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Aquimarina intermedia sp. nov., reclassification of *Stanierella latercula* (Lewin 1969) as *Aquimarina latercula* comb. nov. and *Gaetbulimicrobium brevivitae* Yoon *et al.* 2006 as *Aquimarina brevivitae* comb. nov. and emended description of the genus *Aquimarina*

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A heterotrophic, aerobic, Gram-negative, pigmented and gliding bacterium, strain KMM 6258^T, was isolated from the sea urchin *Strongylocentrotus intermedius* and investigated using a polyphasic taxonomic approach. 16S rRNA gene sequence analysis revealed that the closest relatives of the novel strain are *Aquimarina muelleri*, *Stanierella latercula* and *Gaetbulimicrobium brevivitae*, members of the family *Flavobacteriaceae*, with sequence similarities of 96·3, 96·4 and 96·2 %, respectively. Phylogenetic evidence, supported by chemotaxonomic and phenotypic data, assigned strain KMM 6258^T to the genus *Aquimarina* as *Aquimarina intermedia* sp. nov. (type strain KMM 6258^T=DSM 17527^T=JCM 13506^T=LMG 23204^T). The reclassification of *Stanierella latercula* as *Aquimarina latercula* comb. nov. and *Gaetbulimicrobium brevivitae* as *Aquimarina brevivitae* comb. nov. is proposed.

The genus *Aquimarina*, a member of the family *Flavobacteriaceae* (Bernardet *et al.*, 2002), accommodates heterotrophic, Gram-negative, aerobic, dark-yellow or brownish-coloured, gliding flavobacteria producing flexirubin-type pigments that have been isolated from seawater samples (Nedashkovskaya *et al.*, 2005). Sufficient phylogenetic divergence, as well as a number of phenotypic differences and distinctive fatty acid contents, allowed the separation of the representatives of the single species of the genus, *Aquimarina muelleri*, from their nearest neighbour, [*Cytophaga*] *latercula*. The latter species was reclassified as *Stanierella latercula* in the same study.

In the course of a study of cultured bacteria isolated from the sea urchin *Strongylocentrotus intermedius*, we found a novel reddish-coloured bacterium, strain KMM 6258^T. Phylogenetic analysis of the 16S rRNA gene sequence of strain KMM 6258^T revealed that the closest relatives of the novel isolate were members of the genera *Aquimarina*, *Stanierella* and *Gaetbulimicrobium*. The novel strain occupied an intermediate position between the three genera. Based on the results of phylogenetic, phenotypic and fatty acid analyses, we propose that strain KMM 6258^T be placed in the genus *Aquimarina* as a separate species and that the nearest neighbours, *Stanierella latercula* and *Gaetbulimicrobium brevivitae*, are reclassified as members of the genus *Aquimarina*.

Strain KMM 6258^T was isolated from the sea urchin *Strongylocentrotus intermedius* collected in Troitsa Bay, Gulf of Peter the Great, Sea of Japan. To isolate the strain, 0·1 ml tissue homogenate was transferred onto plates of marine agar 2216 (Difco). After primary isolation and purification, the novel strain was cultivated at 28 °C on the same medium and stored at −80 °C in marine broth 2216 (Difco) supplemented with 20 % (v/v) glycerol.

The almost-complete 16S rRNA gene sequence of strain KMM 6258^T was determined following a previously described procedure (Vancanneyt *et al.*, 2004). This sequence (a continuous stretch of 1476 bp) was aligned with sequences retrieved from EMBL and a phylogenetic tree was constructed by the neighbour-joining method using the BioNumerics software package, version 4.0 (Applied

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Aquimarina intermedia* KMM 6258^T is AM113977.

Maths). Unknown bases were discarded for analyses. Bootstrapping analysis was undertaken to test the statistical reliability of the topology of the neighbour-joining tree using 500 bootstrap resamplings of the data (Fig. 1). The tree topology obtained with the neighbour-joining method was evaluated and confirmed by maximum-parsimony analysis using BioNumerics (data not shown). Strain KMM 6258^T showed 16S rRNA gene sequence similarities of 99·7, 96·4, 96·3 and 96·2 % with its nearest neighbours, [*Flexibacter*] *tractuosus* IFO 15980, *S. latercula* ATCC 23177^T, *A. muelleri* KMM 6020^T and *G. brevivitae* SMK-19^T, respectively (Fig. 1). These observations support the reclassification of *S. latercula* and *G. brevivitae* in the genus *Aquimarina*.

DNA was isolated according to the method of Marmur (1961) and G+C content was determined by using the thermal denaturation method (Marmur & Doty, 1962). The G+C content of the DNA of strain KMM 6258^T was 37·1 mol%.

Analysis of fatty acid methyl esters of strain KMM 6258^T and of the type strains of *A. muelleri* and *S. latercula* grown on marine agar at 28 °C for 24 h was carried out according to the standard protocol of the Sherlock Microbial Identification System (Microbial ID). The major cellular fatty acids for strain KMM 6258^T were: iso-$C_{17:0}$ 3-OH (32·6 %), iso-$C_{15:0}$ (25·9 %), iso-$C_{17:1}\omega 9c$ (12·7 %), iso-$C_{15:1}$ (7·8 %), iso-$C_{15:0}$ 3-OH (7·0 %) and summed feature 3 (5·0 %), comprising $C_{16:1}\omega 7$ and/or iso-$C_{15:0}$ 2-OH (Table 1).

The absorption spectrum of pigments extracted using acetone/methanol 7 : 2 (v/v) was determined at between 300 and 700 nm with a UV spectrophotometer (CE 7250, 7000; CECIL series). Cells of strain KMM 6258^T produced reddish-coloured pigments with maximum absorption at 469·8 nm (Table 2).

Phenotypic analysis was performed using methods described previously (Nedashkovskaya *et al.*, 2003a, b). In order to determine susceptibility to antibiotics, the novel strain was grown on marine agar at 28 °C for 48 h. In addition to the antibiotics tested in previous studies, the susceptibility of the strain to chloramphenicol (30 µg), doxycycline (10 µg) and erythromycin (15 µg) was also determined.

Table 1. Fatty acid content of species of the genus *Aquimarina*

Taxa: 1, *Aquimarina intermedia* sp. nov. KMM 6258^T; 2, *Aquimarina* (*Gaetbulimicrobium*) *brevivitae* SMK-19^T; 3, *Aquimarina* (*Stanierella*) *latercula* LMG 1343^T; 4, *Aquimarina muelleri* LMG 22569^T. Data from Yoon *et al.* (2006) and this study. Values are given as mean percentages of the total. Fatty acids amounting to <1 % in all taxa are not shown. NP, Not present; tr, <1 %.

Fatty acid	1	2	3	4
iso-$C_{13:0}$	tr	NP	tr	1·5
iso-$C_{14:0}$	NP	1·3	NP	NP
$C_{15:0}$	1·4	4·5	5·3	1·0
$C_{15:1}\omega 6$	tr	NP	1·6	NP
$C_{15:0}$ 2-OH	NP	NP	1·1	NP
iso-$C_{15:0}$	25·9	20·2	18·4	22·2
iso-$C_{15:1}$	7·8	5·2	7·3	5·2
iso-$C_{15:0}$ 3-OH	7·0	6·9	7·0	9·7
$C_{16:0}$	NP	1·2	NP	NP
$C_{16:0}$ 3-OH	tr	NP	2·0	1·5
iso-$C_{16:0}$	tr	4·0	2·7	tr
iso-$C_{16:1}$H	tr	1·4	1·4	NP
iso-$C_{16:0}$ 3-OH	tr	2·9	2·5	tr
$C_{17:0}$ 3-OH	NP	1·0	1·1	NP
$C_{17:1}\omega 6c$	tr	1·4	1·6	NP
iso-$C_{17:1}\omega 9c$	12·7	3·7	4·7	9·9
iso-$C_{17:0}$ 3-OH	32·6	31·9	30·5	37·6
$C_{18:1}\omega 5c$	1·0	NP	NP	NP
Summed feature 3*	5·0	3·7	6·4	6·8

*Summed features consist of one or more fatty acids that could not be separated by the Microbial Identification System. Summed feature 3 comprises iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega 7$.

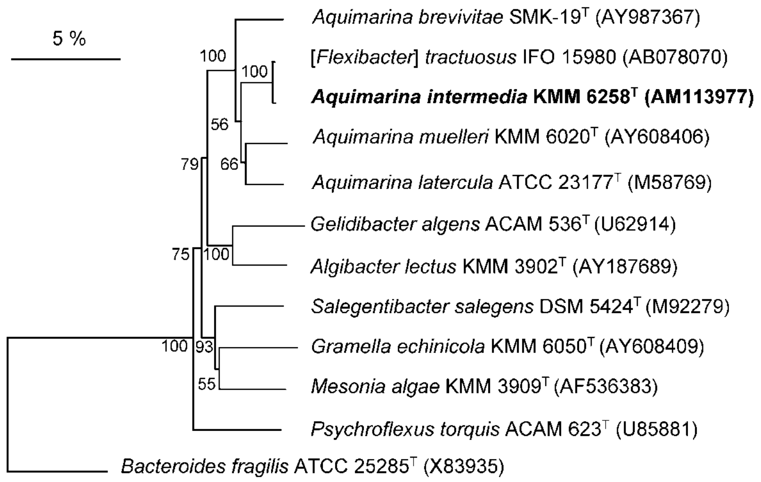

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences of *Aquimarina* species and of representative members of related genera in the family *Flavobacteriaceae*, generated using the neighbour-joining method (Saitou & Nei, 1987). Numbers at nodes indicate bootstrap values (expressed as percentages of 500 replications). Bar, 0·05 substitutions per nucleotide position.

The physiological, biochemical and morphological characteristics of strain KMM 6258^T are given in the species description and in Table 2. Phenotypic examination revealed many common traits between the novel strain and its closest relatives, *A. muelleri*, *S. latercula* and *G. brevivitae*. However, strain KMM 6258^T could be clearly differentiated from these species by its ability to utilize carbohydrates and by a higher DNA G+C content (Table 2). Strain KMM 6258^T could also be differentiated from *A. muelleri* by the absence of chitinase activity, the presence of β-galactosidase activity, hydrogen sulfide production and susceptibility to benzylpenicillin. Some features of strain KMM 6258^T, including catalase and amylase activities, the ability to grow in the presence of 10 % NaCl and the absence of agar and chitin hydrolysis, may be helpful for separating the novel strain from *S. latercula*. A combination of phenotypic properties, such as the ability to produce flexirubin-type pigments and hydrogen sulfide, to utilize L-arabinose, D-mannose and sucrose, a lower temperature range for growth and the inability to form acid from carbohydrates, distinguish strain KMM 6258^T from *G. brevivitae* (Table 2).

Table 2. Differential characteristics of species in the genus *Aquimarina*

Species: 1, *Aquimarina intermedia*; 2, *Aquimarina* (*Gaetbulimicrobium*) *brevivitae*; 3, *Aquimarina* (*Stanierella*) *latercula*; 4, *Aquimarina muelleri*. All strains were positive for the following: respiratory type of metabolism, oxidase and alkaline phosphatase activities, hydrolysis of casein, gelatin and Tweens 20, 40 and 80, susceptibility to ampicillin, chloramphenicol, lincomycin and oleandomycin and resistance to gentamicin, kanamycin, neomycin and polymyxin B. All strains were negative for the following tests: nitrate reduction; cellulose (carboxymethylcellulose and filter paper) and urea degradation and indole and acetoin production. Data from Reichenbach (1989), Nedashkovskaya *et al.* (2005), Yoon *et al.* (2006) and this study. +, Positive; −, negative; ND, not detected.

Characteristic	1	2	3	4
Gliding motility	+	+	−	+
Production of:				
Catalase	+	+	−	+
β-Galactosidase	+	−	+	−
Flexirubin-type pigments	+	−	+	+
Temperature range for growth (°C)	4–36	10–41	4–33	4–34
NaCl range for growth (%)	1–10	1–10	1–5	1–8
Acid formation from carbohydrates	−	+	−	−
Hydrolysis of:				
Agar	−	−	+	−
Starch	+	+	−	+
Chitin	−	ND	+	+
Utilization of:				
L-Arabinose, D-mannose, sucrose	+	−	−	−
D-Glucose	+	+	−	−
H_2S production	+	−	+	−
Susceptible to:				
Benzylpenicillin	+	+	+	−
Carbenicillin	+	+	−	+
Streptomycin	−	+	+	−
Tetracycline	+	−	−	+
Maximum absorption of pigments (nm)	469·8	474; 505	465·0	445·8
DNA G+C content (mol%)	37·1	36	34	31–33

Consequently, on the basis of the combination of significant molecular, genotypic and phenotypic similarities between strain KMM 6258^T and *A. muelleri*, we suggest that strain KMM 6258^T represents a novel species in the genus *Aquimarina*, for which the name *Aquimarina intermedia* sp. nov. is proposed.

Furthermore, the high level of 16S rRNA gene sequence similarity of *S. latercula* and *G. brevivitae* with members of the genus *Aquimarina* (96·2–96·4 %) supports the placement of these two species in the genus *Aquimarina* (Stackebrandt & Goebel, 1994). Taken together with the phylogenetic data, the similarity in fatty acid content of all the strains tested (Table 1) supports the transfer of *Stanierella latercula* and *Gaetbulimicrobium brevivitae* to the genus *Aquimarina* as *Aquimarina latercula* comb. nov. and *Aquimarina brevivitae* comb. nov., respectively. Since gliding motility and catalase production, both characteristics of other members of the genus *Aquimarina*, were not observed among cells of *S. latercula* in this and previous studies (Lewin, 1969; Reichenbach, 1989; Nedashkovskaya *et al.*, 2005), an emended description of the genus *Aquimarina* is proposed.

Emended description of the genus *Aquimarina*

Aquimarina (A.qui.ma.ri′na. L. fem. n. *aqua* water; L. fem. adj. *marina* marine; N.L. fem. n. *aquimarina* an organism of seawater).

Rod-shaped and strictly aerobic cells. Gram-negative. Do not form endospores. Produce non-diffusible carotenoid and/or flexirubin-type pigments. Cells of some species can move by means of gliding. Chemoorganotroph. Cytochrome oxidase- and alkaline phosphatase-positive. The predominant cellular fatty acids are branched-chain saturated and unsaturated and straight-chain unsaturated fatty acids iso-$C_{15:0}$, iso-$C_{15:1}$, iso-$C_{15:0}$ 3-OH, iso-$C_{17:1}\omega 9c$, iso-$C_{17:0}$ 3-OH and summed feature 3 (comprising $C_{16:1}\omega 7$ and/or iso-$C_{15:0}$ 2-OH). The main respiratory quinone is MK-6. 16S rRNA gene sequence analysis indicates that the genus *Aquimarina* is a member of the family *Flavobacteriaceae*, phylum *Bacteroidetes*. The type species is *Aquimarina muelleri*.

Description of *Aquimarina intermedia* sp. nov.

Aquimarina intermedia (in.ter.me′di.a. L. fem. adj. *intermedia* intermediate, referring to the level of 16S rRNA gene sequence similarity with its closest relatives).

The main characteristics are the same as those given for the genus. In addition, cells range from 0·4 to 0·5 μm in width and from 2·1 to 3·2 μm in length and are motile by means of gliding. On marine agar, colonies are 2–3 mm in diameter, circular, shiny with entire edges and reddish-pigmented. Growth is observed at 4–36 °C. Optimal temperature for growth is 25–28 °C. Growth occurs in 1–10 % NaCl, with an optimum of 2–5 % NaCl. β-Galactosidase activity is present. Flexirubin-type pigments are produced. Decomposes casein, gelatin, starch, DNA and Tweens 20, 40 and 80. Does not degrade agar, cellulose (carboxymethylcellulose and filter paper) or chitin. Does not form acid from L-arabinose, D-cellobiose, L-fucose, D-galactose, D-glucose, D-lactose, D-maltose, D-melibiose, L-raffinose, L-rhamnose, L-sorbose, sucrose, DL-xylose, *N*-acetylglucosamine, citrate, adonitol, dulcitol, glycerol, inositol or mannitol. Utilizes L-arabinose, D-glucose, D-mannose and sucrose, but not D-lactose, mannitol, inositol, sorbitol, malonate or citrate. Nitrate is not reduced. Hydrogen sulfide is produced. The results for indole and acetoin (Voges–Proskauer reaction) production are negative. Susceptible to ampicillin, benzylpenicillin, carbenicillin, chloramphenicol, doxycycline, erythromycin, lincomycin, oleandomycin and tetracycline. Resistant to gentamicin, kanamycin, neomycin, polymyxin B and streptomycin. The dominant fatty acids are iso-$C_{17:0}$ 3-OH (32·6 %), iso-$C_{15:0}$ (25·9 %), iso-$C_{17:1}\omega9c$ (12·7 %), iso-$C_{15:1}$ (7·8 %), iso-$C_{15:0}$ 3-OH (7·0 %) and summed feature 3 (5·0 %), comprising $C_{16:1}\omega7$ and/or iso-$C_{15:0}$ 2-OH. The G + C content of the DNA of the type strain is 37·1 mol%.

The type strain, strain KMM 6258^{T} (= DSM 17527^{T} = JCM 13506^{T} = LMG 23204^{T}), was isolated from the sea urchin *Strongylocentrotus intermedius* collected in Troitsa Bay, Gulf of Peter the Great, Sea of Japan.

Description of *Aquimarina latercula* (Lewin 1969) comb. nov.

Aquimarina latercula (la.ter′cu.la. L. masc. dim. n. *laterculus* a small brick; N.L. fem. adj. *latercula* brick-like, brick-red colour).

Basonym: *Cytophaga latercula* Lewin 1969 (Approved Lists 1980).

Synonym: *Stanierella latercula* (Lewin 1969) Nedashkovskaya *et al.* 2005.

The description is as given for the genus and by Nedashkovskaya *et al.* (2005), with the addition that the strain produces flexirubin-type pigments. Gliding motility and catalase activity are not observed. Does not utilize L-arabinose, D-glucose, D-lactose, D-mannose, sucrose, inositol, mannitol, sorbitol, malonate or citrate. The predominant fatty acids are iso-$C_{17:0}$ 3-OH (30·5 %), iso-$C_{15:0}$ (18·4 %), iso-$C_{15:1}$ (7·3 %), iso-$C_{15:0}$ 3-OH (7·0 %), summed feature 3 (6·4 %), comprising $C1_{6:1}\omega7$ and/or iso-$C_{15:0}$ 2-OH, $C_{15:0}$ (5·3 %) and iso-$C_{17:1}\omega9c$ (4·7 %), and The G + C content of the DNA is 34 mol%.

The type strain, LMG 1343^{T} (= ATCC 23177^{T} = NCIMB 1399^{T} = CIP 104806^{T}), was isolated from the outflow of a marine aquarium in La Jolla, California, USA.

Description of *Aquimarina brevivitae* (Yoon *et al.* 2006) comb. nov.

Aquimarina brevivitae (bre.vi.vi.ta′e. L. adj. *brevis* short; L. gen. n. *vitae* of life; N.L. gen. n. *brevivitae* of short life, referring to the short-lived cultures of the type strain).

Basonym: *Gaetbulimicrobium brevivitae* Yoon *et al.* 2006.

The description is as given for *Gaetbulimicrobium brevivitae* by Yoon *et al.* (2006).

The type strain is SMK-19^{T} (= DSM 17196^{T} = KCTC 12390^{T}).

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