Aquibacter zeaxanthinisifaciens gen. nov., sp. nov., a zeaxanthin-producing bacterium of the family Flavobacteriaceae isolated from surface seawater, and emended descriptions of the genera Aestuariibaculum and Gaetbulibacter

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A Gram-stain-negative, strictly aerobic, rod-shaped, non-flagellated, non-spore-forming and gliding marine bacterium, designated strain CC-AMZ-304T, was isolated from coastal surface seawater near Taichung harbour, Taiwan. Strain CC-AMZ-304T predominantly synthesized zeaxanthin and thus formed yellow colonies on marine agar. The novel strain showed an unstable phylogenetic position, although sharing high pairwise 16S rRNA gene sequence similarities of 95.9–94.9, 95.7 and 95.1–93.9 % with Gaetbulibacter species (n=4), Aestuariibaculum suncheonense SC17T and Bizonia species (n=7), respectively. The polar lipid profile of strain CC-AMZ-304T consisted of phosphatidylethanolamine, five unidentified lipids, one unidentified phospholipid, two unidentified aminolipids and one unidentified glycolipid. The major (>5 % of the total) fatty acids were iso-C15:0, iso-C15:1 G, iso-C17:0 3-OH, iso-C15:0 3-OH and C15:1 ω5c. The DNA G+C content was 36.0 mol%. Menaquinone-6 (MK-6) was the sole respiratory quinone and the major polyamine was triamine sym-homospermidine. Phylogenetic distinctiveness, unique polar lipid composition, presence of significant amounts of branched hydroxyl fatty acids (iso-C17:0 3-OH and iso-C15:0 3-OH) and a low amount of anteiso-C15:0, and several additional distinguishing biochemical features clearly discriminated strain CC-AMZ-304T from the type species of the genera Aestuariibaculum and Gaetbulibacter. Thus, based on data from the present polyphasic study, strain CC-AMZ-304T is considered to represent a new species of a new genus within the family Flavobacteriaceae, for which the name Aquibacter zeaxanthinisifaciens gen. nov., sp. nov. is proposed; the type strain of Aquibacter zeaxanthinisifaciens is CC-AMZ-304T (=JCM 18557T=BCRC 80463T). Emended descriptions of the genera Aestuariibaculum and Gaetbulibacter are also proposed.

A number of marine bacterial isolates are known to synthesize xanthophyll carotenoids such as astaxanthin and zeaxanthin (Asker et al., 2007a, b; Hameed et al., 2012; Shahina et al., 2013). The marine members of the family Flavobacteriaceae (phylum Bacteroidetes) represent one of the major components of bacterioplankton, abundant in oceanic environments (Kirchman, 2002; Kirchman et al., 2003). Marine flavobacteria such as Mesoflavibacter zeaxanthinisifaciens (Asker et al., 2007a), Zeaxanthinizibacter enoshimensis (Asker et al., 2007b), Muricauda lutaonensis (Hameed et al., 2011) and Siansivirga zeaxanthinizifaciens (Hameed et al., 2012) have been well characterized for zeaxanthin biosynthesis. In addition, some marine flavobacteria are reported to synthesize rare monocyclic xanthophylls such as saproxanthin and myxol (Shindo et al., 2007). Here, we report the isolation and taxonomic characterization of a marine flavobacterial strain, CC-AMZ-304T, that predominantly synthesizes zeaxanthin.

While exploring carotenoid-producing marine bacterial isolates, strain CC-AMZ-304T was isolated from a surface seawater sample (10 cm depth) collected near Taichung harbour, Taiwan (24.307512° N 120.518572° E) on 26 May 2012. The marine water sample was subject to a standard
dilution-to-extinction plating method using marine agar 2216 (MA; Difco) and incubation at 30 °C for 48–96 h. A yellow colony of strain CC-AMZ-304T was isolated, purified and preserved in marine broth supplemented with 20% glycerol at −80 °C. Taxonomic investigations were carried out according to published guidelines and minimal standards (Tindall et al., 2010; Bernardet et al., 2002). The type strains of the type species of the genera Aestuariibaculum (Aestuariibaculum suncheonense JCM 17789T; Jeong et al., 2013) and Gaetbulibacter (Gaetbulibacter saemankumensis KCTC 12379T; Jung et al., 2005) were used as references for direct comparative analysis. All strains were cultured on MA or in marine broth (MB) for 48 h at 30 °C, unless specified otherwise.

The genomic DNA of strain CC-AMZ-304T was isolated by using the UltraClean Microbial Genomic DNA Isolation kit (MO BIO) according to the manufacturer’s instructions. The partial 16S rRNA gene was amplified via PCR according to Shahina et al. (2013). Gene sequencing was performed by using the Bigdye terminator kit (Heiner et al., 1998) and an automatic DNA sequencer (ABI PRISM 310; Applied Biosystems) (Watts & MacBeath, 2001). Sequence fragments were then assembled using the Fragment Assembly System program from the Wisconsin Package (GGC, 1995). Sequence similarity values were computed using BLAST searches (Altschul et al., 1990) and the EzTaxon-e server (Kim et al., 2012). Sequence data were analysed by MEGA 5 (Tamura et al., 2011), after multiple alignment by CLUSTAL X (Thompson et al., 1997). A distance matrix method (distance options according to the Kimura two-parameter model; Kimura, 1980), including clustering by neighbour-joining (Saitou & Nei, 1987), a discrete character-based maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods, was used. The topologies of the trees were evaluated by using the bootstrap resampling method based on 1000 replications (Felsenstein, 1985). 16S rRNA gene sequence analysis revealed that strain CC-AMZ-304T shared highest pairwise similarity with Gaetbulibacter marinus IMCC 1914T (95.9%), A. suncheonense SC17T (95.7%) and G. saemankumensis SMK-12T (95.6%). The novel strain showed 16S rRNA gene sequence similarities of 95.9–94.9% and 95.1–93.9% with species of the genera Gaetbulibacter (n=4) and Bizonia (n=7), respectively. In the neighbour-joining phylogenetic tree (Fig. 1), strain CC-AMZ-304T formed a distinct phylogenetic lineage associated with A. suncheonense SC17T, although with a very poor bootstrap confidence of the node (10%, not shown). Strain CC-AMZ-304T also exhibited an unstable taxonomic position in the phylogenetic trees constructed based on the maximum-parsimony and maximum-likelihood algorithms, with very poor bootstrap confidence of the nodes (data not shown). It is noteworthy that G. marinus IMCC 1914T (Yang & Cho, 2008), which shared the highest pairwise sequence similarity with strain CC-AMZ-304T, occupied a phylogenetic position closely associated with Gaetbulibacter lutimaris D1-y4T but independent of strain CC-AMZ-304T as well as of the type species of the genus Gaetbulibacter (Fig. 1).

The following phenotypic tests were carried out on strain CC-AMZ-304T only. Colonies were examined for morphological features such as appearance, size, shape, texture and pigmentation. Presence of endospores was assessed by phase-contrast microscopy (model A3000; Zeiss) after malachite-green staining (Smibert & Krieg, 1994) of cells grown on MA for 7 days. Cell morphology including the presence of flagella was determined by placing the cells (1–2 days old) on a carbon-coated copper grid followed by staining with 0.2% uranyl acetate for 5–10 s, brief air-drying and observation under a transmission electron microscope (JEOL JEM-1400). Gram-staining was performed according to Murray et al. (1994). Gliding motility was investigated by using phase-contrast microscopy (model A3000; Zeiss) of a hanging-drop preparation from an MB culture (Bernardet et al., 2002). The presence of flexirubin-type pigments was investigated as described by Reichenbach (1992) and Bernardet et al. (2002). Growth was tested on nutrient agar (NA; Himedia), trypticase soy agar (TSA) and R2A agar (Oxoid). Carbon source utilization was determined using GN2 MicroPlates (Biolog).

The following phenotypic tests were carried out on strain CC-AMZ-304T and A. suncheonense JCM 17789T and G. saemankumensis KCTC 12379T. Growth under anaerobic conditions was tested using MA or MA supplemented with 0.1% KNO3 by incubating the culture plates in an anaerobic chamber (COY). Activity of catalase and oxidase, and hydrolysis of starch (0.2%), egg yolk (1.0%), and Tweens 20 and 80 (1.0%) were tested on MA according to Smibert & Krieg (1994). Degradation of casein (1.0% skimmed milk), colloidal chitin (1.0%), CM-cellulose (1.0%) and xylan (1.0%) was tested on MA. Degradation was revealed by the clear zone formed around colonies either directly or after flooding with appropriate stains (Teather & Wood, 1982). Hydrolysis of L-tyrosine (0.5%) was tested on MA (Barrow & Feltham, 1993). DNase activity was assessed using DNase test agar (Himedia) supplemented with 3.2% sea salts (Sigma). The requirement for NaCl was tested on R2A agar (Difco) supplemented with 0–10% NaCl (at 1% intervals). Growth experiments at pH 4–12 were performed using MB adjusted with 100 mM acetate buffer (pH 4–5), 100 mM NaH2PO4/Na2HPO4 buffer (pH 6–8), 100 mM NaHCO3/Na2CO3 buffer (pH 9–10), Na2HPO4/NaOH buffer (pH 11) and KC1/NaOH buffer (pH 12). The pH 12 medium was filter-sterilized (0.22 μm). Growth at 10, 20, 25, 30, 37, 40, 45, 50 and 55 °C was tested in MB after 72 h of incubation. The three strains were inoculated in API 20 NE, API 20 E, API ZYM and API 50 CH strips (bioMérieux) according to the manufacturer’s instructions, except that the inoculation fluid was supplemented with sterile 3.2% sea salts (final concentration) and that results were recorded after 48 h of incubation at 30 °C.

The phenotypic characteristics of strain CC-AMZ-304T are shown in Fig. S1 (available in IJSEM online) and given in
the genus and species descriptions. The features that distinguished the new isolate from its phylogenetic neighbours are detailed in Table 1.

For cellular fatty acid analysis, the fatty acid methyl esters of strain CC-AMZ-304T and the reference strains were extracted from cells cultivated on MA at 30 °C. Cells were harvested during mid-exponential growth phase, subjected to saponification, methylation and extraction as described by Kämpfer & Kroppenstedt (1996), and analysed by GC (model 7890A; Agilent). Peaks were automatically integrated, and fatty acid names and percentages were determined using the microbial identification standard software package MIDI (version 6) (Sasser, 1990) by adopting the database RTSBA6. The fatty acid profiles of the three strains displayed both qualitative and quantitative differences (Table 2). The major (>5% of the total) fatty acids of strain CC-AMZ-304T were iso-C15:0 (32.5%), iso-C15:1 G (27.6%), iso-C17:0 3-OH (10.7%), iso-C16:0 3-OH (10.1%) and C15:0 2-OH (6.9%). Strain CC-AMZ-304T and the type species of the two closely related genera shared the same predominant unsaturated fatty acid (iso-C15:0) and hydroxyl fatty acid (iso-C15:1 G). Nevertheless, strain CC-AMZ-304T clearly differed from the reference strains by possessing branched hydroxyl fatty acids (iso-C15:0 3-OH and iso-C16:0 3-OH) and lacking significant amounts of anteiso-C15:0, C16:0 and C15:0 2-OH.

The carotenoid pigments of strain CC-AMZ-304T and the reference strains were extracted and analysed according to Hameed et al. (2012). The respiratory quinones of strain CC-AMZ-304T and the reference strains were extracted according to Minnikin et al. (1984) and analysed by reversed-phase HPLC according to Collins (1985) with minor modifications.

**Fig. 1.** Unrooted neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain CC-AMZ-304T and other representatives of the family Flavobacteriaceae. Bootstrap values (>70%) based on 1000 replications are shown at nodes. Bar, 0.005 substitutions per nucleotide position.
Table 1. Differential phenotypic characteristics between strain CC-AMZ-304\textsuperscript{T} and two closely related members of the family Flavobacteriaceae

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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</thead>
<tbody>
<tr>
<td>Gliding motility</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Growth in NaCl (%) (optimum)</td>
<td>2–4 (3)</td>
<td>1–8 (1–2)</td>
<td>1–7 (2–3)</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Acetoin production</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Activity of</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catalase</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tryptophan deaminase</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Casein (skimmed milk), xylan</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Assimilation of (API 20 NE)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>d-Glucose</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>d-Mannitol</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>l-Arabinose</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acid production from (API 50 CH):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythritol</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>D-ribose</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>l-Rhamnose</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Methyl β-d-xlyopyranoside,</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>d-Fructose</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Cellobiose, raffinose, l-fucose</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Lactose, starch, glycogen</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Amygdalin</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Potassium 3-ketogluconate</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Enzyme activity (API ZYM):</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esterase (C4), x-glucosidase</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Esterase lipase (C8), acid phosphatase</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>β-Glucosidase, α-mannosidase</td>
<td>w</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>36.0</td>
<td>46.4</td>
<td>34.8</td>
</tr>
</tbody>
</table>

(Shahina et al., 2013). The polar lipids of strain CC-AMZ-304\textsuperscript{T} and the reference strains were extracted and analysed by two-dimensional TLC (Embley & Wait, 1994). The polyamines of strain CC-AMZ-304\textsuperscript{T} were extracted and analysed by reversed-phase HPLC according to Scherer & Kneifel (1983) with minor modifications (Shahina et al., 2013). For determination of the G+C content, the DNA of strain CC-AMZ-304\textsuperscript{T} was subjected to thermal denaturation followed by enzymic digestion into nucleosides as described by Mesbah et al. (1989). The resultant nucleoside mixture was separated and quantified by reversed-phase HPLC with minor modifications as given by Shahina et al. (2013).

Strain CC-AMZ-304\textsuperscript{T} produced all-trans-zeaxanthin as a predominant (65%) xanthophyll carotenoid. In addition, significant amounts of cis-isomeric zeaxanthin (17%) and some unidentified carotenoids (15%) were detected. Interestingly, similar proportions of these carotenoids were also detected in A. suncheonense JCM 17789\textsuperscript{T} and G.
Table 2. Whole-cell fatty acid profiles (%) of strain CC-AMZ-304T and two closely related members of the family Flavobacteriaceae

Strains: 1, CC-AMZ-304T; 2, Aestuariibaculum suncheonense JCM 17789T; 3, Gaetbulibacter saemankumensis KCTC 12379T. All data are from this study. Fatty acids amounting to <1% of the total fatty acids in all strains are not shown. TR, Trace (<1%); –, not detected. Major components (>5%) are shown in bold type.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td><strong>Saturated</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C14:0</td>
<td></td>
<td>TR</td>
<td>1.3</td>
</tr>
<tr>
<td>C16:0</td>
<td>1.1</td>
<td>6.2</td>
<td>5.5</td>
</tr>
<tr>
<td><strong>Branched saturated</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iso-C13:0</td>
<td>1.5</td>
<td>TR</td>
<td>3.8</td>
</tr>
<tr>
<td>iso-C14:0</td>
<td></td>
<td>TR</td>
<td>1.6</td>
</tr>
<tr>
<td>iso-C15:0</td>
<td>32.5</td>
<td>33.1</td>
<td>24.2</td>
</tr>
<tr>
<td>iso-C16:0</td>
<td>2.7</td>
<td>2.7</td>
<td>0.8</td>
</tr>
<tr>
<td>anteiso-C15:0</td>
<td>1.4</td>
<td>10.3</td>
<td>10.2</td>
</tr>
<tr>
<td><strong>Unsaturated</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C15:0 3-OH</td>
<td>6.9</td>
<td>5.8</td>
<td>5.4</td>
</tr>
<tr>
<td>C15:1 3-OH</td>
<td>–</td>
<td>1.2</td>
<td>TR</td>
</tr>
<tr>
<td>C17:0 3-OH</td>
<td>–</td>
<td>–</td>
<td>TR</td>
</tr>
<tr>
<td>C18:2 9c</td>
<td>TR</td>
<td>1.3</td>
<td>TR</td>
</tr>
<tr>
<td>anteiso-C17:1 9c</td>
<td>TR</td>
<td>–</td>
<td>1.2</td>
</tr>
<tr>
<td><strong>Branched mono-unsaturated</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iso-C15:1 G</td>
<td>27.6</td>
<td>13.3</td>
<td>13.8</td>
</tr>
<tr>
<td>anteiso-C15:1 A</td>
<td>TR</td>
<td>1.6</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Hydroxy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C15:0 2-OH</td>
<td>–</td>
<td>2.2</td>
<td>4.3</td>
</tr>
<tr>
<td>iso-C15:0 3-OH</td>
<td>10.1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>iso-C16:0 3-OH</td>
<td>1.7</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>iso-C17:0 3-OH</td>
<td>10.7</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Summed features</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summed feature 3</td>
<td>4.2</td>
<td>6.0</td>
<td>16.9</td>
</tr>
<tr>
<td>Summed feature 9</td>
<td>–</td>
<td>1.7</td>
<td>1.9</td>
</tr>
</tbody>
</table>

*As indicated by Montero-Calasanz et al. (2013) summed features are groups of two or three fatty acids that are treated together for the purpose of evaluation in the MIDI system and include both peaks with discrete equivalent chain-lengths as well as those where the equivalent chain-lengths are not reported separately. Summed feature 3 was listed as C16:107c and/or C16:106c; summed feature 9 was listed as iso-C17:109c and/or C16:0 10-methyl.

The polyamine profiles of strain CC-AMZ-304T and the reference strains are given in Table S1. All strains possessed triamine sym-homospermidine as the major polyamine besides containing minor to trace amounts of 2-hydroxyputrescine, cadaverine and spermidine. In addition, moderate amounts of putrescine were detected in strain CC-AMZ-304T and A. suncheonense JCM 17789T. The major polyamine is triamine sym-homospermidine; 2-hydroxyputrescine, cadaverine and spermidine are present in minor to trace amounts; the polar lipid profile contains at least one glycolipid in detectable amounts; the predominant carotenoid pigment is all-trans-zeaxanthin.


The description of the genus Gaetbulibacter is as given by Jung et al. (2005) and emended by Yang & Cho (2008), Park et al. (2012) and Jeong et al. (2013). In addition, the type strain of the type species exhibits the following features: the major polyamine is trimeric sym-homospermidine; a moderate amount of putrescine is present; 2-hydroxyputrescine, cadaverine and spermidine are present in minor to trace amounts; the polar lipid profile contains at least one glycolipid in detectable amounts; the predominant carotenoid pigment is all-trans-zeaxanthin.

Description of Aquibacter gen. nov.

Aquibacter (A.qui.bac’ter. L. n. aqua water; N.L. masc. n. bacter from Gr. neut. n. baktron rod; N.L. masc. n. Aquibacter rod isolated from water).
Cells are Gram-stain-negative, strictly aerobic, non-spore-forming, chemoheterotrophic, mesophilic, typically rod-shaped with rounded ends, non-flagellated and motile by gliding. Oxidase-positive and catalase-negative. Carotenoid pigments are present but flexirubin-type pigments are absent. The major isoprenoid quinone is MK-6. The predominant (>25 % of the total) fatty acids are iso-C_{15:0} and iso-C_{15:1} G. The major polar lipids are phosphatidylethanolamine, three unidentified lipids and two unidentified aminolipids. Triamine sym-homospermidine is the major polynamine. As determined by 16S rRNA gene sequence analysis, the genus *Aquibacter* is a member of the family Flavobacteriaceae. The type species is *Aquibacter zeaxanthinifaciens*.

**Description of *Aquibacter zeaxanthinifaciens* sp. nov.**

*Aquibacter zeaxanthinifaciens* (ze.a.xan.thi.ni.fa.ci.ens. N.L. neut. n. zeaxanthinum zeaxanthin; L. part. pres. faciens making/producing; N.L. part. adj. zeaxanthinifaciens zeaxanthin-producing).

Cells are 0.6–0.7 μm in diameter and 1.3–3.9 μm in length. On MA, after 1–2 days of incubation at 30 °C colonies are irregular, convex and yellow, 0.5–1.0 mm in diameter. Growth occurs at 20–35 °C (optimum, 30 °C), at pH 6.0–8.0 (optimum, pH 7.0) and in the presence of 2–4 % NaCl (optimum, 3 %). Growth does not occur on NA, TSA or R2A agar. The predominant carotenoid pigment is all-trans-zeaxanthin. Minor amounts of isomeric zeaxanthin are also present. L-Tyrosine is hydrolysed, whereas chitin, starch, Tween 20 and 80, casein, CM-cellulose, xylan and DNA are not hydrolysed. Black diffusible pigments are not produced on L-tyrosine agar. In GN2 MicroPlates, the following carbon sources are utilized: α-cyclodextrin, N-acetyl-α-galactosamine, celllobiose, L-fucose, D-galactose, gentiobiose, α-D-glucose, α-lactose, lactulose, maltose, α-D-mannose, melibiose, methyl β-D-glucoside, raffinose, sucrose, trehalose, turanose, DL-lactic acid, L-glutamic acid, glycyrl L-aspartic acid, L-ornithine, L-proline, uracil, acylglycine, glycerol, DL-α-glycerol phosphate, D-mannitol, D-xylose, α-D-glucose 1-phosphate; weak reaction for assimilation of D-glucose, l-arginine, DL-α-glucose, glucose 1-phosphate, d Mannose, N-acetylgalactosamine, maltose, potassium gluconate, adic acid, malic acid and trisodium citrate. In API 20 E strips, positive for nitrate reduction, assimilation of D-mannitol, hydrolysis of aesculin and gelatin, and p-nitrophenyl-β-D-galactopyranosidase activity; negative for indole production, glucose fermentation, arginine dihydrolase and urease activities, and assimilation of capric acid and phenylacetic acid; weak reaction for assimilation of D-glucose, L-arabinose, D-mannose, N-acetylgalactosamine, maltose, potassium gluconate, adic acid, malic acid and trisodium citrate. In API 20 E strips, positive for gelatinase activity and fermentation/oxidation of L-arabinose; negative for ONPG, trypsin, D-mannose, D-glucose, D-mannitol, inositol, D-sorbitol, L-rhamnose, sucrose, melibiose and amygdalin. Results obtained with API 20 E for indole production and activity of arginine dihydrolase, urease and gelatinase are similar to those with API 20 NE. In API ZYM strips, positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and N-acetyl-β-glucosaminidase activities; negative for esterase (C4), lipase (C14), trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase and α-fucosidase activities; weak reaction for β-glucosidase and α-mannosidase activities. In API 50 CH strips, acid is produced from glycerol, D-galactose, D-glucose, D-mannose, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, amygdalin, aesculin ferric citrate, celllobiose, maltose, melibiose, sucrose, trehalose, melezitose, raffinose, gentiobiose, turanose, L-fucose and potassium 5-ketogluconate. In addition to the predominant fatty acids listed in the genus description, significant amounts (>5 %) of iso-C_{17:0} and iso-C_{15:0} 2-OH are utilized.

**Fig. 2.** Polar lipid profiles of strain CC-AMZ-304^T^ (a), *Aestuariibaculum suncheonense* JCM 17789^T^ (b) and *Gaetbulibacter saemankumensis* KCTC 12379^T^ (c) as determined by two-dimensional TLC. The total lipids were visualized by spraying the plates with 10 % ethanolic molybdophosphoric acid. PE, phosphatidylethanolamine; L1–6, unidentified lipids; GL, unidentified glycolipid; AL1–3, unidentified aminolipids; PL, unidentified phospholipid.
3-OH, iso-C15:0, 3-OH and C15:10c5c are also present. In addition to the major polar lipids listed in the genus description, moderate to minor amounts of one unidentified phospholipid, one unidentified glycolipid and two unidentified lipids are also present. In addition to a major polyamine given in the genus description, moderate amounts of putrescine, and minor amounts of 2-hydroxyputrescine, cadaverine and spermidine are also present.

The type strain is CC-AMZ-304T (=JCM 18557T = BCRC 80463T), which was isolated from surface seawater (10 cm depth) near Taichung harbour, Taiwan. The DNA G+C content of the type strain is 36.0 mol%.

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References


