Aureitalea marina gen. nov., sp. nov., a member of the family *Flavobacteriaceae*, isolated from seawater

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An irregular rod-shaped, non-gliding, yellow-pigmented bacterium was isolated from seawater from the western North Pacific Ocean near Japan. The strain, designated S1-66^T, was Gram-negative, obligately aerobic, heterotrophic and oxidase-positive. Growth occurred in the presence of 1–4 % NaCl, with optimum growth at 2 % NaCl. The strain grew at 15–30 °C, with optimum growth at 20–25 °C. The G+C content of genomic DNA was 48.1 mol% (HPLC). The predominant isoprenoid quinone was MK-6 and the major cellular fatty acids were iso-C_{15:0} (26.4 %), iso-C_{15:1} (20.3 %) and iso-C_{17:0} 3-OH (14.2 %). Phylogenetic trees generated by using 16S rRNA gene sequences revealed that strain S1-66^T belongs to the family *Flavobacteriaceae* and showed 94.2 % sequence similarity to the most closely related type strain, *Ulvibacter antarcticus* IMCC3101^T. On the basis of phenotypic and phylogenetic features, S1-66^T is classified in a novel genus and species within the family *Flavobacteriaceae*, for which the name *Aureitalea marina* gen. nov., sp. nov. is proposed. The type strain of *Aureitalea marina* is S1-66^T (=NBRC 107741^T =KCTC 23434^T).

The family *Flavobacteriaceae*, belonging to the phylum *Bacteroidetes* (the *Cytophaga–Flavobacterium–Bacteroides* group), was proposed by Reichenbach (1992) and its description was subsequently emended by Bernardet *et al.* (1996, 2002). Currently the family *Flavobacteriaceae* comprises more than 90 genera (<http://www.bacterio.cict.fr>). Most members of the family have been isolated from diverse marine environments, including the deep sea (Romanenko *et al.*, 2007), sediment (Khan *et al.*, 2008), Antarctic water (Macián *et al.*, 2002) and marine plants and animals (Nedashkovskaya *et al.*, 2005; Bae *et al.*, 2007).

An aerobic, yellow-pigmented bacterium, strain S1-66^T, was isolated from seawater samples from the western North Pacific Ocean (30° 11' N 145° 05' E; depth, 100 m), and found to belong to the family *Flavobacteriaceae*. A polyphasic taxonomic analysis of this isolate demonstrated that it represents a novel genus and species in the family.

Strain S1-66^T was isolated from seawater samples collected from the R/V *Mirai* [Japan Agency for Marine-Earth

Science and Technology (JAMSTEC)] on 9 February 2010 (cruise MR10-01). An aliquot of seawater (200 µl) was inoculated onto 1/10-strength ZoBell agar medium (0.5 g peptone, 0.1 g yeast extract, 15 g agar in 1 l 80 % aged natural seawater) and incubated at 15 °C for 30 days. The isolated strain was maintained on 1/2-strength marine agar 2216 (1/2 MA) (Difco) containing 2 % NaCl at 20 °C. Ranges of temperature (5, 10, 15, 20, 25, 30, 37 and 45 °C) and pH (pH 5–10) for growth were determined by incubating the isolate on 1/2 MA containing 2 % NaCl. The NaCl concentration for growth was determined on 1/2 MA (l⁻¹: 15 g agar, 4.4 g MgCl₂, 2.5 g peptone, 1.62 g Na₂SO₃, 0.9 g CaCl₂, 0.5 g yeast extract, 0.27 g KCl, 0.8 g NaHCO₃, 0.5 g ferric citrate, 0.04 g KBr, 0.015 g SrCl₂, 0.01 g H₃BO₃, 4 mg Na₂HPO₄, 2 mg Na₂SiO₃, 1.2 mg NaF, 0.8 mg NH₄NO₃) containing 0–15 % (w/v) NaCl. Gram-staining was performed as described by Murray *et al.* (1994). Cell morphology and motility were observed using light microscopy (BX60; Olympus). Growth under anaerobic conditions was determined after incubation for 4 weeks in an AnaeroPack (Mitsubishi Gas Chemical Co.) on 1/2 MA containing 2 % NaCl. Catalase activity was determined by bubble formation in a 3 % H₂O₂ solution. Oxidase activity was determined using cytochrome oxidase test paper (Nissui Pharmaceutical Co.).

Abbreviations: ML, maximum-likelihood; NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain S1-66^T is AB602429.

A supplementary figure is available with the online version of this paper.

API 20E, API 20NE, API 50CH and API ZYM strips (bioMérieux) were used to determine physiological and biochemical characteristics. Suspension media for the API test strips were supplemented with 2 % (w/v) NaCl (final concentration). API 20E, API 20NE, API 50CH and API ZYM test strips were read after incubation for 5 and 2 days at 20 °C. Determination of the respiratory quinone was carried out as described previously (Xie & Yokota, 2003). A culture grown on 1/2 MA containing 2 % NaCl for 3 weeks at 20 °C was used for the analysis of fatty acid methyl esters. Fatty acid methyl esters were extracted and prepared according to standard protocols provided by the MIDI/Hewlett Packard Microbial Identification system (Sasser, 1990) and then analysed by using the Sherlock 4 software. Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984) and identified by two-dimensional TLC followed by spraying with appropriate detection reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987). Phospholipids were detected with the Zinzadze reagent (Dittmer & Lester, 1964). Whole lipid profiles were detected by spraying with molybdato-phosphoric acid (10 g molybdato-phosphoric acid hydrate in 100 ml ethanol) followed by heating at 120–160 °C (Worliczek *et al.*, 2007). DNA was prepared according to the method of Marmur (1961) from cells grown on 1/2 MA containing 2 % NaCl and the DNA base composition was determined by using the HPLC method of Mesbah *et al.* (1989). A fragment of approximately 1450 bp from the 16S rRNA gene was amplified from the extracted DNA by using bacterial universal primers 27F and 1492R specific to the 16S rRNA gene (Lane, 1991; Weisburg *et al.*, 1991). To ascertain the phylogenetic position of the novel isolate, the 16S rRNA gene sequence of strain S1-66^T was compared with sequences obtained from GenBank (National Center for Biotechnology Information; <http://www.ncbi.nlm.nih.gov>). Multiple alignments of sequences were performed using CLUSTAL_X (version 1.83) (Thompson *et al.*, 1997). Alignment gaps and ambiguous bases were not taken into consideration when 1327 bases of the 16S rRNA gene were compared. Aligned sequences were analysed using MEGA 4 (Tamura *et al.*, 2007). Evolutionary distances [distance options according to Kimura's two-parameter model (Kimura, 1983)] and clustering with the neighbour-joining (NJ; Saitou & Nei, 1987) and maximum-likelihood (ML; Felsenstein, 1981)

methods using PhyML program version 2.4.4 (Guindon & Gascuel, 2003) were determined by using bootstrap values based on 1000 replications (Felsenstein, 1985).

An almost-complete 16S rRNA gene sequence for strain S1-66^T was determined and a FASTA search in GenBank showed that the strain belongs to the family *Flavobacteriaceae*. Species with validly published names exhibited <95 % sequence similarity to the determined sequence. Strain S1-66^T displayed the highest 16S rRNA gene sequence similarity to *Ulvibacter antarcticus* IMCC3101^T (94.2 % similarity), *Ulvibacter litoralis* KMM 3912^T (93.9 %), *Marixanthomonas ophiuræ* KMM 3046^T (92.6 %), *Vitellibacter vladivostokensis* KMM 3516^T (90.1 %), *Aequorivita antarctica* SW49^T (89.7 %) and *Croceibacter atlanticus* HTCC2559^T (87.8 %). Phylogenetic trees obtained by the NJ (Fig. 1) and ML methods revealed a clear affiliation of the novel isolate S1-66^T to the cluster of *U. antarcticus* IMCC3101^T, *U. litoralis* KMM 3912^T, *M. ophiuræ* KMM 3046^T, *V. vladivostokensis* KMM 3516^T, *A. antarctica* SW49^T and *C. atlanticus* HTCC2559^T. Strain S1-66^T formed a cluster with *U. antarcticus* IMCC3101^T and *U. litoralis* KMM 3912^T with bootstrap confidence values of 87 % (NJ) and 76 % (ML).

Cultural, physiological and biochemical characteristics of S1-66^T were compared with those of members of the related genera *Ulvibacter*, *Marixanthomonas*, *Vitellibacter*, *Aequorivita* and *Croceibacter*.

Cells of strain S1-66^T grown on 1/2 MA containing 2 % NaCl at 20 °C for 3 weeks were irregular rod-shaped, yellow-pigmented, approximately 0.5–1 µm wide and 0.5–6 µm long. Spores were not observed microscopically. The yellow pigment that accumulated in the cells was extracted with acetone after freeze-drying treatment of the cells and analysed using a Beckman DU 530 UV-Vis spectrophotometer. The pigment showed a UV-visible spectrum with absorption maxima at 343, 452 and 487 nm. Flexirubin-type pigments were not detected when investigated by using the bathochromic shift test with a 20 % (w/v) KOH solution (Fautz & Reichenbach, 1980; Bernardet *et al.*, 2002). No growth was observed under anaerobic conditions in 1/2 MA containing 2 % NaCl. Growth occurred only under aerobic conditions at 15–30 °C. The optimal temperature and pH were 20–25 °C and pH 7–8. Oxidase

Fig. 1. NJ phylogenetic tree based on 16S rRNA gene sequences showing the position of strain S1-66^T among currently known and related species of the family *Flavobacteriaceae*. Numbers at nodes are bootstrap percentages derived from 1000 replications (NJ/ML). The sequence of *Capnocytophaga ochracea* ATCC 27872^T was used as outgroup. Bar, 0.01 substitutions per nucleotide position.

Table 1. Differential characteristics of strain S1-66^T and members of related genera in the family *Flavobacteriaceae*

Taxa: 1, strain S1-66^T; 2, *Ulvibacter* (data from Nedashkovskaya *et al.*, 2004; Choi *et al.*, 2007); 3, *Marixanthomonas* (Romanenko *et al.*, 2007); 4, *Vitellibacter* (Nedashkovskaya *et al.*, 2003; Kim *et al.*, 2010); 5, *Aequorivita* (Bowman & Nichols, 2002; Park *et al.*, 2009); 6, *Croceibacter* (Cho & Giovannoni, 2003). +, Positive; v, variable; –, negative; ND, no data available.

Characteristic	1	2	3	4	5	6
Flexirubin-type pigment	–	+	–	+	–	–
Gliding motility	–	v	–	–	ND	–
Cell length (µm)	0.5–6	0.5–7.3	2.2–2.8	3–10	0.5–20	1.2–3.1
Temperature for growth (°C)						
Range	15–30	3–36	5–32	4–43	–2 to 40	10–28
Optimum	20–25	21–25	25–28	28–35	20–25	0.5–15
NaCl concentration for growth (% w/v)	1–4	1–6	1–12	1–6	1–8	0.5–15
Catalase	–	+	+	v	+	+
Oxidase	+	+	+	+	–	–
Nitrate reduction	+	v	–	–	–	–
Acid production from glucose	+	–	–	–	–	–
Hydrolysis of:						
Starch	+	v	–	–	v	+
Gelatin	+	+	+	+	+	+
Urea	–	–	+	–	v	ND
Major fatty acids*	i-C _{15:0} , i-C _{15:1} , i-C _{17:0} 3-OH, i-C _{15:0} 2-OH	i-C _{17:0} 3-OH, i-C _{15:0} , i-C _{16:0} , i-C _{16:0} 3-OH, i-C _{15:1}	i-C _{16:0} 3-OH, i-C _{17:0} 3-OH, ai-C _{17:0} 3-OH, C _{16:0} , C _{18:0}	i-C _{15:0} , i-C _{17:0} 3-OH	i-C _{15:1} ω10c, ai-C _{15:1} ω10c, ai-C _{15:0} , i-C _{15:0} , i-C _{16:1} ω6c, i-C _{17:1} ω5c, ai-C _{17:1} ω7c	i-C _{17:0} 3-OH, i-C _{15:0} , i-C _{15:1} , i-C _{17:1} ω9c
Presence of:†						
ai-C _{15:0} and i-C _{15:1}	+	+	–	+	+ / –	+
i-C _{16:1} and i-C _{17:1} ω9c	–	+	–	– / +	–	+
Polar lipids‡	PG, DPG, GLx	ND	SL, PE, lysoPE, APL	ND	ND	ND
DNA G + C content (mol%)	48.1	36.7–38.0	37.3	41.3–48.7	33.5–39.1	34.8

*ai, Anteiso-branched; i, iso-branched.

†When two results are given, they indicate the presence of the first/second fatty acid listed.

‡DPG, Diphosphatidylglycerol; PE, phosphatidylethanolamine; lysoPE, lysophosphatidylethanolamine; SL, sphingolipid; APL, aminophospholipid; GLx, unknown glycolipid.

tested positive but catalase tested negative. The DNA G + C content of strain S1-66^T was 48.1 mol%, which distinguished strain S1-66^T from the related genera *Ulvibacter*, *Marixanthomonas*, *Aequorivita* and *Croceibacter*. Strain S1-66^T required NaCl for growth and grew in 1–4 % NaCl (Table 1). The major cellular fatty acids were iso-C_{15:0} (26.4 %), iso-C_{15:1} (20.3 %), iso-C_{17:0} 3-OH (14.2 %), iso-C_{15:0} 2-OH (7.8 %) and iso-C_{16:0} (5.2 %) (Table 2).

The polar lipid composition of strain S1-66^T contained three major polar lipids, phosphatidylglycerol, diphosphatidylglycerol and an unidentified glycolipid (Fig. S1, available in IJSEM Online), while members of *Marixanthomonas* contained sphingolipid, phosphatidylethanolamine, lyso-phosphatidylethanolamine, aminophospholipid and an unknown lipid. Strain S1-66^T showed no catalase activity, gliding motility or flexirubin-type pigments, while members of the most closely related genus *Ulvibacter* tested positive for these characteristics. DNA G + C contents, acid production from glucose, nitrate reduction and the absence of iso-C_{16:1} and iso-C_{17:1}ω9c fatty acids distinguished strain S1-66^T from members of the genus *Ulvibacter*.

Phenotypic differences among strain S1-66^T and related species are shown in Tables 1 and 2. The data presented showed that strain S1-66^T is sufficiently different from related genera to justify its assignment to a novel genus and species, *Aureitalea marina* gen. nov., sp. nov., in the family *Flavobacteriaceae*.

Description of *Aureitalea* gen. nov.

Aureitalea (Au.re.i.ta'le.a. L. adj. *aureus* golden; L. fem. n. *talea* a rod; N.L. fem. n. *Aureitalea* golden rod).

Cells are yellow-pigmented. Flexirubin-type pigments are not produced. Irregular rod-shaped cells. Do not form endospores. Gram-stain-negative and obligately aerobic. The major respiratory quinone is MK-6. Predominant cellular fatty acids are iso-C_{15:0}, iso-C_{15:1} and iso-C_{17:0} 3-OH. The type species is *Aureitalea marina*.

Description of *Aureitalea marina* sp. nov.

Aureitalea marina (ma.ri'na. L. fem. adj. *marina* of or belonging to the sea, marine).

Exhibits the following properties in addition to those given in the genus description. Cells are 0.4–0.6 µm wide and 0.5–6 µm long. Non-gliding motility is observed. Temperature range for growth is 15–30 °C; optimal temperature 20–25 °C. No growth occurs above 30 °C. Grows at pH 6.0–9.0. NaCl is required for growth and can be tolerated at up to 4 % (w/v). Catalase-negative but oxidase-positive. Production of acetoin and reduction of nitrate to N₂ are observed. Gelatin and starch are hydrolysed but agar is not hydrolysed. Alkaline phosphatase, leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and β-glucosidase

Table 2. Cellular fatty acid compositions of strain S1-66^T and type strains of related species in the family *Flavobacteriaceae*

Strains: 1, strain S1-66^T; 2, *Ulvibacter antarcticus* IMCC3101^T (data from Choi *et al.*, 2007); 3, *Ulvibacter litoralis* KMM 3912^T (Nedashkovskaya *et al.*, 2004); 4, *Marixanthomonas ophiuræ* KMM 3046^T (Romanenko *et al.*, 2007); 5, *Vitellibacter vladivostokensis* KMM 3516^T (Kim *et al.*, 2010); 6, *Aequorivita antarctica* SW49^T (Bowman & Nichols, 2002); 7, *Croceibacter atlanticus* HTCC2559^T (Cho & Giovannoni, 2003). Values are percentages of total fatty acids; –, not detected/not reported.

Fatty acid	1	2	3	4	5	6	7
C _{15:0}	4.6	1.2	3.9	2.4	4.3	–	3.9
C _{16:0}	0.9	–	–	6.9	1.7	1.9	0.6
C _{18:0}	–	–	–	5.2	–	–	0.2
iso-C _{15:0}	26.4	15.3	21.3	2.9	32.4	7.6	13.3
anteiso-C _{15:0}	3.8	1.3	2.3	–	3.8	15.7	1.6
iso-C _{15:1}	20.3	3.9	12.5	–	2.4	–	9.2
iso-C _{15:1} ω10c	–	–	–	–	–	9.5	–
anteiso-C _{15:1} ω10c	–	–	–	–	–	16.0	–
iso-C _{16:0}	5.3	11.0	2.8	1.8	2.7	1.4	1.5
iso-C _{16:1}	–	5.4	4.2	–	–	–	1.7
iso-C _{15:0} 3-OH	3.3	4.1	3.3	–	3.5	5.4	4.3
iso-C _{16:0} 3-OH	2.9	9.2	8.7	41.2	1.4	9.2	4.4
iso-C _{17:0} 3-OH	14.2	19.4	17.2	16.4	23.2	2.0	28.0
anteiso-C _{17:0} 3-OH	–	–	–	5.1	–	2.9	–
iso-C _{17:1} ω9c	–	2.2	3.7	–	9.0	–	9.4
Summed feature 3*	7.8†	5.9	5.7	–	6.9	–	5.1

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI System. Summed feature 3 comprised C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

†Identified as iso-C_{15:0} 2-OH.

activities are present, but esterase (C4), esterase lipase (C8), lipase (C14), *N*-acetyl-β-glucosaminidase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucuronidase, α-mannosidase and α-fucosidase activities are absent. Acid is produced from glycerol, ribose, glucose, fructose, *N*-acetylglucosamine, aesculin, maltose, D-tagatose, starch, xylitol, sucrose and trehalose but not from galactose, melibiose, mannose, turanose, 5-ketogluconate, D- or L-arabinose, D- or L-xylose, methyl β-D-xylopyranoside, rhamnose, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, amygdalin, arbutin, salicin, cellobiose, lactose, melezitose, inulin, raffinose, gentiobiose, D- or L-fucose, erythritol, adonitol, sorbose, dulcitol, inositol, mannitol, sorbitol, glycogen, D- or L-arabitol, gluconate and 2-ketogluconate. Major cellular fatty acids include iso-C_{15:0} 2-OH and iso-C_{16:0}. The DNA G + C content of the type strain is 48.1 mol%. The type strain contains three major polar lipids, phosphatidylglycerol, diphosphatidylglycerol and an unidentified glycolipid.

The type strain, S1-66^T (=NBRC 107741^T =KCTC 23434^T), was isolated from seawater of the western North Pacific Ocean near Japan.

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