibacter sp. nov. (data from this study); 2, Saccharicrinis (this study, [11, 12]); 3, Carboxylicivirga [11, 26]; 4, Geofilum [8]; 5, Marinilabilia [10]; 6, Mangrovilexus [4]; 7, Alkaliflexus [2]; 8, Alkalitalea [3]; 9, Anaerophaga [5]; 10, Natronoflexus [6]; 11, Thermophagus [7].</th>
<th>Positive</th>
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<tbody>
<tr>
<td>Colony colour</td>
<td>Orange</td>
<td>Yellow</td>
<td>Pink</td>
<td>White</td>
<td>Orange</td>
<td>Yellow</td>
<td>Pink</td>
<td>White</td>
<td>Orange</td>
<td>Yellow</td>
<td>Pink</td>
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<td>O2 metabolism</td>
<td>F</td>
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<td>Growth at 10°C</td>
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<td>+</td>
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<tr>
<td>Growth at 40°C</td>
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<td>–</td>
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</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>35–37</td>
<td>37.5–40</td>
<td>40–44.5</td>
<td>42.9</td>
<td>37</td>
<td>44.0</td>
<td>44.3</td>
<td>39.5±0.9</td>
<td>41.8</td>
<td>40.6</td>
<td>38.7</td>
</tr>
</tbody>
</table>

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of strain HQYD1T and some other phylogenetically related taxa. Bootstrap values >70% (based on 1000 replicates) from neighbour-joining, maximum-likelihood and maximum parsimony methods, respectively, are indicated at branch points; values <70% are indicated by a dash. GenBank accession numbers are shown in parentheses. Bar, 0.02 substitutions per nucleotide position.
glucosaminidase, α-mannosidase and β-fucosidase are absent. Acid is produced from D-ribose, D-xylene, D-galactose, D-glucose, aesculin, cellobiose, lactose, amygdalin, starch, glycerogen, D-gentiobiose and 5-keto-potassium gluco-
nate. The following substrates are oxidized: maltose, cellobi-
ose, gentiobiose, α-D-lactose, N-acetyl-β-D-mannosamine,
D-mannose, D-mannitol, myo-inositol, gelatin, L-histidine,
D-galacturonic acid, citric acid and α-ketoglutaric acid. The
type strain is susceptible to acetylsyrinamycin, erythromycin,
penicillin, cefotaxime, ceftriaxone, clindamycin, lincomycin,
chloramphenicol, sulfoxamethoxy hedgeazide and rifampicin.
The major polar lipids are an unidentified lipid and phos-
pholipid. In addition, phosphatidylethanolamine, an amino-
phospholipid and three unidentified lipids are present in
moderate to minor amounts in the polar lipid profile.

The type strain, HQYDI*T (=MCCC 1K02304T=KCTC 42583T), was isolated from a sea squirt (Styela clava) col-
lected from the coastal area of Weihai, China. The DNA
G+C content of the type strain is 35.1 mol%.

**DESCRIPTION OF LABILIBACTER MARINUS COMB. NOV.**

*Labililabacter marinus* (ma.r’i’nas. L. masc. adj. marinus from the sea).

Basonym: *Saccharicrinis marinus* Liu et al. 2015, 3429 [25].

The description remains as given by Liu et al. [25] with the 
following emendations. The major cellular fatty acids are iso-C15:0 anteiso-C15:0 and C16:0. The DNA G+C content
is 36.1 mol%.

The type strain, Y11*T (=CICC 10837T = KCTC 42400T), was isolated from marine sediment at Weihai in China.

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**Conflicts of interest**

The authors declare that they have no conflict of interest.

**Ethical statement**

This article does not contain any studies with animals performed by
any of the authors. Informed consent was obtained from all individual
participants included in the study.

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