Aquimarina mytili sp. nov., isolated from the gut microflora of a mussel, Mytilus coruscus, and emended description of Aquimarina macrocephali

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An orange, rod-shaped, gliding bacterium, designated strain PSC33T, was isolated from the gut microflora of a mussel collected from Gwangyang Bay, South Sea (Republic of Korea). Cells were Gram-reaction-negative, strictly aerobic, and catalase- and oxidase-positive. The major fatty acids were iso-C₁₅:0, iso-C₁₇:0 3-OH, iso-C₁₆:1 G, C₁₅:0 3-OH and iso-C₁₇:1α9c. The only isoprenoid quinone was menaquinone-6 (MK-6). The DNA G+C content of strain PSC33T was 37.9 mol%. A phylogenetic tree based on 16S rRNA gene sequences showed that strain PSC33T formed an evolutionary lineage within the radiation encompassing members of the genus Aquimarina with Aquimarina macrocephali JAMB N27T as its nearest neighbour (96.3% sequence similarity). A number of phenotypic characteristics distinguished strain PSC33T from recognized members of the genus Aquimarina. On the basis of the data presented in this study, strain PSC33T is considered to represent a novel species of the genus Aquimarina, for which the name Aquimarina mytili sp. nov. is proposed. The type strain is PSC33T (=KCTC 23302T=JCM 17454T). An emended description of A. macrocephali is also provided.

The genus Aquimarina belongs to the family Flavobacteriaceae (phylum ‘Bacteroidetes’) and, at the time of writing, comprised seven recognized species: Aquimarina muelleri (type species), A. latercula, A. brevivitae, A. intermedi (Nedashkovskaya et al., 2005, 2006), A. macrocephali (Miyazaki et al., 2010), A. spongiae (Yoon et al., 2011) and A. addita (Yi & Chun, 2011), all isolated from marine environments such as a marine aquarium, marine invertebrates, marine sediment and seawater. Aquimarina species are Gram-reaction-negative, strictly aerobic, chemo-organotrophic and pigmented bacteria. Here, we report the description of an Aquimarina-like strain, and show that this represents a novel species of the genus.

Strain PSC33T was isolated from a sample of gut content obtained from a mussel (Mytilus coruscus) collected from Gwangyang Bay in the South Sea (34° 53’ N 127° 46’ E), Republic of Korea, in February 2009. Isolation was achieved by using the standard dilution plating technique on marine agar (MA; Becton Dickinson) (Yang et al., 2006) incubated at 25°C for 2 weeks. The isolate was routinely cultured on MA and preserved at −80°C as a suspension in marine broth (MB; Becton Dickinson) containing glycerol (20%, w/v). The following were used as reference strains for all phenotypic tests: A. brevivitae KCTC 12390T, A. intermedi JCM 13506T, A. latercula JCM 8515T, A. macrocephali JCM 15542T, A. muelleri KCTC 12285T and A. spongiae A6T.

Bacterial DNA preparation, and PCR amplification and sequencing of the 16S rRNA gene were carried out as described by Chun & Goodfellow (1995). Identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarities were achieved by using the EzTaxon server (http://www.eztaxon.org/; Chun et al., 2007). Related sequences and the novel sequence were aligned by using CLUSTAL X (Thompson et al., 1997), and the alignment was refined by using PHYLIP version 3.1 (http://plaza.snu.ac.kr/~jchun/phydit/). Phylogenetic analysis was performed by using the computer packages PHYLIP (Felsenstein, 1993) and PAUP* 4.0 (Swofford, 1998). Phylogenetic trees were inferred by using the neighbour-joining (Saitou & Nei, 1987), Fitch–Margoliash (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1993) and maximum-parsimony (Fitch, 1971) algorithms. A distance matrix of the neighbour-joining dataset was generated according to the model of Jukes & Cantor (1969). The robustness of the topology in the neighbour-joining phylogenetic tree was evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

Preliminary comparisons with 16S rRNA gene sequences held in GenBank indicated that the novel isolate was closely related to members of the genus Aquimarina. The newly
determined sequence (1429 nt) was then aligned manually against representatives of the genus *Aquimarina*. Strain PSC33T showed highest 16S rRNA gene sequence similarity to *A. macrocephali* JAMB N27T (96.3%) and the type strains of other members of the genus *Aquimarina* (94.4–95.8%). The neighbour-joining tree (Fig. 1) showed that strain PSC33T was grouped with members of the genus *Aquimarina* and formed a distinct phyletic line. The topology of the other phylogenetic trees was essentially the same (data not shown). On the basis of 16S rRNA gene sequence similarity data and phylogenetic inference, it is clear that the isolate represents a novel genomic species within the genus *Aquimarina* (Wayne *et al.*, 1987).

As levels of 16S rRNA gene sequence similarity between strain PSC33T and the type strains of recognized *Aquimarina* species were below the level indicative of relatedness at the species level (97%; Tindall *et al.*, 2010), no DNA–DNA hybridization experiments were needed to define novel species status.

Growth was tested on nutrient agar (NA; Becton Dickinson), trypticase soy agar (TSA; Becton Dickinson), R2A agar (Becton Dickinson) and glucose-yeast extract agar (GYEA, containing per litre distilled water: 10 g yeast extract, 10 g glucose and 15 g agar). Cell morphology was observed by phase-contrast microscopy (TMS-F; Nikon) and scanning electron microscopy (S-4800; Hitachi) of cells of exponentially growing cultures. Gliding motility was examined by observing cells grown in wet mounts by using phase-contrast microscopy (DS-Fi1; Nikon). Cells of strain PSC33T and the six reference strains grown on MA at 25 °C for 3 days were used for physiological and biochemical tests. Growth was assessed in MB at 4, 10–50 (at 5 °C intervals), 37 and 42 °C. Growth was assessed in MB adjusted to pH 4.0–10.0 (at 1 pH unit intervals) by using 100 mM acetate buffer (pH 4–5), 100 mM NaH2PO4/Na2HPO4 buffer (pH 6–8) and 100 mM NaHCO3/Na2CO3 buffer (pH 9–10) (Yumoto *et al.*, 2004). The requirement for and tolerance to NaCl were determined in NaCl-free artificial seawater medium (ASW, containing per litre distilled water: 5.9 g MgCl2.6H2O, 3.24 g MgSO4.7H2O, 1.8 g CaCl2.2H2O, 0.55 g KCl, 0.16 g NaHCO3, 0.08 g KBr, 0.034 g SrCl2.6H2O, 0.022 g H2BO3, 0.008 g Na2HPO4, 0.004 g Na2SiO3, 0.0024 g NaF and 0.0016 g NH4NO3) supplemented with 5.0 g peptone, 1.0 g yeast extract and various concentrations of NaCl (Sigma) (final concentration 0–10%, using increments of 1%) (Yang & Cho, 2008). Anaerobic growth was tested on MA in a jar containing an AnaeroPak (Mitsubishi Gas Products Co., Ltd., Japan).

![Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain PSC33T, the type strains of recognized *Aquimarina* species and representatives of other genera in the family *Flavobacteriaceae*. Evolutionary distances, generated using the model of Jukes & Cantor (1969), are based on 1112 unambiguously aligned nucleotides. Numbers at nodes are bootstrap values (percentages of 1000 resamplings); only values ≥ 70% are shown. *Helicobacter pylori* ATCC 43504T (U01330) was used as an outgroup (not shown). Filled circles indicate that the corresponding nodes were also recovered in trees generated with the Fitch–Margoliash, maximum-parsimony and maximum-likelihood algorithms. Bar, 0.01 substitutions per nucleotide position.](http://ijs.sgmjournals.org)
Chemical), which works as an oxygen absorber and CO₂ generator, for up to 14 days at 25°C. Acid production from sugars was tested at 25°C by using phenol red broth base (Becton Dickinson) with a modification that 2% NaCl solution was used instead of distilled water. The presence of flexirubin-type pigments was determined by the bathochromic shift test by using 20% (w/v) KOH solution (Bernardet et al., 2002; McCammon & Bowman, 2000). The presence of catalase activity was revealed by the production of oxygen bubbles by the cells when mixed with 3% (v/v) H₂O₂ on a glass slide. The presence of oxidase activity was determined by oxidation of a 1% (w/v) N₅N₅N₇N₇-tetramethyl-p-phenylenediamine solution. Indole production was determined with Kovacs’ indole reagent on SIM agar (Becton Dickinson) supplemented with 2% NaCl. H₂S production was determined on Kligler iron agar (Becton Dickinson) supplemented with 2% NaCl. Degradation of the following macromolecules was tested by using MA as the basal medium and incubation at 25°C for 10 days (all w/v; all Sigma): carboxymethylcellulose (CM-cellulose) (1%), casein (2% skimmed milk), starch (0.2%), Tween 20 (1%) and Tween 80 (1%). Degradation was revealed by formation of clear zones around the colonies either directly or after flooding with adequate staining solutions (Smibert & Krieg, 1994). Hydrolysis of L-tyrosine (0.5%, w/v; Sigma) and xylan (1%, w/v; Sigma) was tested by using MA as the basal medium (Barrow & Feltham, 1993). Hydrolysis of gelatin (1%, w/v; Sigma) and urea (2%, w/v; Sigma) was determined as described by Lányi (1987) with a modification that 2% NaCl solution was used instead of distilled water. DNase activity was determined with DNase test agar (Becton Dickinson) supplemented with 2% NaCl. Other biochemical tests and enzyme activity tests were performed by using the API 20NE and API ZYM kits (bioMérieux) prepared for cellular fatty acid analysis, strain PSC33T and the six reference strains except for the presence of C₁₅ : 0 3- OH and the absence of iso-C₁₅ : 0 3- OH (Table 2). The only isoprenoid quinone of strain PSC33T was menaquinone-6 (MK-6; 100%); MK-6 is the only or major respiratory quinone in all Aquimarina species and all members of the family Flavobacteriaceae. The polar lipid compositions of strain PSC33T and A. macrocephali JCM 15542T were very similar, with an unknown amino lipid (AL1) and two unknown polar lipids (L1–L2) as the major components (Fig. 2). Strain PSC33T differed from A. macrocephali JCM 15542T by a large amount of phosphatidylethanolamine and the presence of two additional unidentified aminolipids (Fig. S2). The DNA G+C content of strain PSC33T was 37.9±0.3 mol% (mean ± SD of 3 determinations), a value in the range of recognized Aquimarina species (Table 1).

Phylogenetic inference and phenotypic data clearly indicate that strain PSC33T represents a novel species of the genus Aquimarina, for which the name Aquimarina mytili sp. nov. is proposed. On the basis of new data obtained in this study, an emended description of A. macrocephali is also presented.

**Description of Aquimarina mytili sp. nov.**

Aquimarina mytili (my’i.li. L. n. mytilus -i a kind of mussel, sea mussel, an edible mussel, and also a scientific genus name; L. gen. n. mytili of a sea mussel, Mytilus, reflecting isolation of the type strain from Mytilus coruscus).

Cells are Gram-reaction-negative, strictly aerobic rods, motile by gliding, approximately 0.3–0.4 μm in diameter and 1.2–3.0 μm in length. Colonies on MA are circular with entire margins, convex, smooth, opaque, orange-pigmented and approximately 1.0–2.0 mm in diameter after 3 days at 25°C (pH 7). Growth occurs on MA, but not on GYEa, NA, R2A agar or TSA. Growth occurs with 1–4% (w/v) NaCl (optimum, 2%), at pH 7–9 and at 15–37°C (optimum, 25–30°C). Oxidase- and catalase-positive. Flexirubin-type pigments are absent (KOH-test-negative). Nitrate is reduced. Arginine dihydrolase activity is absent.
H₂S and indole are not produced. Acid is not produced from D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannitol, L-rhamnose, sucrose or D-xylose. Hydrolyses aesculin, casein, gelatin, starch and Tween 80, but not agar, CM-cellulose, DNA, Tween 20, L-tyrosine, urea or xylan. In API ZYM strips, positive for N-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, esterase (C4), leucine arylamidase, and naphthol-AS-BI-phosphohydrolase and valine arylamidase activities, but negative for α-chymotrypsin, cystine arylamidase, esterase lipase (C8), α-fucosidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucuronidase, lipase (C14), α-mannosidase and trypsin activities. Cells are sensitive to (µg per disc, unless otherwise indicated) amikacin (30), gentamicin (10), kanamycin (30), nalidixic acid (30), polymyxin B (300 IU), streptomycin (10) and tetracycline (30), but resistant to ampicillin (10), chloramphenicol (30), erythromycin (15), penicillin (10 IU) and vancomycin (30). The major fatty acids (>10% of the total) are iso-C₁₅:₀, iso-C₁₇:₀ 3-OH and iso-C₁₅:₁ G. The complete fatty acid
Table 2. Fatty acid compositions (%) of strain PSC33\textsuperscript{T} and the type strains of recognized Aquimarina species

<table>
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<th>Fatty acid</th>
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<td>Tr</td>
<td>Tr</td>
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<td>3.8</td>
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<td>1.0</td>
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<td>3.9</td>
<td>10.1</td>
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<td>C\textsubscript{18}:0</td>
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<td>Tr</td>
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<td>6.7</td>
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<td>–</td>
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<td>Tr</td>
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<td>–</td>
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<tr>
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<td>2.5</td>
<td>1.6</td>
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<tr>
<td>C\textsubscript{15}:0 3-OH</td>
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<td>–</td>
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<td>–</td>
<td>–</td>
<td>Tr</td>
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<td>1.1</td>
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<td>8.9</td>
<td>8.2</td>
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<td>Tr</td>
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<td>–</td>
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</table>

*a Summed feature 3 comprises C\textsubscript{16}:1\textsuperscript{a} and/or iso-C\textsubscript{15}:0 2-OH.
†ECL, Equivalent chain-length.

Emended description of \textit{Aquimarina macrocephali} Miyazaki et al. 2010

The description is as given by Miyazaki et al. (2010) with the following addition. Polar lipids are phosphatidylethanolamine, an unknown aminolipid and four unknown polar lipids.

Acknowledgements

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References


Aquimarina mytili sp. nov.


