**Belliella marina** sp. nov., isolated from seawater

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A Gram-stain-negative, rod-shaped bacterium, strain SW112ᵀ, was isolated from a seawater sample collected from the Indian Ocean. The strain was strictly aerobic and catalase- and oxidase-positive. Strain SW112ᵀ grew at 4–42 °C (optimum 30 °C), at pH 5.5–9.5 (optimum pH 7.5) and in the presence of 0–9.0 % (w/v) NaCl (optimum 2.0–3.0 %). The predominant cellular fatty acids were iso-C₁₅:₀ (29.7 %), iso-C₁₇:₀-3-OH (14.3 %) and summed feature 3 (comprising C₁₆:₁ω7c and/or C₁₆:₁ω6c, 15.1 %). The major menaquinone was menaquinone-7 and the major polar lipid was phosphatidylethanolamine. The genomic DNA G+C content of strain SW112ᵀ was 39 mol %. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain SW112ᵀ was related to members of the genus *Belliella*, showing the highest similarity with *Belliella aquatica* TS-T86ᵀ and *Belliella baltica* DSM 15883ᵀ (96.5 % and 96.4 % sequence similarity, respectively). On the basis of phylogenetic inference and phenotypic characteristics, it is proposed that strain SW112ᵀ represents a novel species of the genus *Belliella*, for which the name *Belliella marina* sp. nov. is proposed. The type strain is SW112ᵀ (=CGMCC 1.15180ᵀ=KCTC 33694ᵀ).

The genus *Belliella* was proposed by Brettar et al. (2004) within the class ‘Sphingobacteria’ and the family ‘Flexibacteriaceae’, and the genus description was subsequently emended by Anil Kumar et al. (2012). Members of the genus are aerobic, Gram-stain-negative rods, containing iso-C₁₅:₀ as the major fatty acid, menaquinone-7 (MK-7) as the major respiratory quinone and phosphatidylethanolamine as the predominant polar lipid. At the time of writing, the genus comprises four recognized species: *Belliella baltica* (type species; Brettar et al., 2004), *Belliella pelovolcani* (Arun et al., 2009), *Belliella kenyensis* (Akhwale et al., 2015) and *Belliella aquatica* (Zhong et al., 2015). During an investigation of the microbial community in a seawater sample collected from the North-West Indian Ocean (3° 42′ 29″ N 63° 49′ 52″ E) at a water depth of 1500 m, a novel potential member of the genus *Belliella* was isolated. The characterization and taxonomic position of the strain was studied in this work.

Strain SW112ᵀ was isolated using the serial dilution and plate screening method on marine agar 2216 (Difco; Becton Dickinson). Culture purity was checked by microscopic examinations. The cultures were preserved at −80 °C in filtered water with 10 % (v/v) glycerol. Strain SW112ᵀ was routinely cultivated at 30 °C on marine agar 2216 (Difco) or in the corresponding broth (Difco).

Genomic DNA was extracted and purified by using a Puregene DNA isolation kit (Gentra Systems) in accordance with the manufacturer’s instructions. The almost-complete 16S rRNA gene of strain SW112ᵀ was amplified by PCR, sequenced as described previously (Chen & Dong, 2004). The obtained consensus sequence was submitted to the GenBank database to search for similar sequences using the BLAST algorithm and also on the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/) by using identity analysis (Kim et al., 2012). For reconstruction of phylogenetic trees, related sequences were selected from the RDP and NCBI databases. All sequences were aligned using the CLUSTAL X program (version 1.83) as described by Thompson et al. (1997). On the basis of a consensus sequence of the 16S rRNA gene (1349 bp) of strain SW112ᵀ, a phylogenetic tree was reconstructed using the neighbour-joining method implemented in MEGA software version 4.0 (Tamura et al., 2007). Similar results were obtained using the maximum-likelihood and maximum-parsimony algorithms (Fig. 1). The resultant tree topologies were evaluated...
Strain SW112T was incubated in an anaerobic jar to test the tolerance to 0.5 % NaCl as described by Zhong et al. (2014). Final NaCl concentrations of 0–10.0 % (w/v, at intervals of 0.5 %) were examined in modified marine 2216 broth with 0.5 % NaCl. The requirement for oxygen using the Atmosphere Generation System (Oxoid) was tested at pH 5.0–10.5 (at 0.5 pH unit intervals) according to Dong & Cai (2001). Growth was measured at 2, 4, 10, 15, 20, 25, 30, 35, 37, 40, 42, 43 and 45 °C. Growth at different initial pH values was tested at pH 5.0–10.5 (at 0.5 pH unit intervals) according to Dong & Cai (2001). The requirement for NaCl was examined in modified marine 2216 broth with final NaCl concentrations of 0–10.0 % (w/v, at intervals of 0.5 %) as described by Zhong et al. (2014). Strain SW112T was incubated in an anaerobic jar to test the requirement for oxygen using the Atmosphere Generation System (Oxoid). Other physiological and biochemical tests were carried out at 30 °C using the GEN III Microplate (Biolog) and the API 20E, API 20NE and API ZYM systems (bioMérieux) according to the manufacturers’ instructions.

Strain SW112T was strictly aerobic, catalase- and oxidase-positive. Colonies were circular, smooth transparent and pink after cultivation at 30 °C for 3 days. Cells of strain SW112T were Gram-stain-negative, non-spore-forming, non-motile rods. The physiological and biochemical characteristics of strain SW112T are given in the species description and in Table 1.

For analysis of cellular fatty acids, cells of strain SW112T, together with the two reference strains, were incubated in marine 2216 broth (Difco) at 30 °C for 3–4 days and harvested in the late exponential phase. Cellular fatty acids were saponified, methylated and extracted according to Miller (1982) and Kuykendall et al. (1988), and analysed by using the standard MIDI 6.0 system (Sasser, 1990). An Agilent GC 6890 gas chromatograph was used and fatty acids were identified using the TSBA 6.0 database. Respiratory quinones of strain SW112T were extracted and purified as described by Collins (1985) and analysed by using reversed-phase HPLC (Wu et al., 1989). Polar lipids of SW112T were extracted and analysed by two-dimensional TLC, as described by Tindall (1990). Total polar lipids were detected with 10 % ethanolic molybdophosphoric acid, ninhydrin was used for detection of aminolipids, molybdenum blue for phospholipids and α-naphthol for glycolipids, as described by Embly & Wait (1994).

The cellular fatty acids of strain SW112T were mainly comprised of iso-fatty acids, predominantly iso-C_{15:0}.
Table 1. Differential characteristics of strain SW112\textsuperscript{T} and closely related type strains

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tbody>
<tr>
<td>Colony pigmentation</td>
<td>Pink</td>
<td>Red</td>
<td>Pink</td>
<td>Pink/red</td>
<td>Red</td>
</tr>
<tr>
<td>Tolerance to 8 % NaCl</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Assimilation of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>l-Arabinose</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>l-Rhamnose</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>NR</td>
<td>-</td>
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<tr>
<td>D-Galactose</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>NR</td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>NR</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>NR</td>
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<tr>
<td>Cellobiose</td>
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<td>+</td>
<td>+</td>
<td>NR</td>
</tr>
<tr>
<td>N-Acetyl D-glucosamine</td>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>NR</td>
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<td>Hydrolysis of:</td>
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<tr>
<td>Tween 40</td>
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<td>-</td>
<td>-</td>
<td>+</td>
<td>NR</td>
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<tr>
<td>Tyrosine</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Gelatin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Casein</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Aesculin</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Pectin</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>NR</td>
<td>NR</td>
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<td>Enzyme activity (API ZYM)</td>
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<tr>
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<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Esterase lipase (C8)</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>β-Galactosidase</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>β-Glucosidase</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>β-Mannosidase</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>39</td>
<td>35\textsuperscript{a}</td>
<td>35\textsuperscript{b}</td>
<td>40</td>
<td>38</td>
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</tbody>
</table>

\*Data from: a, Zhong et al. (2015); b, Brettar et al. (2004).

The chemotaxonomic features of strain SW112\textsuperscript{T} as well as its DNA G+C content are in accordance with those of species of the genus \textit{Belliella}. However, the sequence divergence indicated that strain SW112\textsuperscript{T} cannot be affiliated to any recognized species of the genus \textit{Belliella}. In addition to the large sequence divergence, the novel strain also showed distinct phenotypic features that enabled it to be distinguished from the representative members of the genus \textit{Belliella}. Strain SW112\textsuperscript{T} could be differentiated from \textit{B. aquatica} by the latter’s complex polar lipid profile, phosphatidylethanolamine, two unidentified phospholipids, two unidentified aminolipids, one unidentified aminophospholipid and two other unidentified polar lipids (Fig. 2).

DNA–DNA hybridization was not performed in this study since the 16S rRNA gene sequence similarity of strain SW112\textsuperscript{T} to related species was less than 97 % (Tindall et al., 2010). The G+C content of the genomic DNA of strain SW112\textsuperscript{T} was determined using HPLC (Mesbah et al., 1989) with the modification of Lee et al. (2005), and was 39 mol%. This value was higher than those of the two reference strains [35 mol% for \textit{B. aquatica} CGMCC 1.12479\textsuperscript{T} (Zhong et al., 2015) and 35.3 mol% for \textit{B. baltica} DSM 15883\textsuperscript{T} (Brettar et al., 2004)] and within the range reported for species of the genus \textit{Belliella} (35–40 mol%).

Table 2. Cellular fatty acid contents of strain SW112\textsuperscript{T} and closely related type strains

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
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<tbody>
<tr>
<td>iso-C\textsubscript{15}:1 G</td>
<td>1.7</td>
<td>22.9</td>
<td>8.1</td>
</tr>
<tr>
<td>iso-C\textsubscript{15}:0</td>
<td>29.7</td>
<td>12.6</td>
<td>25.2</td>
</tr>
<tr>
<td>anteiso-C\textsubscript{15}:0</td>
<td>6.0</td>
<td>1.5</td>
<td>5.1</td>
</tr>
<tr>
<td>C\textsubscript{15}:0\textsubscript{106c}</td>
<td>TR</td>
<td>2.0</td>
<td>1.5</td>
</tr>
<tr>
<td>C\textsubscript{16}:0\textsubscript{105c}</td>
<td>1.2</td>
<td>2.5</td>
<td>2.1</td>
</tr>
<tr>
<td>C\textsubscript{16}:0</td>
<td>TR</td>
<td>1.3</td>
<td>TR</td>
</tr>
<tr>
<td>iso-C\textsubscript{17}:0 3-OH</td>
<td>8.8</td>
<td>11.2</td>
<td>9.6</td>
</tr>
<tr>
<td>iso-C\textsubscript{17}:0</td>
<td>TR</td>
<td>1.8</td>
<td>TR</td>
</tr>
<tr>
<td>C\textsubscript{17}:0\textsubscript{108c}</td>
<td>TR</td>
<td>1.1</td>
<td>TR</td>
</tr>
<tr>
<td>C\textsubscript{17}:0\textsubscript{106c}</td>
<td>3.5</td>
<td>4.6</td>
<td>5.4</td>
</tr>
<tr>
<td>iso-C\textsubscript{17}:0 3-OH</td>
<td>14.3</td>
<td>13.1</td>
<td>11.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Summed features*</th>
<th>3</th>
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<tbody>
<tr>
<td>NR</td>
<td>15.1</td>
<td>12.9</td>
</tr>
<tr>
<td>NR</td>
<td>7.3</td>
<td>2.8</td>
</tr>
<tr>
<td>NR</td>
<td>20.0</td>
<td>6.6</td>
</tr>
</tbody>
</table>

\*Summed features are groups of two or three fatty acids that cannot be separated by GLC using the MIDI System. Summed feature 3 comprised C\textsubscript{16}:1\textsubscript{ω7c} and/or C\textsubscript{16}:1\textsubscript{ω6c}, summed feature 4 comprised iso-C\textsubscript{17}:1\textsubscript{i} and/or anteiso-C\textsubscript{17}:1 B.

\*Data from: a, Zhong et al. (2015); b, Brettar et al. (2004).
as well as the different biochemical traits like nitrate reduction and the spectrum of substrates utilized for growth. Features that differentiate strain SW112T from *B. baltica* are tolerance to NaCl and several biochemical traits including hydrolysis of gelatin and casein (Table 1). Additional differential characteristics of strain SW112T and the two closely related type strains are given in Table 1.

On the basis of the distant phylogenetic relationship with related taxa, unique chemotaxonomic characteristics, DNA G+C content and physiological and biochemical traits described above, it is evident that strain SW112T represents a novel species of the genus *Belliella*, for which the name *Belliella marina* sp. nov. is proposed.

### Description of *Belliella marina* sp. nov.


Cells are strictly aerobic, Gram-stain-negative, non-spore-forming, non-motile rods. Colonies are circular, smooth, transparent and pink after cultivation at 30 °C for 3 days. Growth occurs at 4–42 °C (optimum 30 °C), at pH 5.5–9.5 (optimum pH 7.5) and in the presence of 0–9.0 % (w/v) NaCl (optimum 2.0–3.0 %). Oxidase and catalase activities are present, but arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase activities are absent. Nitrate is reduced to nitrite. Indole is not produced from tryptophan and H₂S is not produced from thiosulfate. The Voges–Proskauer test is negative. Positive for hydrolysis of aesculin, gelatin, casein, DNA, starch, pectin and Tween 40, but negative for hydrolysis of chitin. D-Glucose, D-fructose, D-galactose, D-mannose, L-rhamnose, sucrose, lactose, cellubiose, maltose, trehalose, glycerol, acetic acid, l-lactic acid, l-aspartic acid, l-glutamic acid, L-serine and N-acetyl D-glucosamine are oxidized, but D-sorbitol, trehalose D-mannitol, D-serine, citric acid, formic acid and propionic acid are not. Alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine aminopeptidase, valine arylamidase, cystine, trypsin, a-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphoamidase, a-galactosidase, b-galactosidase, a-glucosidase, b-glucosidase, N-acetyl-b-glucosaminidase and a-mannosidase activities are present. The predominant cellular fatty acids are iso-C₁₅ : ₀, iso-C₁₇ : ₀ and summed feature 3 (comprising C₁₆ : ₁ω₇c and/or C₁₆ : ₁ω₆c). The major menaquinone is MK-7 and the major
polar lipids consist of phosphatidylethanolamine, two unidentified phospholipids, two unidentified aminolipids, one unidentified aminophospholipid and two other unidentified polar lipids.

The type strain is SW112T (=CGMCC 1.15180T=KCTC 33694T), isolated from a seawater sample collected from the north-west Indian Ocean. The genomic DNA G+C content of the type strain is 39 mol% (HPLC).

Acknowledgements

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References


