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SHORT COMMUNICATION

Flavobacterium ahnfeltiae sp. nov., a new marine polysaccharide-degrading bacterium isolated from a Pacific red alga

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Abstract A Gram-negative, aerobic, rod-shaped, motile by gliding and yellow-pigmented bacterium, designated strain 10Alg 130^T, that displayed the ability to destroy polysaccharides of red and brown algae, was isolated from the red alga *Ahnfeltia tobuchiensis*. The phylogenetic analysis based on 16S rRNA gene sequence placed the novel strain within the genus *Flavobacterium*, the type genus of the family *Flavobacteriaceae*, the phylum *Bacteroidetes*, with sequence similarities of 96.2 and 95.7 % to *Flavobacterium jumunjiense* KCTC 23618^T and *Flavobacterium ponti* CCUG 58402^T, and 95.3–92.5 % to other recognized *Flavobacterium* species. The prevalent fatty acids of strain 10Alg 130^T were iso-$C_{15:0}$, iso-$C_{15:0}$ 3-OH, iso-$C_{17:0}$ 3-OH, $C_{15:0}$ and iso-$C_{17:1}\omega9c$. The polar lipid profile consisted of phosphatidylethanolamine, two unknown aminolipids and three unknown lipids. The DNA G+C content of the type strain was 34.3 mol%. The new isolate and the type strains of recognized species of the genus *Flavobacterium* could strongly be distinguished by a number of phenotypic characteristics. A combination of the genotypic and phenotypic data showed that the algal isolate represents a novel species of the genus *Flavobacterium*, for which the name *Flavobacterium ahnfeltiae* sp. nov. is proposed. The type strain is 10Alg 130^T (=KCTC 32467^T = KMM 6686^T).

Keywords *Flavobacterium ahnfeltiae* · *Flavobacteriaceae* · Marine bacteria · Red alga *Ahnfeltia tobuchiensis*

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Introduction

Marine flavobacteria are widespread in various marine environments and often colonize the surface of the coastal red algae (Nylund et al. 2010; Fernandez et al. 2012; Miranda et al. 2013). They are characterized by the extraordinary species diversity and can play a sufficient role in the degradation of organic matter as other representatives of this taxonomic group. A comprehensive analysis of genomes of marine flavobacteria revealed the presence of numerous polysaccharide-degrading enzymes which can be involved in the degradation of the cell walls of the host organisms (Bauer et al. 2006; Mann et al. 2013). However, little is known about adaptation of members of the genus *Flavobacterium* to living on algal surfaces in the maritime environments using high molecular weight organic compounds. During the investigation of the taxonomic structure and metabolic potential of the microbial community of the red alga *Ahnfeltia tobuchiensis*, a yellow-pigmented

strain, designated 10Alg 130^T, showing polysaccharase activities, was isolated. Phylogenetic analysis based on the 16S rRNA gene sequencing and a comparative phenotypic analysis revealed that the algal isolate represents a novel species within the genus *Flavobacterium* that is the type genus of the extremely taxonomically diverse family *Flavobacteriaceae* of the phylum *Bacteroidetes* (Bernardet and Bowman 2011). At the time of writing, the genus *Flavobacterium* comprises 110 species with validly published names recovered from a wide range of habitats (http://www.bacterio.net). Among them, only 11 species were isolated from marine environments (Fu et al. 2011; Humphry et al. 2001; Joung et al. 2013; Kaur et al. 2012; McCammon and Bowman 2000; Miyashita et al. 2010; Nogi et al. 2005; Nupur et al. 2013; Song et al. 2013; Vela et al. 2007; Yoon et al. 2011). The majority of the species were free-living organisms, and only *Flavobacterium ceti* and *Flavobacterium algicola* were associated with the beaked whales and the unidentified algae, respectively (Vela et al. 2007; Miyashita et al. 2010). In this paper, we describe the isolation and detailed characterization of the first representative of the genus *Flavobacterium* associated with red alga *A. tobuchiensis* for which the name *Flavobacterium ahnfeltiae* sp. nov. is proposed.

Materials and methods

Strain isolation

Strain 10Alg 130^T was isolated from the red alga *A. tobuchiensis* collected near Island Paramushir, Kuril Isls, Okhotsk Sea, by a standard dilution plating method. The sample of algal fronds (5 g) was homogenized in 10 mL sterile seawater in a glass homogenizer, and 0.1 mL homogenate was spread onto marine agar 2216 (MA, Difco) plates. The novel isolate was obtained from a single colony after incubation of the plate at 28 °C for 7 days. After primary isolation and purification, the strain was cultivated at 28 °C on the same medium and stored at −80 °C in marine broth (Difco) supplemented with 20 % (v/v) glycerol.

Phylogenetic analysis

Genomic DNA extraction, PCR and sequencing of the 16S rRNA gene followed the procedures given in Bakunina et al. (2013). To establish the taxonomic position of strain 10Alg 130^T, 1435 nt of its 16S rRNA gene sequence was determined. Sequence alignments were performed using the SILVA (http://www.arb-silva.de/aligner) considering the secondary structure of rRNA gene (Pruesse et al. 2007). Evolutionary distances were calculated using the Kimura two-parameter model (Kimura 1983). Phylogenetic trees were reconstructed based on the maximum likelihood (Felsenstein 1981), neighbor-joining (Saitou and Nei 1987) and minimum evolution (Rzhetsky and Nei 1992) methods by using the MEGA5 program (Tamura et al. 2011). Bootstrapping analysis with 1,000 resampling was undertaken to test the statistical reliability of the topology of the neighbor-joining tree (Felsenstein 1985). Pairwise sequence similarities were calculated by EzTaxon-e (Kim et al. 2012).

Determination of DNA G+C content

The G+C content of genomic DNA was determined according to the method described by Gonzalez and Saiz-Jimenez (2002).

Whole cell fatty acid, polar lipid and lipoquinone composition

For whole-cell fatty acid and polar lipid analysis of strain 10Alg 130^T and *Flavobacterium dongtanense* KCTC 22671^T, *Flavobacterium jumunjiense* KCTC 23618^T and *Flavobacterium ponti* CCUG 58402^T were grown under optimal physiological conditions (at 28 °C for 48 h on MA). Cellular fatty acid methyl esters (FAMEs) were prepared according to the methods described by Sasser (1990) using the standard protocol of Sherlock Microbial Identification System (version 6.0, MIDI) and analyzed on a Shimadzu GC-21A gas chromatograph equipped with a flame ionization detector, using a fused silica capillary column (Supelcowax-10, 30 m × 0.25 mm, Supelco) at 210 °C. FAMEs were identified by using equivalent chain length values and comparing the retention times to those of authentic standards. Identification was confirmed by GC–MS (QP5050A; Shimadzu) fitted with a MDN-5S capillary column (30 m × 0.25 mm, Supelco); the temperature program was from 140 to 250 °C, at a rate of 2 °C min^{-1}. The polar lipids of strain 10Alg 130^T and the reference strains *F. dongtanense* KCTC 22671^T, *F. jumunjiense* KCTC 23618^T and *F. ponti* CCUG 58402^T were extracted using the chloroform/methanol (1:2, by vol). Two-dimensional TLC of polar lipids was carried out on silica gel 60 F254 (6 × 6 cm; Merck) using chloroform/methanol/water (65:25:4, by vol.) in the first dimension and chloroform/methanol/acetic acid/water (80:12:15:4, by vol.) in the second dimension (Collins and Shah 1984). The spray reagents used to reveal the spots were molybdenum reagent, ninhydrin and ceric ammonium molybdate (CAM). Isoprenoid quinones were extracted according to the method Komagata and Suzuki (1987) and analyzed by reversed-phase HPLC (Shimadzu LC-10A) with a Supelcosil LC-18 (150 × 4.6 mm) column. The column was

kept at 40 °C. Menaquinones were detected by monitoring at 270 nm and were identified by comparison with known quinones from reference strain *F. dongtanense* KCTC 22671^T.

Morphological, biochemical and physiological characterization

The physiological, morphological and biochemical properties of strain 10Alg 130^T and the reference strains *F. dongtanense* KCTC 22671^T, *F. jumunjiense* KCTC 23618^T and *F. ponti* CCUG 58402^T were studied using the standard methods. The novel isolate was also examined in the API 20E, API 20NE and API ZYM galleries (bioMérieux, France) according to the manufacturer's instructions, except that the galleries were incubated at 28 °C. Gram staining was performed as recommended by Gerhardt et al. (1994). Oxidative or fermentative utilization of glucose was determined on Hugh and Leifson's medium modified for marine bacteria (Lemos et al. 1985). Catalase activity was tested by addition of 3 % (v/v) H_2O_2 solution to a bacterial colony and observation for the appearance of gas. Oxidase activity was determined by using tetramethyl-*p*-phenylenediamine. Degradation of agar, starch, casein, gelatin, chitin, DNA and urea, growth at different pH values and production of acid from carbohydrates, hydrolysis of Tweens 20, 40 and 80, nitrate reduction, production of hydrogen sulfide, acetoin (Voges–Proskauer reaction) and indole, and presence of β-galactosidase and alkaline phosphatase activities were tested according to standard methods (Gerhardt et al. 1994). The temperature range for growth was assessed on MA. Tolerance to NaCl was assessed in medium containing 5 g bactopeptone (Difco), 2 g bactoyeast extract (Difco), 1 g glucose, 0.02 g KH_2PO_4 and 0.05 g $MgSO_4 \cdot 7H_2O$ per liter of distilled water with 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3, 4, 5, 6, 8 and 10 % (w/v) of NaCl. Carbon source utilization was tested (1) using commercial API 20E (bioMérieux) identification strip following the instructions of the manufacturer, and (2) using a medium that contained 0.2 g $NaNO_3$, 0.2 g NH_4Cl, 0.05 g yeast extract (Difco) and 0.4 % (w/v) carbon source per liter of artificial seawater. Susceptibility to antibiotics was examined by the routine disc diffusion plate method. Discs were impregnated with the following antibiotics: ampicillin (10 μg), benzylpenicillin (10 U), carbenicillin (100 μg), cefalexin (30 μg), cefazolin (30 μg), chloramphenicol (30 μg), erythromycin (15 μg), doxycycline (10 μg), gentamicin (10 μg), kanamycin (30 μg), lincomycin (15 μg), nalidixic acid (30 μg), neomycin (30 μg), ofloxacin (5 μg), oleandomycin (15 μg), oxacillin (10 μg), polymyxin B (300 U), rifampicin (5 μg), streptomycin (30 μg), tetracycline (5 μg) and vancomycin (30 μg).

Enzyme hydrolysis assay

For enzyme activities evaluation, bacteria were cultivated on shaker at 150 rev/min for 24 h at 25 °C in 250-cm^3 flasks, containing 50 mL of the following composition (g/L): bactopepton 5.0, yeast extract 2.0, glucose 1.0, KH_2PO_4 0.02 and MgSO4·7H_2O 0.05 in 50 % natural seawater. For bacterial extract preparation, the cells were separated from the culture medium by centrifugation at 3,000*g*. The bacterial biomass was frozen at −20 °C. The weighed portion of the frozen raw biomass was resuspended in 0.01 M Na^+ phosphate buffer solution, pH 7.3, on an ice bath up to concentration of 0.2 g/mL. Cells were homogenized by sonification at a frequency of 22 kHz and a current of 0.4 A five times for 20 s at intervals of 20 s. The cell suspension was incubated at 4 °C for 3 h; the homogenate was then centrifuged at 11,000*g* for 30 min. The pellet was discarded; the protein concentration in the extracts was determined according to the Bradford (1976) using bovine serum albumin (Sigma) as the standard. The polysaccharide hydrolase and lyase activities were measured in 0.05 M Na^+ phosphate buffer, pH 7.3, at 20 °C. The reaction mixture contained 0.05 mL of 0.2 g/mL bacterial cell extracts and 0.2 mL of 0.1 % corresponding substrate solution in 0.05 M Na^+ phosphate and 0.1 M NaCl, pH 7.3. The reaction was terminated by adding Nelson reagent. One unit of activity was defined as the quantity of reducing sugars released from substrate using corresponding monosaccharide as a standard Somogyi–Nelson method (Nelson 1944). Dextran, water-soluble agar, amylose, carboxymethyl cellulose (Sigma, USA), polyguluronic acid (British Drug Houses, UK), carrageenan from red alga *Chondrus* sp., fucoidans from brown algae *Fucus evanescence* (α-1,3;1,4-L-fucan sulfate) free of polyphenol or alginic acid (Zvyagintseva et al. 2003), pustulan from lichen *Umbilicaria rossica* (β-1,6-D-glucan) (Kusaykin et al. 2008), laminaran from *Laminaria cichorioides* (branched β-1,3;1,6-D-glucan), polymannuronic acid from *Alaria fistulosa* (β-1,4-glycoside-bound mannuronic acid) (Zvyagintseva et al. 2005) and pullulan from *Aureobasidium pullulans* (α-1,4;1,6-glucan) were used as substrates for polysaccharide-degrading enzymes dextranase, agarase, amylase, cellulose, polyguluronan lyase, carrageenase, fucoidanases, pustulanase, laminaranase, polymannuronase and pullulanase, respectively.

Results and discussion

16S rRNA gene sequences and phylogenetic analysis

The phylogenetic position of strain 10Alg 130^T was determined from its almost complete 16S rRNA gene

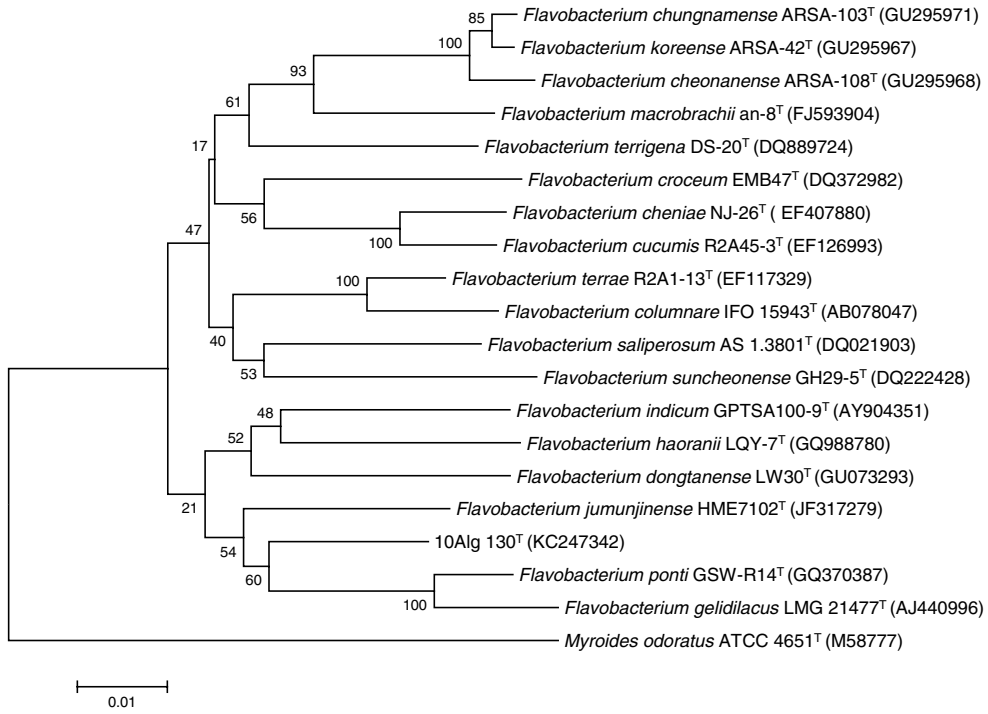

Fig. 1 Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic position of *F. ahnfeltiae* sp. nov. 10Alg 130^T and related members of the family *Flavobacteriaceae*. Bootstrap values are based on 1,000 replications. *Bar*, 0.01 substitutions per nucleotide position

sequence (1,435 nt). Phylogenetic analysis clearly indicated that the novel strain belongs to the genus *Flavobacterium* (Fig. 1). The novel strain shared highest 16S rRNA gene sequence similarity with *F. jumunjiense* KCTC 23618^T and *F. ponti* CCUG 58402^T (96.2 and 95.7 %, respectively). The 16S rRNA gene sequence similarity between strain 10Alg 130^T and other recognized species of the genus *Flavobacterium* was less than 95.3 %. The phylogenetic tree topology constructed using the neighbor-joining method indicated that the algal isolate formed a robust cluster with *F. jumunjiense* KCTC 23618^T and *Flavobacterium gelidilacus* LMG 21477^T (Fig. 1). This result was evaluated and confirmed by maximum likelihood and minimum evolution analyses (see Supplementary Figs. S1 and S2 available in Archives of Microbiology Online).

The DNA G+C content

The DNA G+C content of strain 10Alg 130^T was 34.3 mol%, which was higher than that of *F. ponti* CCUG 23618^T (31.4 mol%), *Flavobacterium gelidilacus* LMG 24277^T (30.4 mol%) and *F. dongtanense* KCTC 22671^T (30.0 mol%), and lower than that of *Flavobacterium jumunjinense* KCTC 23618^T (36.5 mol%) (Table 2).

Chemotaxonomic characteristics

The prevalent fatty acids of strain 10Alg 130^T were iso-$C_{15:1}$ (24.0 %), iso-$C_{15:0}$ (17.2 %), iso-$C_{15:0}$ 3-OH (15.4 %), iso-$C_{17:0}$ 3-OH (12.3 %), $C_{15:0}$ (10.2 %) and iso-$C_{17:1}\omega9c$ (6.6 %) (Table 1). The amount of unsaturated fatty acids in strain 10Alg 130^T was 82.2 % of total. The fatty acid profile of the novel isolate was in line with those of the reference strains and others reported for *Flavobacterium* species (Van Trappen et al., 2003; Zhang et al. 2010). The polar lipid profile of the algal isolate consisted of phosphatidylethanolamine, two unidentified aminophospholipids and three unidentified lipids (see Supplementary Fig. S3 available in Archives of Microbiology Online) and was similar to those of its closest relatives. The major respiratory quinone was MK-6 that is characteristic for members of the family *Flavobacteriaceae*.

Morphological, biochemical and physiological characterization

Strain 10Alg 130^T was Gram stain negative, strictly aerobic, heterotrophic, rod shaped and motile by gliding organism that formed yellow-pigmented and smooth colonies with regular edges on marine agar. A detailed description of the physiological, biochemical and morphological characteristics of

Table 1 Fatty acid composition of strain 10Alg 130^T and the type strain of its closely related species

Fatty acid	1	2	3	4	5
iso-$C_{14:0}$	–	–	4.0	–	–
iso-$C_{15:0}$	17.2	23.0	12.0	26.3	17.1
anteiso-$C_{15:0}$	–	2.1	8.0	2.3	1.4
iso-$C_{15:1}$	24.0	11.5	10.0	16.5	13.4
anteiso-$C_{15:1}$	–	–	1.0	–	–
iso-$C_{16:0}$	4.5	4.9	8.0	1.2	2.2
iso-$C_{16:1}$	–	–	4.0	–	–
iso-$C_{17:1}$ ω9c	6.6	8.5	2.0	4.1	2.8
$C_{15:0}$	10.2	11.2	10.0	8.1	16.4
$C_{15:1}$ ω6c	2.8	1.3	6.0	5.8	6.7
$C_{15:1}$ ω8c	1.1	1.2	–	1.0	1.3
$C_{16:0}$	1.1	1.7	–	–	–
$C_{17:0}$	–	1.0	–	–	–
$C_{17:1}$ ω6c	–	–	3.0	1.2	2.6
$C_{18:1}$ ω5c	–	–	1.0	–	–
iso-$C_{15:0}$ 3-OH	15.4	16.6	6.0	11.9	8.9
iso-$C_{16:0}$ 3-OH	2.2	1.0	10.0	1.6	3.0
iso-$C_{17:0}$ 3-OH	12.3	10.9	6.0	13.4	11.5
$C_{15:0}$ 3-OH	2.0	1.4	1.0	tr	1.5
$C_{15:0}$ 2-OH	–	–	2.0	–	–
$C_{16:0}$ 3-OH	1.4	1.9	–	1.2	1.3
$C_{17:0}$ 3-OH	1.1	1.0	–	1.0	1.3
Summed feature 3*	–	2.2	–	1.1	1.8

Strains: 1, 10Alg 130^T (this study); 2, *F. dongtanense* KCTC 22671^T (this study); 3, *F. gelidilacus* LMG 21477^T (data from Van Trappen et al., 2003); 4, *F. jumunjinense* HME7102^T (this study); and 5, *F. ponti* GSW-R14^T (this study). For fatty acid analysis, all strains were cultivated under similar conditions; – <1 % of the total fatty acid content; fatty acids amounting <1 % in all strains are not shown

* Summed feature 3 consists of $C_{16:1}\omega7c$ and iso-$C_{15:0}$ 2-OH fatty acids which could not be separated by the Microbial Identification System

the novel isolate is given in the species description, Table 2 and in Supplementary Table S1 available in Archives of Microbiology Online. Strain 10Alg 130^T was found to be clearly different from the nearest phylogenetic neighbor, *F. jumunjiense* KCTC 23618^T, because of its ability to produce urease, esterase (C4), lipase (C14), and hydrogen sulfide and to utilize D-lactose, inositol, and mannitol (Table 2). The presence of lipase (C14), gliding motility and utilization of inositol can be helpful for discrimination of the novel isolate from *F. dongtanense* and *F. ponti*. A set of phenotypic traits including maximum growth temperature (35 vs. 25 °C), oxidase activity, hydrogen sulfide production and hydrolysis of casein, DNA and urea discriminated the novel strain from *F. geledilacus*. Other biochemical and physiological characteristics that distinguish the algal isolate from its closest relatives are shown in Table 2. Strain 10Alg 130^T demonstrated enzyme activities against carrageen (22 mU/mg protein) and fucoidan (20 mU/mg protein), which are structural sulfated polysaccharides of cell walls of red and brown alga, respectively. Only trace amounts of such β-glucanases as cellulase (5 mU/mg protein) and pustulanase (4 mU/mg protein) were marked in the isolate extract under conditions studied (pH 7,3; at 25 °C). These data can suggest participation of the novel strain in degradation of algal polysaccharides for acquisition of nutrient compounds as necessary sources for energy and carbon; perhaps the above-mentioned hydrolytic features are used for attachment of flavobacterial cells to algal surfaces and for providing other members of microbial communities including *Alphaproteobacteria* (especially SAR11) and *Gammaproteobacteria* by labile nutrient compounds as it was recently reported for planctonic flavobacteria (Williams et al. 2013). However, the further experiments are yet to be carried out to understand the real role of novel isolate and other members of the genus *Flavobacterium* in the lifestyles of other members of the algal microbial community and the host organism.

The phylogenetic evidence taken together with the genotypic, phenotypic and chemotaxonomic data obtained from this study clearly indicates that the novel strain represents a novel species of the genus *Flavobacterium*, for which the name *F. ahnfeltiae* sp. nov. is proposed.

Description of *Flavobacterium ahnfeltiae* sp. nov.

Flavobacterium ahnfeltiae [ahn.fel'ti.a.e. N.L. fem. gen. n. *Ahnfeltia*, the genus of the red alga, referring to the source from which the strain has been isolated].

Cells are 0.3–0.6 μm in diameter and 0.9–3.7 μm in length, Gram negative, strictly aerobic, rod shaped and non-motile. On marine agar, colonies are 2–3 mm in diameter, circular, with entire edges, shiny and yellow in color. Growth occurs at 4–35 °C (optimum, 25–28 °C), at pH 5.0–10.0 (optimum, pH 7.5–8.0) and with 0–5 % NaCl (optimum, 1.0–2.0 %). Catalase and oxidase activities are present. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase activities are absent. Casein, gelatin, DNA, tweens 20, 40 and 80, and urea are hydrolyzed but aesculin, agar, starch and chitin are not hydrolyzed. No acid is produced from L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, D-lactose, maltose, mannose, melibiose, raffinose, L-rhamnose, ribose, sorbose, sucrose, D-xylose, *N*-acetylglucosamine, glycerol, inositol, mannitol, sorbitol and citrate. Cellobiose, D-galactose, D-glucose, D-lactose, maltose, mannose, melibiose, raffinose, trehalose, D-xylose, inositol, mannitol, adipate and gluconate are utilized as sole carbon sources, but L-arabinose, L-rhamnose, sucrose, sorbitol, *N*-acetylglucosamine, L-alanine, L-histidine, L-leucine, methionine, valine, phenylalanine, L-prolin, triptophane, treonine, tyrosin, caprate, citrate,

Table 2 Differential phenotypic characteristics of strain between strain 10Alg 130^T and closest related members of the genus *Flavobacterium*

Characteristic	1	2	3	4	5
Source of isolation	Red alga *Ahnfeltia tobuchiensis*	Seawater	Seawater	Lake microbial mat	Wetland reed
Type of metabolism	A[a]	A	F	A	A
Gliding motility	+[b]	+	−	+	−
Temperature range for growth (°C)	4–35	15–30	10–35	5–25	4–37
Salinity range for growth (% NaCl)	0–5	0–3	0–6	0–3	0–3
Oxidase	+	+	+	−	+
Catalase	+	+	+	+	−
H_2S production	+	−	−	−	+
Indole production	−	+	−	−	−
Hydrolysis of:					
Casein	+	+	+	−	+
DNA	+	+	+	−	−
Starch	−	−	+	−	−
Tyrosine	−	+	+	−	−
Urea	+	−	−	−	−
Acid formation from carbohydrates	−	−	+	−	−
Utilization of:					
Arabinose	−	+	−	+	+
D-Glucose	+	+	+	+	−
Lactose	+	−	−	+	+
Sucrose	−	−	−	+	−
Inositol	+	−	−	−	−
Mannitol	+	−	−	−	+
Malate, citrate, phenylacetate	−	+	−	−	−
N-Acetylglucosamine	−	+	−	−	+
API ZYM test					
Cystine arylamidase	−	+	+	+	−
Esterase (C4)	+	−	+	−	−
Lipase (C14)	+	−	−	−	−
Trypsin	+	+	−	+	−
α-Chymotrypsin	−	−	−	−	−
Acid phosphatase	+	+	−	+	+
Naphtol-AS-BI-phosphohydrolase	+	+	−	+	−
α-Glucosidase	−	−	+	+	−
DNA G+C content (mol%)	34.3	36.5	31.4	30.4	30.0

Species: 1, strain 10Alg 130^T (this study); 2, *F. jumunjinense* KCTC 23618^T (this study); 3, *F. ponti* CCUG 58402^T (this study); 4. *F. gelidilacus* LMG 21477^T (data from Van Trappen et al., 2003; Joung et al, 2013); and 5, *F. dongtanense* KCTC 22671^T (this study). Data on the DNA G+C contents of the reference strains from Joung et al. (2013), Xiao et al. (2011) and Yoon et al. (2011). All strains were positive for gelatin hydrolysis, alkaline phosphatase, esterase lipase (C8), leucine arylamidase and valine arylamidase activities, and mannose utilization. All strains were negative for aesculin hydrolysis, nitrate reductase activity, flexirubin-type pigments production and α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities

[a] A aerobic type of metabolism; F fermentative type of metabolism

[b] + positive reaction; −negative reaction

malate, malonate and phenyl acetate are not utilized. Growth is observed on L-asparagine and glutamic acid, but no growth occurred on L-alanine, L-histidine, L-leucine, DL-methionine, L-proline, L-threonine, L-tryptophan, L-tyrosine or L-valine. None of substrates of the API 50CH gallery are oxidized. In the API ZYM gallery, alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, trypsin, acid phosphatase and naphtol-AS-BI-phosphohydrolase activities are present; but cystine arylamidase, α-chymotrypsin, α-galactosidase, *β*-galactosidase,

β-glucuronidase, α-glucosidase, β-glucosidase *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. Nitrate is not reduced to nitrite. Hydrogen sulfide is produced, but indole and acetoin are not produced. The type strain is susceptible to ampicillin, benzylpenicillin, carbenicillin, cefalexin, cefazolin, chloramphenicol, doxycycline, erythromycin, lincomycin, nalidixic acid, ofloxacin, oleandomycin, rifampicin, tetracycline and vancomycin; and resistant to gentamicin, neomycin, streptomycin, kanamycin, oxacillin and polymyxin B. The prevalent fatty acids are iso-$C_{15:1}$, iso-$C_{15:0}$, iso-$C_{15:0}$ 3-OH, iso-$C_{17:0}$ 3-OH, $C_{15:0}$ and iso-$C_{17:1}$ ω9c. The polar lipid profile consists of phosphatidylethanolamine, two unidentified aminophospholipids and three unidentified lipids. The major respiratory quinone is MK-6. The DNA G+C content of the type strain is 34.3 mol%. The type strain, 10Alg-130^T (=KCTC 32467^T = KMM 6686^T), was isolated from the red alga *A. tobuchiensis* collected from the Sea of Okhotsk, Pacific Ocean, Russia. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *F. ahnfeltiae* 10Alg 130^T is KC247342.

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