**Aquimarina megaterium** sp. nov., isolated from seawater

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A novel Gram-stain-negative, rod-shaped, non-flagellated, strictly aerobic strain with gliding motility, designated XH134<sup>T</sup>, was isolated from surface seawater of the South Pacific Gyre (45° 58′ S 163° 11′ W) during the Integrated Ocean Drilling Program Expedition 329. The major respiratory quinone of strain XH134<sup>T</sup> was MK-6. The dominant fatty acids of strain XH134<sup>T</sup> were iso-C<sub>15 : 0</sub>, iso-C<sub>15 : 1</sub> G, C<sub>16 : 1</sub>ω6c and/or C<sub>16 : 1</sub>ω7c, iso-C<sub>17 : 0</sub> 3-OH, iso-C<sub>15 : 0</sub> 3-OH and 10-methyl C<sub>16 : 0</sub> and/or iso-C<sub>17 : 1</sub>ω9c. The polar lipids of strain XH134<sup>T</sup> comprised phosphatidylethanolamine, one unknown aminolipid and three unknown polar lipids. The DNA G+C content of strain XH134<sup>T</sup> was 32.4 mol%. Phylogenetic analyses based on 16S rRNA gene sequences showed that the novel strain was related most closely to *Aquimarina macrocephali* JAMB N27<sup>T</sup> with 96.9% sequence similarity. A number of phenotypic characteristics distinguished strain XH134<sup>T</sup> from described members of the genus *Aquimarina*. On the basis of combined phenotypic and phylogenetic analyses, strain XH134<sup>T</sup> represents a novel species of the genus *Aquimarina*, for which the name *Aquimarina megaterium* sp. nov. is proposed. The type strain is XH134<sup>T</sup> (=CGMCC 1.12186<sup>T</sup>=JCM 18215<sup>T</sup>).

The genus *Aquimarina*, a member of the family *Flavobacteriaceae*, was proposed by Nedashkovskaya et al. (2005) for accommodation of heterotrophic, Gram-negative, aerobic, dark-yellow or brownish-coloured, gliding flavobacteria producing flexirubin-type pigments (Nedashkovskaya et al., 2005). Later, on the basis of phylogenetic data based on 16S rRNA gene sequences and cellular fatty acid composition, *Staniellia latercula* (Lewin, 1969; Nedashkovskaya et al., 2005) and *Gaetbulimicrobium brevivitae* (Yoon et al., 2006) were reclassified within this genus as *Aquimarina latercula* and *Aquimarina brevivitae*, respectively, together with *Aquimarina intermedia* as a novel species (Nedashkovskaya et al., 2006). *Aquimarina macrocephali* (Miyazaki et al., 2010), *Aquimarina spongiae* (Yoon et al., 2011), *Aquimarina addita* (Yi & Chun, 2011), *Aquimarina agarlytica* (Lin et al., 2012), *Aquimarina mytili* (Park et al., 2012), *Aquimarina salinaria* (Chen et al., 2012), *Aquimarina longa* (Yu et al., 2013) and *Aquimarina gracilis* (Park et al., 2013) were classified in the genus on the basis of 16S rRNA gene sequence and morphological, physiological and biochemical characteristics. Twelve species of the genus *Aquimarina* have been recognized at the time of writing, all from marine environments: *A. addita*, *A. longa*, *Aquimarina muelleri* and *A. mytili* were isolated from seawater, *A. latercula* from the outflow of a marine aquarium, *A. intermedia* from a sea urchin, *A. brevivitae* from tidal flat sediment, *A. macrocephali* from the marine sediment adjacent to sperm whale carcasses, *A. spongiae* from a marine sponge, *A. agarlytica* from the surface of the marine red alga *Porphyra haitanensis*, *A. salinaria* from a saltpan and *A. gracilis* from mussel. A novel bacterial strain, designated XH134<sup>T</sup>, was isolated from surface seawater in the South Pacific Gyre at station U1371 (45° 58′ S 163° 11′ W) during the Integrated Ocean Drilling Program Expedition 329. The aim of the present study was to determine the exact taxonomic position of strain XH134<sup>T</sup> using a polyphasic taxonomic approach.

Seawater samples were spread on marine agar 2216 (MA; BD) plates and incubated at 28 °C. Strain XH134<sup>T</sup>, which formed irregular colonies with non-entire margins that were convex, orange-pigmented and opaque on MA after culturing for 2–3 days, was purified by streaking three times on MA. Cultures were maintained on MA plates at 28 °C and stocks were preserved in sterile 0.85% (w/v) saline supplemented with 15% (v/v) glycerol at −80 °C. *A. muelleri* LMG 22569<sup>T</sup>, obtained from the Belgian Co-ordinated Collections of Microorganisms (BCCM), *A. macrocephali* JCM 15542<sup>T</sup>, *A. latercula* JCM 8515<sup>T</sup> and *A. addita* JCM 17106<sup>T</sup> obtained from the Japan Collection of Microorganisms (JCM) and *A. longa* SW024<sup>T</sup> obtained from our own laboratory, which were used as reference strains, were cultured as for strain XH134<sup>T</sup> [MA/marine broth 2216 (MB; BD), 28 °C], unless otherwise specified.

The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of *Aquimarina megaterium* is XH134<sup>T</sup> is KC476292.

Two supplementary figures are available with the online version of this paper.
Gram staining and flagellum staining were investigated using standard methods (Beveridge et al., 2007). Cell morphology was determined by transmission electron microscopy (JEM-1200EX; JEOL) after cells had been negatively stained with 1 % (w/v) phosphotungstic acid. Growth under anaerobic conditions was determined on MA after incubation in an anaerobic jar which was filled with nitrogen and a packet of AnaeroPack-Anaero (Mitsubishi Gas Chemical) at 28 °C for 1 month. The temperature range for growth was determined on MB by incubating cultures at 4–42 °C (4, 8, 16, 28, 30, 34, 37 and 42 °C) for 5 days and at 0 °C on MA for 30 days. Salt tolerance was investigated using synthetic marine ZoBell broth [5 g Bacto peptone, 1 g yeast extract and 0.1 g FePO₄ in 1 litre modified artificial seawater (Lyman & Fleming, 1940) supplied with various concentrations of NaCl (0–15 %, w/v, at intervals of 1 %)]. In the modified artificial seawater, all of the Na⁺ was replaced by appropriate K⁺. Growth with NaCl as the sole salt was investigated on MA (distilled water instead of seawater) supplemented with 0–15 % (w/v) NaCl. The pH range for growth was determined in MB at pH 2–10 at intervals of 1 pH unit. The presence of gliding motility and the production of flexirubin-type pigments were investigated using the methods described by Bernardet et al. (2002). Using strains cultured for 3 days on MA at 28 °C, the absorption spectrum of pigments extracted using acetone/methanol (7:2, v/v) was determined at 300–700 nm with a UV–visible spectrophotometer (TU-1810; Beijing Purkinje General Instrument) (Tindall et al., 2007).

Standard protocols (Tindall et al., 2007) were used to assess catalase and oxidase activities, degradation of casein, urea, starch, gelatin and Tweens 20, 40 and 80, agar corrosion, nitrate reduction and H₂S production from thiosulfate with the modification that sterile seawater was used. DNase activity was examined by using DNase agar (Qingdao Hope Bio-technology) with sterile seawater, according to the manufacturer’s instructions. Degradation of chitin was examined on chitin agar with sterile seawater (Hsu & Lockwood, 1975). Utilization of substrates as sole carbon and energy sources was tested for 2 weeks in artificial seawater supplemented with 0.2 % (w/v) NaNO₃ and 1 % (w/v) of the substrate. Activities of constitutive enzymes and other physiological properties were determined after growth on MA at 28 °C for 2 days by using API 20E, API 20NE, API 50CH and API ZYM strips (bioMérieux) according to the manufacturer’s instructions except that sterile seawater was used to prepare the inocula. Susceptibility to antibiotics was investigated on MA plates by using discs (Hangzhou Microbiology Reagent) containing different antibiotics. The morphological, physiological and biochemical characteristics of strain XH134T are given in the species description, Table 1 and Fig. 1.

For fatty acid analysis, cell masses of strains XH134T, A. longa SW024T, A. Muelleri LMG 22569T, A. macrocephali JCM 15542T, A. latercula JCM 8515T and A. addita JCM 17106T were obtained after cultivation on MA at 28 °C for 2–3 days when the bacterial communities reached the late exponential stage of growth. Extraction of fatty acid methyl esters and separation by GC were performed by using the Instant FAME method of the Microbial Identification System (MIDI) version 6.1 and the RTSBA6 6.10 database (Sasser, 1990). The respiratory quinones of strain XH134T were extracted with chloroform/methanol (2:1, v/v), separated by TLC and identified by HPLC as described by Collins (1994). Polar lipids were analysed by using standard procedures (Minnikin et al., 1984). Extracted lipids were separated by two-dimensional TLC and identified by spraying with appropriate detection reagents (Minnikin et al., 1984). The G+C content of the chromosomal DNA of strain XH134T was determined according to the methods described by Mesbah & Whitman (1989) using reversed-phase HPLC. The dominant cellular fatty acids of strain XH134T were iso-C₁₅₅₀ (33.5 %), iso-C₁₅₂₀ G (12.6 %), C₁₆₅₀t and/or C₁₆₂₀t7c (11.7 %), iso-C₁₇₃₀ 3-OH (8.6 %), iso-C₁₅₅₀ 3-OH (7.3 %) and 10-methyl C₁₆₅₀ and/or iso-C₁₇₅₀ 9c (7.0 %). The fatty acid profile of strain XH134T was essentially similar to those of the five reference strains except for the proportions of some fatty acids (Table 2). In accordance with other members of the family Flavobacteriaceae, strain XH134T contained menaquinone 6 (MK-6) as the major respiratory quinone. The polar lipid composition of strain XH134T was phosphatidylethanolamine, one unknown aminolipid (AL) and three unknown polar lipids (L1–L3) (Fig. S2, available in IJSEM Online), which was very similar to that of A. macrocephali JCM 15542T, except that A. macrocephali has four unknown polar lipids (L1–L4) (Park et al., 2012). The G+C content of the DNA of strain XH134T was 32.4 mol%, a value in the range of those for recognized species of the genus Aquimarina (Table 1).

For 16S rRNA gene sequencing, DNA was extracted from strain XH134T and purified using standard methods (Ausubel et al., 1995). The 16S rRNA gene was amplified by PCR using two universal primers (8F, 5′-AGAGTTTGATCCTGGCTCAG-3′; B1510, 5′-GTTACCTGTAGGACCTT-3′). The PCR product was purified using the TIANgel Midi Purification kit (TIANGEN Biotech), cloned into pUCm-T (TaKaRa) and sequenced using an automated DNA sequencer (model ABI3730; Applied Biosystems) at BGI, Qingdao, China. The nearly complete 16S rRNA gene sequence (1485 nt) of strain XH134T was submitted to GenBank, and the identification of phylogenetic neighbours and the calculation of pairwise 16S rRNA gene sequence similarities were achieved using the EzTaxon server (http://eztaxon-e.ezbiocloud.net/; Kim et al., 2012). Sequences were aligned using CLUSTAL X1.8 (Thompson et al., 1997). Phylogenetic trees were reconstructed using the neighbour-joining (Fig. 2) and maximum-likelihood (Fig. S1) methods with Kimura two-state parameter model analyses (Kimura, 1980) implemented in the program MEGA version 5 (Tamura et al., 2011). In each case, bootstrap values were calculated based on 1000 replications.
Phylogenetic analysis according to the nearly complete 16S rRNA gene sequence showed that strain XH134\(^T\) belonged to the genus *Aquimarina*, and showed 96.9 % sequence similarity to *A. macrocephali* JAMB N27\(^T\), 96.3 % to *A. muelleri* KMM 6020\(^T\), 95.7 % to *A. latercula* ATCC 23177\(^T\), 95.7 % to *A. addita* JC2680\(^T\), 95.3 % to *A. gracilis* PSC32\(^T\), and 94.6 % to *A. longa* SW024\(^T\).
95.2 % to *A. agarilytica* ZC1\(^T\), 94.9 % to *A. intermedia* KMM 6258\(^T\), 94.7 % to *A. longa* SW024\(^T\), 94.7 % to *A. spongiae* A6\(^T\), 94.6 % to *A. mytili* PSC33\(^T\), 94.6 % to *A. salinaria* antisso-27\(^T\) and 93.4 % to *KMM 6258T*, 94.7 % to *A. brevivitae* SMK-19\(^T\) and 93.4 % to *A. brevivitae* SMK-19\(^T\).

However, the low levels of sequence similarity to the type strains of recognized species of the genus *Aquimarina* implied that strain XH134\(^T\) may represent a novel species (Stackebrandt & Goebel, 1994). Moreover, a number of phenotypic characteristics (Table 1), namely cell size, differences in production of hydrolytic enzymes, utilization of carbon sources and susceptibility to antibiotics, as well as the fatty acid (Table 2) and polar lipid compositions, clearly differentiated the new isolate from recognized species of the genus *Aquimarina*.

On the basis of phenotypic characteristics and phylogenetic inference, strain XH134\(^T\) is assigned to the genus *Aquimarina* as a representative of a novel species, for which the name *Aquimarina megaterium* sp. nov. is proposed.

**Description of *Aquimarina megaterium* sp. nov.**

*Aquimarina megaterium* (me.ga.te'i.um. Gr. adj. megalos large; Gr. n. therion, monster, beast; N.L. neut. n. megaterium big beast).

Cells are Gram-stain-negative, strictly aerobic, long rods, 0.5 μm in width and 5.4–77.8 μm in length, non-flagellated and motile by gliding. Colonies on MA are orange, opaque, convex, irregular with non-entire margin and 1.0–1.5 mm in diameter after 2–3 days at 28 °C. Growth occurs at 8–37 °C (optimum 28–30 °C). NaCl alone does not support growth; requires seawater or artificial seawater for growth. Growth occurs in MB with 2–4 % (w/v) NaCl. The pH range for growth is pH 6–8. Produces pigment with maximum absorption at 473 nm. Flexirubin-type pigments are formed. Oxidase and catalase are positive. Acetoin, H\(_2\)S and indole are not produced. Nitrate is reduced to nitrite. Chitin, gelatin, DNA, starch and Treen 20, 40 and 80 are hydrolysed, but not ascinul, agar, casein or urea. Acid is produced from fucose, starch and glycogen, but not from other compounds in the API 50CH system. According to carbon source experiments, citrate, leucine and lysine can be utilized, but D-mannose, fructose, x-lactose, glucose, raffinose, rhamnose, sucrose, xylose and ornithine cannot be utilized. In the API ZYM system, alkaline phosphatase, acid phosphatase, leucine arylamidase and valine arylamidase activities are present, esterase (C4), esterase lipase (C8), cystine arylamidase, trypsin, \(\beta\)-chymotrypsin and naphthol-AS-BI-phosphohydrolase activities are weakly present, but lipase (C14), \(\beta\)-galactosidase, \(\beta\)-glucuronidase, \(\alpha\)-glucosidase, \(\beta\)-glucosidase, N-acetyl-\(\beta\)-glucosaminidase, \(\alpha\)-mannosidase and \(\alpha\)-fucosidase activities are absent. Susceptible to (mg per disc unless otherwise stated) ciprofloxacin (5), erythromycin (15), vancomycin (30), norfloxacin (10), furazolidone (300), clindamycin (2), chloramphenicol (30) and rifampicin (5), and intermediately susceptible to piperacillin (100) and rocephin. Resistant to benzylpenicillin (10 IU), ampicillin (10), carbenicillin (100), cephalosporin V (30), cefuroxime (30), polymyxin B (300 U), gentamicin (10), kanamycin (30), neomycin (30), tetracycline (30), minocycline (30),

However, the low levels of sequence similarity to the type strains of recognized species of the genus *Aquimarina* implied that strain XH134\(^T\) may represent a novel species (Stackebrandt & Goebel, 1994). Moreover, a number of phenotypic characteristics (Table 1), namely cell size, differences in production of hydrolytic enzymes, utilization of carbon sources and susceptibility to antibiotics, as well as the fatty acid (Table 2) and polar lipid compositions, clearly differentiated the new isolate from recognized species of the genus *Aquimarina*.

On the basis of phenotypic characteristics and phylogenetic inference, strain XH134\(^T\) is assigned to the genus *Aquimarina* as a representative of a novel species, for which the name *Aquimarina megaterium* sp. nov. is proposed.

**Table 2.** Cellular fatty acid compositions of strain XH134\(^T\) and other members of the genus *Aquimarina*

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>iso-C(_{13:0})</td>
<td>1.1</td>
<td>5.6</td>
<td>1.6</td>
<td>TR</td>
<td>TR</td>
<td>3.8</td>
</tr>
<tr>
<td>C(_{13:1}) at 12-13</td>
<td>TR</td>
<td>3.4</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
</tr>
<tr>
<td>C(_{14:0})</td>
<td>1.0</td>
<td>1.5</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
</tr>
<tr>
<td>C(_{15:0}) 2-OH</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>1.3</td>
<td>1.5</td>
<td>TR</td>
</tr>
<tr>
<td>iso-C(_{15:0})</td>
<td>33.5</td>
<td>20.4</td>
<td>26.7</td>
<td>22.5</td>
<td>24.2</td>
<td>34.8</td>
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<tr>
<td>iso-C(_{15:1}) G</td>
<td>12.6</td>
<td>6.6</td>
<td>8.3</td>
<td>11.8</td>
<td>9.1</td>
<td>10.5</td>
</tr>
<tr>
<td>C(_{15:1O6c})</td>
<td>–</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
<td>TR</td>
</tr>
<tr>
<td>iso-C(_{15:0}) 3-OH</td>
<td>7.3</td>
<td>5.7</td>
<td>10.0</td>
<td>5.4</td>
<td>4.8</td>
<td>7.5</td>
</tr>
<tr>
<td>C(_{16:0})</td>
<td>5.4</td>
<td>4.8</td>
<td>1.1</td>
<td>2.4</td>
<td>1.8</td>
<td>1.4</td>
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<tr>
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<td>7.6</td>
<td>–</td>
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<td>2.1</td>
<td>3.3</td>
</tr>
<tr>
<td>anteiso-C(_{16:0})</td>
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<td>–</td>
<td>–</td>
<td>2.6</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>iso-C(_{16:1}) H</td>
<td>–</td>
<td>3.2</td>
<td>TR</td>
<td>–</td>
<td>1.6</td>
<td>1.7</td>
</tr>
<tr>
<td>iso-C(_{16:1}) G</td>
<td>1.1</td>
<td>–</td>
<td>3.1</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>C(_{16:0}) 3-OH</td>
<td>1.4</td>
<td>1.7</td>
<td>–</td>
<td>2.2</td>
<td>1.1</td>
<td>TR</td>
</tr>
<tr>
<td>iso-C(_{17:06c}) 3-OH</td>
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<td>TR</td>
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<td>1.1</td>
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<tr>
<td>C(_{17:06c})</td>
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<td>TR</td>
<td>2.9</td>
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<td>iso-C(_{17:06c}) 3-OH</td>
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<td>18.9</td>
<td>12.0</td>
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<tr>
<td>C(_{20:09c})</td>
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<td>TR</td>
<td>TR</td>
<td>2.0</td>
<td>TR</td>
<td>TR</td>
</tr>
<tr>
<td>iso-C(<em>{17:06c}) and/or C(</em>{16:1O7c})</td>
<td>11.7</td>
<td>15.1</td>
<td>11.6</td>
<td>9.0</td>
<td>10.6</td>
<td>4.3</td>
</tr>
<tr>
<td>10-Methyl C(<em>{16:0}) and/or C(</em>{16:1O7c})</td>
<td>7.0</td>
<td>7.5</td>
<td>13.9</td>
<td>6.3</td>
<td>12.3</td>
<td>9.8</td>
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</table>

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The major respiratory quinone is menaquinone 6 (MK-6). The dominant cellular fatty acids are iso-C15 : 0, iso-C15 : 1 \(\omega_{6c}\) and/or \(\omega_{7c}\), iso-C17 : 0 \(3\)-OH, iso-C15 : 0 \(3\)-OH and 10-methyl C16 : 0 and/or iso-C17 : 1 \(\omega_{9c}\). The polar lipid profile comprises phosphatidylethanolamine, one unknown aminolipid and three unknown polar lipids.

The type strain, XH134 \(^{\mathrm{T}}\) (=CGMCC 1.12186 \(^{\mathrm{T}}\)=JCM 18215 \(^{\mathrm{T}}\)), was isolated from surface seawater of the South Pacific Gyre (45° 58′ S 163° 11′ W). The DNA G+C content of the type strain is 32.4 mol%.

### Acknowledgements

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### References


Aquimarina megaterium sp. nov.


