

## *Antarcticimonas flava* gen. nov., sp. nov., Isolated from Antarctic Coastal Seawater

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A marine bacterium, designated IMCC3175<sup>T</sup>, was isolated from a seawater sample collected off the Antarctic coast. The strain was Gram-negative, obligately aerobic, carotenoid pigment-containing, and rod-shaped bacterium that divided by binary fission. As determined by 16S rRNA gene sequence comparisons, the most closely related genera were *Formosa* (92.9~93.3%), *Bizionia* (91.6~93.2%), *Gaetbulibacter* (91.5~92.8%), *Sediminibacter* (92.7%), *Yeosuana* (92.6%), *Subsaximicrobium* (92.1~92.2%), and *Gillisia* (89.5~92.2%). Phylogenetic analysis based on 16S rRNA gene sequences showed that the strain formed a monophyletic clade together with the genera *Sediminibacter* and *Subsaximicrobium* but represented an independent phyletic line in this clade of the family *Flavobacteriaceae*. The DNA G+C content of the strain was 37.3 mol%. The major respiratory quinone was MK-6 and the predominant cellular fatty acids were C<sub>16:1</sub> ω7c and/or iso-C<sub>15:0</sub> 2-OH (12.8%), anteiso-C<sub>15:0</sub> (9.4%), and iso-C<sub>16:1</sub> (9.4%). Low 16S rRNA gene sequence similarity, formation of a distinct phylogenetic branch, and several phenotypic characteristics, including a narrow range of temperature and salinity for growth, differentiated strain IMCC3175<sup>T</sup> from other related genera in the family *Flavobacteriaceae*. Therefore the name *Antarcticimonas flava* gen. nov., sp. nov. is proposed, with strain IMCC3175<sup>T</sup> (=KCCM 42713<sup>T</sup> =NBRC 103398<sup>T</sup>) as the type strain.

**Keywords:** *Antarcticimonas flava* gen. nov., sp. nov., *Flavobacteriaceae*, Antarctic, seawater, phylogenetic analysis

The family *Flavobacteriaceae* (Reichenbach, 1989; Bernardet *et al.*, 2002) is one of the major groups within the phylum *Bacteroidetes* (Garrity and Holt, 2001) that generally comprise 6~30% of the total bacterial communities in marine systems, as determined by fluorescence *in situ* hybridization or 16S rRNA gene cloning analyses (Glöckner *et al.*, 1999; Eilers *et al.*, 2000). This family includes many marine species that form a well-defined clade in 16S rRNA gene sequence phylogenetic trees (Bowman and Nichols, 2005) and recent taxonomic studies have led to a rapid increase in the descriptions of novel members of the family.

Members of the family *Flavobacteriaceae* are characterized as short to moderately long rods, Gram-negative, non-spore-forming, and non-motile or motile by gliding. Menaquinone 6 is either the only or the major respiratory quinone and the DNA G+C contents range from 27 to 44 mol% (Bernardet *et al.*, 2002). The family is currently composed of 85 well-defined genera and many psychrophilic genera have been isolated from the Arctic or Antarctic environments, including *Gelidibacter* and *Psychroserpens* (Bowman *et al.*, 1997a), *Polaribacter* (Gosink *et al.*, 1998), *Psychroflexus* (Bowman *et al.*, 1998), *Salegentibacter* (McCannon and Bowman, 2000), *Aequorivita* (Bowman and Nichols, 2002), *Gillisia* (Van Trappen *et al.*, 2004), *Lacinutrix*, *Subsaxibacter*, and *Subsaximicro-*

*bium* (Bowman and Nichols, 2005), and *Sejongia* (Yi *et al.*, 2005). Psychrophilic members of the family *Flavobacteriaceae* seem to play important roles for organic carbon mineralization in cold marine environments (Bowman *et al.*, 1997b) and some species contain rare carotenoids that are candidates for pharmaceuticals or food additives (Shindo *et al.*, 2007).

In this study, a yellow-pigmented bacterial strain, designated IMCC3175<sup>T</sup>, was isolated from Antarctic coastal seawater and subjected to taxonomic studies including 16S rRNA gene phylogeny, phenotypic characterization, and chemotaxonomic analyses. Based on these taxonomic approaches, strain IMCC3175<sup>T</sup> represented a new genus and novel species in the family *Flavobacteriaceae*, for which the name *Antarcticimonas flava* gen. nov., sp. nov. is proposed.

### Materials and Methods

#### Isolation and culture of bacterial strain

An Antarctic seawater sample was collected off the coast of the King George Island, Weaver Peninsula, Antarctica (62°08'07"S, 58°28'13"W). A yellow colony-forming strain, IMCC3175<sup>T</sup>, was isolated using a standard dilution-plating method on an oligotrophic medium, 1/10 R2A [modified R2A (Reasoner and Geldreich, 1985), diluted 1:10 in aged Antarctic seawater] (Cho and Giovannoni, 2004), after incubating the plates at 20°C for 1 month. Strain IMCC3175<sup>T</sup>, initially isolated on 1/10R2A, was further purified on marine agar 2216 (MA; BD Difco) after growing the strain at 20°C

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for one week. After its optimum growth temperature and medium were determined, cultures of strain IMCC3175<sup>T</sup> were maintained routinely on MA at 25°C and preserved as a glycerol suspension (10%, v/v) at -80°C.

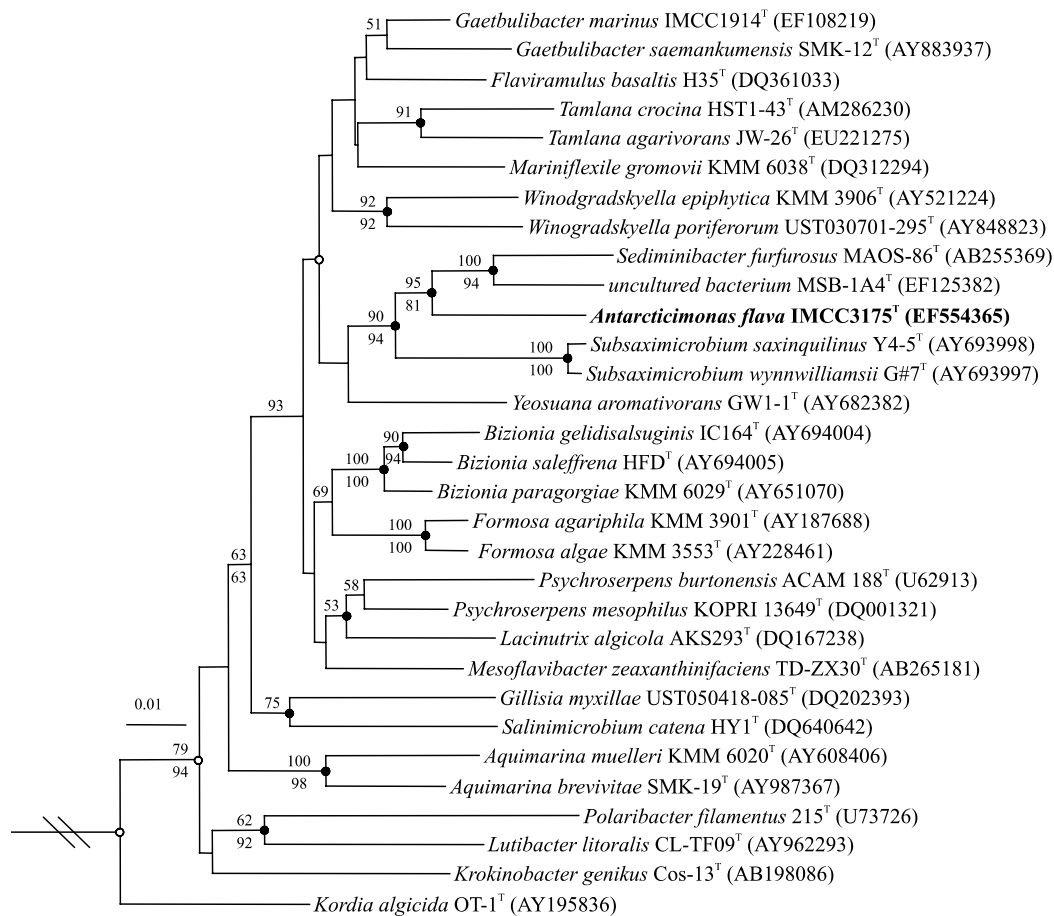
### Phylogenetic analysis based on 16S rRNA gene sequences

DNA extraction, PCR, and sequencing of the 16S rRNA gene were performed according to the methods described in a previous study (Yang *et al.*, 2007). The resultant almost complete 16S rRNA gene sequence of strain IMCC3175<sup>T</sup> was aligned automatically with the nearest neighbors using a PT-server implemented in the ARB software package (Ludwig *et al.*, 2004) and its alignment was checked manually with considering the secondary structure of 16S rRNA molecules. The 16S rRNA gene sequence similarity was calculated based on this sequence alignment in the ARB software package and closely related type species were determined by using the EzTaxon server (Chun *et al.*, 2007). To reveal the phylogenetic position of the strain, unambiguously aligned 1,284 nucleotide positions were used for phylogenetic analyses in PAUP\* 4.0 beta 10 (Swofford, 2002). Phylogenetic

trees were generated by neighbor-joining (Saitou and Nei, 1987) with the Jukes-Cantor distance (Jukes and Cantor, 1969), maximum parsimony (Fitch, 1971), and maximum likelihood (Felsenstein, 1981). Tree topologies from neighbor-joining and maximum-parsimony were evaluated by bootstrap analyses based on 1,000 re-samplings.

### Phenotypic and biochemical characteristics

Cell morphology and size were examined by transmission electron microscopy (CM200, Philips, Netherlands) and phase-contrast microscopy (Nikon 80i, Nikon, Japan) using 4-day culture on MA at 25°C. Colony morphology, size, and color were examined from cultures grown aerobically on MA for 7 days. Motility was examined using wet mounts, made from fresh cultures grown on MA at 25°C for 4 days. Gliding motility was determined by phase-contrast microscopy with 17 h-incubated cells on microscopic slides coated with 0.7% agar (Bowman, 2000). Cellular pigments of the strain were extracted with acetone/methanol (1:1, v/v) and their absorption spectra were determined using a scanning UV/visible spectrophotometer (Optizen 2120UV, Mechasis Co., Korea). The presence of flexirubin-type pigments was determined



**Fig. 1.** Neighbor-joining phylogenetic tree, based on 16S rRNA gene sequences, showing relationships between strain IMCC3175<sup>T</sup> and representatives of the family *Flavobacteriaceae*. Bootstrap percentages (above 50%) from both neighbor-joining (above nodes) and maximum parsimony (below nodes) are shown. Filled and open circles at each node indicate nodes recovered by all three treeing methods or by two treeing methods, respectively. Scale bar, 0.01 substitutions per nucleotide position.

by the bathochromic shift (Bernardet *et al.*, 2002). Temperature range and optimum for cellular growth were measured at 3–42°C (3, 10, 15, 20, 25, 30, 35, 37, and 42°C). The pH range and optimum for growth were tested at pH values of 4–12 (at 0.5 pH unit intervals). The NaCl concentrations and optimum for growth were determined in NaCl-free artificial seawater medium (ASW; basic formula, per liter: 5.9 g MgCl<sub>2</sub>·6H<sub>2</sub>O, 3.24 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 1.8 g CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.55 g KCl, 0.16 g NaHCO<sub>3</sub>, 0.08 g KBr, 0.034 g SrCl<sub>2</sub>·6H<sub>2</sub>O, 0.022 g H<sub>3</sub>BO<sub>3</sub>, 0.008 g Na<sub>2</sub>H<sub>2</sub>PO<sub>4</sub>, 0.004 g Na<sub>2</sub>SiO<sub>3</sub>, 0.0024 g NaF, 0.0016 g KN<sub>4</sub>NO<sub>3</sub>), supplemented with 5.0 g peptone, 1.0 g yeast extract, and different concentrations of NaCl (0–15%, w/v). The catalase test was performed by addition of 3% hydrogen peroxide to exponential phase colonies and oxidase activity was determined using the oxidase reagent (bioMérieux). Other biochemical tests and carbon source oxidation tests were carried out in API 20NE and API ZYM strips (bioMérieux) and in GN2 microplates (Biolog), according to the manufacturer's instructions except for inoculating with bacterial suspensions in ASW. Degradation of macromolecules was tested by incubating the bacterial cultures on MA containing macromolecules for 3 weeks. The following macromolecules were tested: starch (0.2%, w/v), casein (10% skim milk, w/v), elastin (0.5%, w/v), chitin (0.5%, w/v), agar (1.5%, w/v), and carboxymethyl cellulose (0.2%, w/v). Hydrolysis was determined by formation of clear zones around the colonies either by non-staining or after flooding adequate staining solutions (Teather and Wood, 1982). Susceptibility to 10 different antimicrobial agents [tetracycline (30 µg), ampicillin (10 µg), kanamycin (30 µg), chloramphenicol (25 µg), erythromycin (15 µg), gentamicin

(10 µg), penicillin G (10 µg), streptomycin (10 µg), vancomycin (30 µg) and rifampicin (50 µg)] was determined using the diffusion disc method (Jorgensen *et al.*, 1999).

#### Determination of G+C content, cellular fatty acids, and respiratory quinones

The DNA G+C content of strain IMCC3175<sup>T</sup> was determined by using HPLC with a reverse-phase C18 column (Mesbah *et al.*, 1989). Cellular fatty acid methyl esters were extracted and prepared from cultures grown on MA at 25°C for 4 days, and analyzed according to the standard protocol of the MIDI Microbial Identification System. Respiratory quinone content was analyzed using HPLC with a reverse-phase C18 column (Komagata and Suzuki, 1987).

#### Nucleotide sequence accession number

The 16S rRNA gene sequence of strain IMCC3175<sup>T</sup> has been deposited in GenBank under the accession number EF554365.

## Results and Discussion

#### Phylogenetic basis for a new genus delineation

A total of 1,455 nucleotides of the 16S rRNA gene sequence were determined for strain IMCC3175<sup>T</sup>. Results of preliminary BLASTN searches and insertion of the sequence into the ARB tree showed that the strain belonged to the family *Flavobacteriaceae* in the phylum *Bacteroidetes*. The strain was considered to be unique in its sequence because it was most closely related to uncultured bacterium clone NBDTU7 (GenBank accession no., FJ529927), showing only

**Table 1.** Differential characteristics of strain IMCC3175<sup>T</sup> and other genera in the family *Flavobacteriaceae*

Genera : 1, IMCC3175<sup>T</sup>; 2, *Sediminibacter* (Khan *et al.*, 2007); 3, *Subsaximicrobium* (Bowman and Nichols, 2005); 4, *Bizionia* (Bowman and Nichols, 2005; Nedashkovskaya *et al.*, 2005b); 5, *Flaviramulus* (Einen and Øvreås, 2006); 6, *Formosa* (Ivanova *et al.*, 2004; Nedashkovskaya *et al.*, 2006); 7, *Gaetbulibacter* (Jung *et al.*, 2005; Yang *et al.*, 2007); 8, *Lacinutrix* (Bowman and Nichols, 2005; Nedashkovskaya *et al.*, 2008); 9, *Psychroserpens* (Bowman *et al.*, 1997a; Kwon *et al.*, 2006b); 10, *Tamlana* (Lee, 2007; Yoon *et al.*, 2008); 11, *Winogradskyella* (Lau *et al.*, 2005; Nedashkovskaya *et al.*, 2005a; Nedashkovskaya *et al.*, 2009; Romanenko *et al.*, 2009); 12, *Yeosuana* (Kwon *et al.*, 2006a). +, positive; –, negative; V, variable; W, weakly positive; ND, not determined

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Pigments production	Yellow	Light brown	Orange Yellow	Yellow	Orange Yellow	Yellow	Yellow	Yellow	Orange Yellow	Saffron, Yellow	Yellow	Yellowish-brown
Gilding motility	–	–	–	–	+	+	V	–	–	–	+	–
Temperature range for growth (°C)	10-25	10-37	-2-25	-2-36	-2-34	4-34	3-40	-2-37	0-34	10-38	4-44	23-39
Salinity range for growth (%)	0.5-2.5	1-6	1.5-12	1-17.6	0.3-6	0-8	0.5-7	0.6-12	0.5-8	0.5-6	0.5-9.0	0.5-4
Oxidase	–	+	ND	+	–	+	V	ND	V	+	+	–
Nitrate reductase	–	–	–	–	ND	V	V	–	V	+	–	–
Anaerobic growth	–	ND	–	–	–	V	V	ND	– <sup>a</sup>	–	–	–
Hydrolysis of												
Casein	–	+	–	+	ND	–	V	–	–	–	V	–
Gelatin	+	+	+	+	+	+	–	+	+	–	+	+
Starch	+	+	–	–	+	V	–	–	–	–	V	–
DNA G+C content (mol%)	37.3	39.0	39.0-41.0	35.0-45.0	31.4	34.0-36.0	34.7-38.1	34.7-37.0	27.0-29.8	35.8-36.8	32.8-36.1 <sup>b</sup>	51.4
Major Quinone(s)	MK-6	MK-6	MK-6	MK-6	MK-6	MK-6	MK-6	MK-6	MK-6	MK-6	MK-6	MK-5, MK-6

<sup>a</sup> Data only available for *P. burtonensis*

<sup>b</sup> Data not available for *W. arenosi*

**Table 2.** Cellular fatty acid profiles of IMCC3175<sup>T</sup> and other genera of the family *Flavobacteriaceae*. Genera: 1, IMCC3175<sup>T</sup>; 2, *Sediminibacter* (Khan et al., 2007); 3, *Subsaximicrobium* (Bowman and Nichols, 2005); 4, *Bizionia* (Bowman and Nichols, 2005; Nedashkovskaya et al., 2005b); 5, *Flaviramulus* (Einen and Øvreås, 2006); 6, *Formosa* (Ivanova et al., 2004; Nedashkovskaya et al., 2006); 7, *Gaetbulibacter* (Jung et al., 2005; Yang et al., 2007); 8, *Lacinutrix* (Bowman and Nichols, 2005; Nedashkovskaya et al., 2008); 9, *Psychroserpens* (Bowman et al., 1997a; Kwon et al., 2006b); 10, *Tamlana* (Lee, 2007; Yoon et al., 2008); 11, *Winogradskyella* (Lau et al., 2005; Nedashkovskaya et al., 2005a; Nedashkovskaya et al., 2009; Romanenko et al., 2009); 12, *Yeosuana* (Kwon et al., 2006a). Percentage ranges are for all the species in the genus. Fatty acids representing >3.0% of the total cellular fatty acids from at least one of the species are shown. br, branched fatty acid but branching position is unclear. -, not detected; tr, trace

Fatty acid	1	2	3	4	5	6	7	8	9	10	11	12
C <sub>12:0</sub>	-	-	-	-	-	-	-	-	-	6.0	-	-
C <sub>15:0</sub>	4.4	-	-	2.3-4.3	-	8.7-27.2	3.9	1.6-6.7	5.6-10.1	5.0-12.1	1.2-7.9	5.3
C <sub>15:1</sub> <sup>a</sup>	-	-	-	-	-	6.0-13.0	-	-	3.5-7.6	-	1.1-6.5	-
C <sub>16:0</sub>	-	2	-	1.2-3.4	-	-	-	-	-	3.4-8.5	-	-
C <sub>16:1</sub> <sup>b</sup>	-	-	9.5-13.4	2.6-5.5	-	3.3-6.7	-	-	0.3-6.9	-	-	10.9
C <sub>17:1</sub> <sup>c</sup>	-	-	-	-	-	0.8-3.2	-	-	-	-	-	-
C <sub>18:0</sub>	-	-	-	-	-	-	-	-	-	12.7	-	-
iso-C <sub>13:0</sub>	-	-	-	tr-3.0	-	-	-	-	-	-	-	-
iso-C <sub>14:0</sub>	-	-	-	-	-	-	-	1.4-4.1	4.1	-	1.1-4.5	-
iso-C <sub>15:0</sub>	5.9	39	7.4-10.4	3.1-13.2	19.4	12.7-22.0	20.6-24.3	10.6-19.9	10.4-31.4	13.7-25.9	6.7-25.6	21.7
Anteiso-C <sub>15:0</sub>	9.4	2	9.9-17.1	10.1-20.8	6.2	1.6-9.2	4.0-10.8	4.6-18.2	10.4-10.9	3.2-4.4	4.9-15.9	14.9
iso-C <sub>15:1</sub> <sup>d</sup>	6.2	25	6.7-13.4	3.1-13.2	21.2	6.5-20.9	12.5-32.1	7.2-12.0	7.1-14.1	8.2-11.8	10.4-20.9	-
anteiso-C <sub>15:1</sub> <sup>e</sup>	-	-	5.9-14.7	5.8-14.0	-	-	3.4	1.2-14.2	8.4	-	0.9-6.3	14.8
iso-C <sub>16:0</sub>	4.7	-	-	1.3-5.8	-	-	-	2.2-7.1	7.3	-	0.3-5.7	-
iso-C <sub>16:1</sub> <sup>f</sup>	9.4	-	-	-	-	-	-	-	2.7-9.1	-	0.5-4.7	-
br-C <sub>16:1</sub>	-	-	1.9-4.7	1.2-5.4	-	-	-	3.1-7.2	-	-	-	-
anteiso-C <sub>17:0</sub>	-	-	-	-	-	-	-	-	3.0	-	-	4.2
iso-C <sub>17:1</sub> <sup>g</sup>	4.7	-	3.5-5.1	1.7-15.1	-	-	-	-	-	-	-	-
anteiso-C <sub>17:1</sub>	6.7	3	2.1-3.8	1.5-4.6	-	-	-	-	-	-	-	-
C <sub>15:0</sub> 2-OH	-	-	-	-	-	-	-	-	-	-	1.0-3.6	-
C <sub>15:0</sub> 3-OH	-	-	-	-	-	2.4-4.0	-	-	-	-	-	-
iso-C <sub>15:0</sub> 3-OH	-	6	2.4-5.4	1.2-9.3	13.6	6.7-10.5	4.8-8.6	3.1-12.2	-	3.5-5.0	2.6-17.0	-
anteiso-C <sub>15:0</sub> 3-OH	-	-	3.6-8.7	3.4-22.9	-	-	-	-	-	-	3.4	-
iso-C <sub>16:0</sub> 3-OH	6.4	-	8.8-13.7	1.3-8.5	4.5	3.1-8.9	5.5	2.7-14.4	-	2.1-3.2	3.2-18.1	-
C <sub>17:0</sub> 2-OH	5.2	-	-	-	-	-	-	tr-3.9	-	-	0.3-5.2	-
iso-C <sub>17:0</sub> 3-OH	4.7	9	1.0-3.4	0.4-4.3	13.3	8.5-10.7	7.8-16.0	2.3-7.6	-	8.0-9.9	5.4-10.2	-
anteiso-C <sub>17:0</sub> 3-OH	-	-	2.7-5.3	-	-	-	-	-	-	-	8.5	-
C <sub>16:1</sub> 10 methyl	-	-	-	-	-	-	-	-	3.2	-	6.3	4.7
C <sub>17:1</sub> cyclo	-	-	-	-	-	2.7-7.4	-	-	-	-	-	-
Summed feature 3 <sup>h</sup>	12.8	9	-	6.3	4.9	5.9-15.8	4.5-10.4	2.8-9.2	-	5.0	4.2-10.6	-

<sup>a</sup> Contains several of the following fatty acids: C<sub>15:1</sub> ω4c, C<sub>15:1</sub> ω6c, C<sub>15:1</sub> ω8c, and C<sub>15:1</sub> ω11c

<sup>b</sup> Contains several of the following fatty acids: C<sub>16:1</sub> ω5c, C<sub>16:1</sub> ω6c, C<sub>16:1</sub> ω7c, and C<sub>16:1</sub> ω11c

<sup>c</sup> Contains C<sub>17:1</sub> ω6c and/or C<sub>17:1</sub> ω8c

<sup>d</sup> Contains iso-C<sub>15:1</sub> ω10c

<sup>e</sup> Contains anteiso-C<sub>15:1</sub> ω6c and/or anteiso-C<sub>15:1</sub> ω10c

<sup>f</sup> Contains iso-C<sub>16:1</sub> ω11c

<sup>g</sup> Contains iso-C<sub>17:1</sub> ω9c

<sup>h</sup> Contains C<sub>16:1</sub> ω7c and/or iso-C<sub>15:0</sub> 2-OH

93.4% 16S rRNA gene sequence similarity. In comparative sequence analyses with the validly published bacterial species, strain IMCC3175<sup>T</sup> showed the highest 16S rRNA gene sequence similarity with *Formosa algae* (93.3%), followed by *Bizionia paragorgiae* (93.2%), *Formosa agariphila* (92.9%), and *Gaetbulibacter marinus* (92.8%). The genera showing over 92.0% 16S rRNA gene sequence similarity to strain IMCC3175<sup>T</sup> were *Formosa* (92.9–93.3%), *Bizionia* (91.6–93.2%), *Gaetbulibacter* (91.5–92.8%), *Sediminibacter* (92.7%), *Yeosuana* (92.6%), *Subsaximicrobium* (92.1–92.2%), and *Gillisia* (89.5–92.2%). Because it was impossible to assume the taxonomic position of the strain solely based on 16S

rRNA gene sequence similarities, phylogenetic analyses were performed using three treeing algorithms. In all phylogenetic trees (Fig. 1), strain IMCC3175<sup>T</sup> formed a monophyletic clade together with the genera *Sediminibacter* and *Subsaximicrobium* but represented an independent phyletic line in this clade of the family *Flavobacteriaceae*. The relationship between strain IMCC3175<sup>T</sup> and members of the genera *Sediminibacter* and *Subsaximicrobium* was supported by high bootstrap values. The low sequence similarities (<93.3%) and distant relationships between strain IMCC 3175<sup>T</sup> and other genera suggested that the strain represented a novel genus and species in the family *Flavobacteriaceae*.

### Phenotypic and biochemical characteristics

Cells of strain IMCC3175<sup>T</sup> was Gram-negative, non-motile, obligately aerobic, and straight rods that divide by binary fission. The colonies are yellow-colored and contained carotenoid pigment. However, flexirubin-type pigment was absent in the cells. The strain hydrolyzed starch and gelatin, and oxidized various kinds of carbohydrates, showing typical characteristics of chemoheteroorganotrophs. Other detailed phenotypic and biochemical characteristics of strain IMCC 3175<sup>T</sup> are fully described in the genus and species descriptions. As shown in Table 1, strain IMCC3175<sup>T</sup> differed from other genera of the family *Flavobacteriaceae* in several phenotypic characteristics, including a narrow range of temperature and salinity for growth and hydrolysis of macromolecules.

### Chemotaxonomic characteristics

The DNA G+C content of strain IMCC3175<sup>T</sup> was 37.3 mol%, as determined by the HPLC method, which was coincided with the range of DNA G+C content of the closely related members of the family *Flavobacteriaceae*. The major cellular fatty acids were iso-C<sub>15:0</sub> 2-OH and/or C<sub>16:1</sub> ω7c (12.8%), anteiso-C<sub>15:0</sub> (9.4%), iso-C<sub>16:1</sub> (9.4%), anteiso-C<sub>17:1</sub> ω9c (6.7%), iso-C<sub>16:0</sub> 3-OH (6.4%), and iso-C<sub>15:1</sub> (6.2%), showing a difference from other related members in proportions of several fatty acids (Table 2). The major respiratory quinone of strain IMCC3175<sup>T</sup> was MK-6, a major quinone found in members of the family *Flavobacteriaceae*.

### Taxonomic conclusions

As shown by the low 16S rRNA gene sequence similarity (<93.4%) with other validly published genera in the family *Flavobacteriaceae*, formation of an independent phyletic line in the phylogenetic trees (Fig. 1), and several differential phenotypic characteristics (Table 1), strain IMCC3175<sup>T</sup> could not be assigned to any other known genera in the family *Flavobacteriaceae*. For these reasons, strain IMCC3175<sup>T</sup> should be classified as a novel species in a new genus, for which the name *Antarcticimonas flava* gen. nov., sp. nov. is proposed.

### Description of *Antarcticimonas* gen. nov.

*Antarcticimonas* (an.tarc.ti.ci.mo'nas. L. adj. *antarcticus* Antarctic; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Antarcticimonas* an Antarctic monad, a bacterium isolated from the Antarctic sea water).

Cells are Gram-negative, non-motile, non-gliding, chemoheterotrophic, obligately aerobic rods. Carotenoid pigment is present but flexirubin type pigment is absent. Oxidase-negative and catalase-positive. Degrades gelatin and starch and oxidizes various kinds of carbon substrates. The major fatty acids are iso-C<sub>15:0</sub> 2-OH and/or C<sub>16:1</sub> ω7c, anteiso-C<sub>15:0</sub> and iso-C<sub>16:1</sub>. The major respiratory quinone is MK-6 and the DNA G+C content of the type species is 37.3 mol%. The genus is a member of the family *Flavobacteriaceae*. The type species is *Antarcticimonas flava*.

### Description of *Antarcticimonas flava* sp. nov.

*Antarcticimonas flava* (fla'va. L. fem. adj. *flava* yellow, referring to the color of the colony).

Cells are 0.8~2.0 μm long and 0.4~0.7 μm wide. Colonies are yellow-colored, circular with entire edges, convex, and butyrous with a diameter of 0.4~1.3 mm after growing on marine agar 2216 for 7 days. Growth occurs at 10~25°C (optimum, 20°C), at pH 7~10 (optimum, pH 7), and with 0.5~2.5% NaCl (optimum, 2.0%). Absorption spectral peak of the carotenoid pigment is observed at 450 nm. Degrades gelatin and starch, but not casein, elastin, and chitin. In API 20 NE strips, positive for aesculin and gelatin hydrolysis and β-galactosidase activity but negative for nitrate reduction, indole production, acid production from glucose, arginine dihydrolase, and hydrolysis of urea. In API ZYM system, activities for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, α-chymotrypsin, acid phosphatase, and naphthol-AS-BI-phosphohydrolase are present, but esterase (C4), lipase (C14), trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase activities are absent. Oxidizes the following carbon substrates (Biolog GN2 microplates): α-cyclodextrin, dextrin, glycogen, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, L-arabinose, D-cellobiose, i-erythritol, D-fructose, L-fucose, gentiobiose, α-D-glucose, m-inositol, α-D-lactose, maltose, D-mannitol, β-methyl-D-glucoside, D-psicose, D-raffinose, D-sorbitol, sucrose, D-trehalose, turanose, xylitol, succinic acid mono-methyl-ester, acetic acid, D-galactonic acid lactone, D-galacturonic acid, D-glucuronic acid, α-keto glutaric acid, α-keto glutaric acid, D,L-lactic acid, Malonic acid, succinic acid, L-alanyl-glycine, L-aspartic acid, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-histidine, L-ornithine, L-proline, urocanic acid, inosine, uridine, thymidine, pheylethyl-amine, 2-amino-ethanol, 2,3-butanediol, DL-α-glycerol phosphate, and α-D-glucose-1-phosphate. Susceptible to chloramphenicol, erythromycin, penicillin G, and rifampicin, but resistant to ampicillin, gentamicin, kanamycin, streptomycin, tetracycline, and vancomycin. The major cellular fatty acids are iso-C<sub>15:0</sub> 2-OH and/or C<sub>16:1</sub> ω7c, anteiso-C<sub>15:0</sub>, iso-C<sub>16:1</sub>, anteiso-C<sub>17:1</sub> ω9c, iso-C<sub>16:0</sub> 3-OH, iso-C<sub>15:1</sub>, iso-C<sub>15:0</sub>, and C<sub>17:0</sub> 2-OH. The type strain is IMCC3175<sup>T</sup> (=KCCM 42713<sup>T</sup> =NBRC 103398<sup>T</sup>), isolated from a surface seawater of the Maxwell Bay, King George Island, West Antarctica (62°08'07"S, 58°28'13"W).

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