Description of Tropicibacter oceani sp. nov, isolated from the intertidal zone sediment of Chinese Yellow Sea

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Abstract

In this study, we reported a Gram-stain-negative, rod-shaped, atrichous, and aerobic bacterial strain named YMD87\textsuperscript{T}, which was isolated from the intertidal zone sediment of Chinese Yellow Sea. Growth of strain YMD87\textsuperscript{T} occurred at 10.0–40.0°C (optimum, 25–30°C), pH 4.0–12.0 (optimum, 8.0) and with 0–6.0% (w/v) NaCl (optimum, 0.0–2.0%). Phylogenetic tree analysis based on 16S rRNA gene sequence indicated that strain YMD87\textsuperscript{T} belonged to the genus \textit{Tropicibacter} and was closely related to \textit{Tropicibacter alexandrii} LMIT003\textsuperscript{T} (97.2% sequence similarity). Genomic analysis indicated that strain YMD87\textsuperscript{T} contains a circular chromosome of 3,932,460 bp with G + C content of 63.8% and three circular plasmids of 116,492 bp, 49,209 bp and 49,673 bp, with G + C content of 64.3%. The predominant respiratory quinone of YMD87\textsuperscript{T} was ubiquinone-10 (Q-10). The major polar lipids of YMD87\textsuperscript{T} contained phosphatidylglycerol, phosphatidylethanolamine, five unidentified lipids, five unidentified phospholipids, phosphatidylcholine, unidentified glycolipid and five unidentified aminolipids. The major fatty acids of strain YMD87\textsuperscript{T} contained C\textsubscript{12:1}\textsubscript{3-OH}, C\textsubscript{16:0}, and summed feature 8 (C18:1 \textit{ω}7\textsubscript{c} or/and C18:1 \textit{ω}6\textsubscript{c}). Phylogenetic, physiological, biochemical and morphological analyses suggested that strain YMD87\textsuperscript{T} represents a novel species of the genus \textit{Tropicibacter}, and the name \textit{Tropicibacter oceani} sp. nov is proposed. The type strain is YMD87\textsuperscript{T} (= MCCC 1K08473\textsuperscript{T} = KCTC 92856\textsuperscript{T}).

Introduction

The genus \textit{Tropicibacter}, belonging to the family \textit{Rhodobacteraceae}, was first proposed by Harwati et al. in 2009 (Harwati et al, 2009). At the time of writing, there are only two species in this genus, \textit{Tropicibacter naphthalenivorans}, isolated from seawater obtained from Semarang Port in Indonesia (Harwati et al, 2009), and \textit{Tropicibacter alexandrii} LMIT003\textsuperscript{T}, isolated from a liquid culture of the dinoflagellate \textit{Alexandrium minutum} (Wang et al., 2020). Four species previously affiliated with this genus were reclassified into other genera based on genomic analyses. \textit{Tropicibacter multivorans} was reclassified in the genus \textit{Epibacterium}, while \textit{Tropicibacter mediterraneus}, and \textit{Tropicibacter litoreus} were reclassified in the genus \textit{Ruegeria} (Witth et al., 2018), and \textit{Tropicibacter phthalicus} was reclassified in the genus \textit{Pelagimonas} (Hordt et al., 2020). Strains of the genus \textit{Tropicibacter} are Gram-stain-negative, aerobic, oxidase-positive and rod-shaped. Chemotaxonomic characteristics include the predominant lipoquinone ubiquinone-10 (Q-10), and a G + C content of 61.9–64.6 mol% (Harwati et al, 2009, Wang et al., 2020). The common major fatty acids identified in this genus include C\textsubscript{16:0} and summed feature 8 (C\textsubscript{18:1}\textit{ω}7\textsubscript{c} or/and C\textsubscript{18:1}\textit{ω}6\textsubscript{c}) (Harwati et al, 2009, Wang et al., 2020). In this study, we proposed a novel species of the genus \textit{Tropicibacter}, strain YMD87\textsuperscript{T}, isolated from the intertidal sediment obtained from the intertidal zone sediment of Chinese Yellow Sea.

Materials and methods

Isolation and culture conditions
Strain YMD87\textsuperscript{T} was isolated from the sediment collected from the intertidal zone of Chinese Yellow Sea (37°23′0″N, 121°36′0″E). For bacterial isolation, the sediment sample was tenfold serially diluted with sterilized seawater, and spread onto the marine 2216EA (Haibo, Qingdao, China) plates. After incubation at 28 °C for 7 days, the pure culture of YMD87\textsuperscript{T} was obtained after three successive transfers to new 1116EA plates. The strain was stored at –80 °C in marine 2216EB (Haibo) medium supplemented with 20% (v/v) glycerol.

**16S rRNA phylogeny and genomic sequencing**

For 16S rRNA gene and genomic sequencing, genomic DNA of YMD87\textsuperscript{T} was extracted using a bacterial genomic DNA extraction kit (Tiangen, Beijing, China). The partial 16S rRNA gene sequence of YMD87\textsuperscript{T} was amplified by universal primers 27F (5'-AGAGTTTGATCCTGGCTCA-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') (Buchan et al., 2005). The PCR product was further cloned into the pEASY-T1 simple vector (TransGen, Beijing, China), and an almost complete 16S rRNA gene sequence of strain YMD87\textsuperscript{T} was obtained and submitted to GenBank (accession number OP942226). The 16S rRNA gene sequence was analyzed using EzTaxon-eserver (http://eztaxon-e.ezbiocloud.net/) (Kim et al., 2012). The phylogenetic tree was generated using MEGA 7.0 software by neighbour-joining (Saitou et al., 1987), minimum-evolution (Rzhetsky et al., 1992) and maximum-likelihood (Guindon et al., 2003) and algorithms with bootstrap values (1000 replications) (Kumar et al., 2016).

The genome of strain YMD87\textsuperscript{T} was sequenced in Novogene Bioinformatics Technology Co., Ltd. (Beijing, China) using PacBio Sequel and Illumina NovaSeq PE150 platforms. A total of 367,182 reads were obtained, which were further assembled, corrected and authenticity ensured as described previously (Xu et al., 2021). The genome component and gene function were further predicted by NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova et al., 2016). The secretory proteins were predicted with SignalP (Version 4.1) and TMHMM (Version 2.0c) (Petersen et al., 2011). The secondary metabolic gene clusters were analyzed with antiSMASH (version 2.0.2) (Medema et al., 2011). The genome sequence of *T. alexandrii* LMIT003\textsuperscript{T} (accession number GCA_003667315.2) was obtained from NCBI database, and the genome was used to analyze the genomic difference with strain YMD87\textsuperscript{T}. ChunLab’s online ANI calculator (www.ezbiocloud.net/tools/ani) (Lee et al., 2016) was used to determine the average nucleotide identity (ANI). DNA-DNA relatedness was estimated using the Genome-to-Genome Distance Calculator (GGDC) 2.1 provided by the DSMZ (http://ggdc.dsmz.de/distcalc2.php) (Meier-Kolthoff et al., 2013).

**Physiological and chemotaxonomic characterization**

The type strain *T. alexandrii* LMIT003\textsuperscript{T} (KCTC 62895\textsuperscript{T}) was obtained from Korean Collection for Type Cultures (KCTC, Korean), and used as a reference strain for biochemistry and fatty acid analysis. For cell morphology analysis, strain YMD87\textsuperscript{T} was grown on marine 2216EB for 24 h at 28°C, after harvesting and washing with distilled water, cells were negatively stained with 1% phosphotungstic acid and observed by transmission electron microscopy HT-7700 (Hitachi, Tokyo, Japan). Gram staining was performed using a
Gram-staining kit (Haibo). Growth temperature of 4, 10, 15, 20, 25, 30, 35, 37, 40 and 45°C were evaluated on 2216EB to measure the growth range of YMD87<sup>T</sup>. The NaCl range for growth was tested by using NaCl-free 2216EB with different NaCl concentrations (0–15.0%, at intervals of 1.0%) at 28°C. The optimal pH was determined by growing at pH 4.0–12.0 (at intervals of 1.0 pH unit) using the buffer system described by Xu et al. (Xu et al., 2005). In order to investigate anaerobic growth, strain YMD87<sup>T</sup> was cultured at 28°C on 2216EB and supplemented with resazurin (0.02%, w/v) as an indicator of anaerobic condition. The plates were incubated in an anaerobic incubator YQX (Yuejin, Shanghai, China) filled with nitrogen. Oxidase activity was evaluated with the oxidase reagent (Haibo), and catalase activity was tested by the production of oxygen bubbles in 3% (v/v) H<sub>2</sub>O<sub>2</sub> solution. Hydrolysis of starch, casein, tween 20, and 80 was examined on 2216EA plates with the corresponding substrate. Nitrate reductase, gelatinase, urease activities and other enzyme productions were examined using the API 20NE and API ZYM system (bioMérieux, Marcy-l'Étoile, France) in 28°C, according to the instructions. Acid production from different carbohydrates was performed using the API 50CH system (bioMérieux). For fatty acid methyl esters (FAME) analysis, bacteria were grown on 2216EA until the late of the exponential growth phase (2 days) at 28°C, and the cells were harvested and analyzed as reported by Sasser (Sasser et al., 1990). Polar lipids were extracted according to the protocol of Minnikin et al. and examined using two-dimensional TLC (Minnikin et al., 1984). Respiratory quinones of strain YMD87<sup>T</sup> was analyzed using freeze-dried cells as reported previously (Sasser et al., 1990). Antibiotic susceptibility was determined on 2216 EA plates using antibiotic discs containing the following (μg per disc unless otherwise stated): polymyxin B (300 IU), furazolidone (300), piperacillin (100), cefoperazone (75), midecamycin (30), minocycline (30), doxycycline (30), tetracycline (30), neomycin (30), amikacin (30), ceftriaxone (30), ceftazidime (30), cefuroxime (30), cefradine (30), cefamezin (30), cephalaxin (30), penicillin (10 U), gentamicin (10), norfloxacin (10), ciprofloxacin (5), ofloxacin (5), clindamycin (2), sulfamethoxazole (1.25).

Results and discussion

Phylogeny analysis

The 16S rRNA gene sequence analysis showed that strain YMD87<sup>T</sup> belonged to the genus *Tropicibacter* and was most closely related to *T. alexandrii* LMIT003<sup>T</sup> with 97.18% sequence similarities, and the identities between strain YMD87<sup>T</sup> and other close members were all below 97.0%. The NJ phylogenetic tree showed that strain YMD87<sup>T</sup> formed a group with *T. alexandrii* LMIT003<sup>T</sup> (Fig. 1). The corresponding ML and ME trees showed similar topologies (Fig. 1), which supported the proposal that strain YMD87<sup>T</sup> belonged to the genus *Tropicibacter*. The G + C content of YMD87<sup>T</sup> is 63.8%, which is higher than the related reference strain *T. alexandrii* LMIT003<sup>T</sup> (Table 1). The ANI value and the digital DDH value between strain YMD87<sup>T</sup> and *T. alexandrii* LMIT003<sup>T</sup> are 76.5% and 21.3%, respectively. These values were much lower than the threshold values for prokaryotic species delineation, which are 95–96% for ANI and
70% for DDH (Kim et al., 2014; Wayne et al., 1987). These results indicated that strain YMD87\(^T\) represented a novel *Tropicibacter* species.

Table 1  
Differential characteristics of strain YMD87\(^T\) from the reference strain. Strains: 1, YMD87\(^T\); 2, *Tropicibacter alexandrii* LMIT003\(^T\) (KCTC 62895\(^T\)). Data for strains 1, 2 are from this study, unless otherwise indicated. +, positive; w, weakly positive; −, negative. All strains are positive for the following: activity of oxidase, catalase, alkaline phosphatase, leucine arylamidas, and \(\alpha\)-glucosidase. All strains are negative for the following: motility; hydrolysis of tween 20, 40, 60, 80, starch; activity of lipase C14, trypsin, chymotrypsin, \(\alpha\)-galactosidase, \(\beta\)-galactosidase, N-acetyl-\(\beta\)-glucosaminidase, and \(\alpha\)-mannosidase.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
</tr>
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<tbody>
<tr>
<td><strong>Cell size (µm)</strong></td>
<td>3.4–6.8×1.2–2.4</td>
<td>0.8–3.4×0.8–1.3*</td>
</tr>
<tr>
<td><strong>Temperature (°C) range</strong></td>
<td>10–40</td>
<td>15–35*</td>
</tr>
<tr>
<td><strong>pH range</strong></td>
<td>4–12</td>
<td>4–12*</td>
</tr>
<tr>
<td><strong>NaCl (% w/v) range</strong></td>
<td>0–6</td>
<td>0–6*</td>
</tr>
<tr>
<td><strong>Hydrolysis of:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gelatin</td>
<td>-</td>
<td>+*</td>
</tr>
<tr>
<td>Urea</td>
<td>+</td>
<td>-*</td>
</tr>
<tr>
<td><strong>Enzyme activity:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esterase(C4)</td>
<td>+</td>
<td>-*</td>
</tr>
<tr>
<td>Esterase lipase (C8)</td>
<td>+</td>
<td>-*</td>
</tr>
<tr>
<td>Valine arylamidase</td>
<td>+</td>
<td>-*</td>
</tr>
<tr>
<td>Cystine arylamidase</td>
<td>+</td>
<td>-*</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>+</td>
<td>-*</td>
</tr>
<tr>
<td>Naphthol-AS-BI-phosphohydrolase</td>
<td>+</td>
<td>-*</td>
</tr>
<tr>
<td>(\beta)-glucosidas</td>
<td>-</td>
<td>+*</td>
</tr>
<tr>
<td>(\beta)-fucosidase</td>
<td>nd</td>
<td>-*</td>
</tr>
<tr>
<td><strong>DNA G + C content (mol%)</strong></td>
<td>63.8</td>
<td>61.9*</td>
</tr>
</tbody>
</table>

*Data from Wang et al., 2020 [2].
Genomic analysis

The complete genome and three plasmid sequences of strain YMD87T was obtained and submitted to GenBank (accession number CP124616-CP124619). Genomic analysis indicated that strain YMD87T contains a circular chromosome of 3,932,460 bp with G + C content of 63.8% and three circular plasmids of 116,492 bp with G + C content of 63.09%, 49,209 bp with G + C content of 66.34% and 49,673 bp with G + C content of 65.3%, respectively. The predicted numbers of 5S rRNA, 16S rRNA, 23S rRNA, and tRNA sequences were 3, 3, 3, and 49, respectively (Table S1). In addition, 10 genomics islands, 3 prophages, 2 CRISPR, and 341 secreted proteins were also detected in the genome of strain YMD87T (Table S1). Furthermore, secondary metabolites analysis revealed 8 gene clusters, i.e., hserlactone (2 clusters), NRPS, RvPP-like, terpene, betalactone, ectoine, NRPS-like, and T1PKS, including a total of 186 genes (Table S1).

Physiological and chemotaxonomic analysis

The morphological, cultural, physiological and biochemical characteristics of strain YMD87T are given in the species descriptions (Table 1, S2, and Fig. 2). FAME analysis showed that the major fatty acid (≥ 5% of the total fatty acids) detected in strain YMD87T was C16:0 (9.5%), C12:1 3-OH (5.8%), and summed feature 8 (72.4%) (Table 2). The reference strain, T. alexandrii LMIT003T showed different profile of FAME with strain YMD87T in some aspects, and contained C18:0 (9.5%) as the major fatty acids, but C16:0 and C12:1 3-OH were not the major fatty acids (Table 2). The polar lipids of YMD87T contained phosphatidylglycerol (PG), phosphatidylethanolamine (PE), five unidentified lipids (L), five unidentified phospholipids (PL), phosphatidylcholine (PC), unidentified glycolipid (GL), and five unidentified aminolipids (AL) (Fig. S1). The PC, PE, PG, AL, and PL were also detected in T. alexandrii LMIT003T, but L and GL were not detected. The antibiotic resistance test show that, strain YMD87T was susceptible to penicillin, piperacillin, cephalexin, cefamezin, cefradine, cefuroxime, ceftazidime, ceftriaxone, cefoperazone, amikacin, neomycin, tetracycline, doxycycline, minocycline, midecamycin, norfloxacin, ofloxacin, ciprofloxacin, polymyxin B, sulfamethoxazole, furazolidone, weekly susceptible to clindamycin, resistant to gentamicin.
Table 2

Cellular fatty acid compositions of strain YMD87<sup>T</sup> and the reference strain *Tropicibacter alexandrii* LMIT003<sup>T</sup> (KCTC 62895<sup>T</sup>). Strains: 1, YMD87<sup>T</sup>; 2, KCTC 62895<sup>T</sup>. Data for strains 1, 2 are from this study. Only fatty acids accounting for at least 0.5% of the total acid content are listed. Fatty acids that represent >5.0% are indicated as bold. -, Not detected.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
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<tbody>
<tr>
<td><strong>Saturated</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;10:0&lt;/sub&gt;</td>
<td>-</td>
<td>0.7</td>
</tr>
<tr>
<td>C&lt;sub&gt;16:0&lt;/sub&gt;</td>
<td>9.5</td>
<td>2.9</td>
</tr>
<tr>
<td>C&lt;sub&gt;17:0&lt;/sub&gt;</td>
<td>1.2</td>
<td>3.2</td>
</tr>
<tr>
<td>C&lt;sub&gt;18:0&lt;/sub&gt;</td>
<td>3.7</td>
<td>9.5</td>
</tr>
<tr>
<td><strong>Unsaturated</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;19:0&lt;/sub&gt; cyclo ω8c</td>
<td>-</td>
<td>4.1</td>
</tr>
<tr>
<td>11-Methyl C&lt;sub&gt;18:1&lt;/sub&gt; ω7c</td>
<td>2.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Iso-C&lt;sub&gt;18:0&lt;/sub&gt;</td>
<td>1.8</td>
<td>2.0</td>
</tr>
<tr>
<td><strong>Hydroxy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C&lt;sub&gt;12:1&lt;/sub&gt; 3-OH</td>
<td>5.8</td>
<td>4.8</td>
</tr>
<tr>
<td><strong>Summed features:</strong></td>
<td>#</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3.4</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>72.4</td>
<td>71.0</td>
</tr>
</tbody>
</table>

#Summed Features are fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature with a single percentage of the total. Summed feature 3 consists of C<sub>16:1</sub> ω7c and/or C<sub>16:1</sub> ω6c; summed feature 8 consists of C<sub>18:1</sub> ω7c or/and C<sub>18:1</sub> ω6c.

**Conclusion**

Strain YMD87<sup>T</sup> phylogenetically formed a distinct group with *T. alexandrii* LMIT003<sup>T</sup>, and the chemotaxonomic profiles of strain YMD87<sup>T</sup> were generally similar to *T. alexandrii* LMIT003<sup>T</sup>. However, some physiological and biochemical properties, such as enzyme activities, acid production substrates and antibiotic resistance, distinguished strain YMD87<sup>T</sup> from *T. alexandrii* LMIT003<sup>T</sup>. Therefore, strain YMD87<sup>T</sup> represents a novel species of the genus *Tropicibacter*, for which the name *Tropicibacter oceani* sp. nov. is proposed.
Description of *Tropicibacter oceani* sp. nov.

*Rhodophyticola* (o.ce.a’ni. L. gen. n. *oceani*, of the ocean).

Cells of strain YMD87\textsuperscript{T} are Gram-stain-negative, rod-shaped, atrichous, and aerobic, 1.2-2.4 μm in width and 3.4-6.8 μm in length. Colonies of YMD87\textsuperscript{T} on 2216EA are circular, smooth, white, and approximately 1-2 mm in diameter after incubation for 7 days at 28 °C. Growth of strain YMD87\textsuperscript{T} occurred at 10.0-40.0 °C (optimum, 25-30 °C), pH 4.0-12.0 (optimum, 8.0) and with 0-6.0 % (w/v) NaCl (optimum, 0-2.0 %). Activities of oxidase, catalase and urease are positive, but tween-20, 40, 60, 80, starch and gelatin cannot be hydrolyzed. API ZYM reaction tests were positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidas, valine arylamidase, cystine aramidase, acid phosphatase, Naphthol-AS-BI-phosphohydrolase, α-glucosidase, negative for lipase C14, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, N-acetyl-β-glucosaminidase, β-uronidase, β-glucosidas, α-fucosidase and α-mannosidase. Acid cannot be produced. The G + C content of the genomic DNA of strain YMD87\textsuperscript{T} is 63.8 %. The principal respiratory quinone is Q-10. The polar lipids of YMD87\textsuperscript{T} contained phosphatidylglycerol, phosphatidylethanolamine, five unidentified lipids, five unidentified phospholipids, phosphatidylcholine, unidentified glycolipid, and five unidentified amino lipids. The major fatty acids of strain YMD87\textsuperscript{T} contained C\textsubscript{12:1} 3-OH, C\textsubscript{16:0}, and summed feature 8 (C18:1 ω7c or/and C18:1 ω6c).

The type strain is YMD87\textsuperscript{T} (= MCCC 1K08473\textsuperscript{T} = KCTC 92856\textsuperscript{T}), which was isolated from the intertidal sediment obtained from the intertidal zone sediment of Chinese Yellow Sea. The GenBank accession numbers of the 16S rRNA gene sequence and the complete genome sequence of strain YMD87\textsuperscript{T} are OP942226 and CP124616, respectively.

Author contributions DDZ: investigation, conceptualization, writing original draft. XDX: isolated the bacterium. BZZ and JXF: phylogenetic and genomic characterisation. JZ: supervision and writing-reviewing and editing, funding acquisition. All authors read and approved the manuscript.

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Data availability The 16S rRNA gene and the complete genome sequences of strain YMD87\textsuperscript{T} have been deposited under the GenBank accession numbers OP942226 and CP124616, respectively.
Conflicts of interest The authors declare that they have no competing interests.

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

References


**Figures**
The neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic position of *Tropicibacter Oceani* YMD87\(^T\). Bootstrap values (greater than 50\%) based on 1000 replications are shown at branching points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum likelihood and minimum-evolution algorithms. *Agaricicola taiwanensis* CC-SBABM117\(^T\) (GenBank accession NR_125534.1) was used as outgroup. Scale bar, 0.01 substitutions per nucleotide position.
Figure 2

Transmission electron micrograph of the cell of strain YMD87\textsuperscript{T}. Bar, 2.0 μm.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarydata.docx