

# *Flavirhabdus iliipiscaria* gen. nov., sp. nov., isolated from intestine of flounder (*Paralichthys olivaceus*) and emended descriptions of the genera *Flavivirga*, *Algibacter*, *Bizionia* and *Formosa*

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A Gram-stain-negative, orange-coloured, rod-shaped bacterium, designated strain Th68<sup>T</sup>, was isolated from the intestine of flounder (*Paralichthys olivaceus*). The isolate required sea salts for growth. Gliding motility was not observed. Flexirubin-type pigments were present. 16S rRNA gene sequence analysis indicated that strain Th68<sup>T</sup> represented a distinct phyletic line within the family *Flavobacteriaceae* with less than 96.1 % similarity to members of the recognized genera of the family. The DNA G + C content was 33.0 mol%. The major fatty acids were iso-C<sub>15:0</sub>, iso-C<sub>15:1</sub> G, iso-C<sub>17:0</sub> 3-OH and iso-C<sub>15:0</sub> 3-OH. The major polar lipids were phosphatidylethanolamine, two unidentified aminolipids and two unidentified polar lipids. Menaquinone 6 (MK-6) was the only respiratory quinone. On the basis of the phenotypic, chemotaxonomic and phylogenetic data, strain Th68<sup>T</sup> represents a novel species of a new genus in the family *Flavobacteriaceae*, for which the name *Flavirhabdus iliipiscaria* gen. nov., sp. nov. is proposed. The type strain of *Flavirhabdus iliipiscaria* is Th68<sup>T</sup> (=JCM 18637<sup>T</sup>=KCTC 32141<sup>T</sup>).

The family *Flavobacteriaceae* was derived from the etymology of its type genus *Flavobacterium*, which means yellow-coloured bacterium (Bernardet & Nakagawa, 2006). One of the important characteristics of members of this family is the presence of carotenoids or flexirubin-type pigments or both, although unpigmented flavobacteria also occur (Bernardet *et al.*, 1996, 2002). At the time of writing, the bacterial family *Flavobacteriaceae* comprises 114 genera with validly published names (<http://eztaxon-e.ezbiocloud.net>). An additional yellow-pigmented flavobacterial strain, Th68<sup>T</sup>, isolated from the intestine of flounder was studied according to the minimal standards for describing new taxa in the family *Flavobacteriaceae* (Bernardet *et al.*, 2002). On the basis of evidence derived from a polyphasic taxonomic approach, the isolate is considered to represent a novel species of a new genus in the family *Flavobacteriaceae*.

Strain Th68<sup>T</sup> was isolated from the intestine of cultured flounder (*Paralichthys olivaceus*) in 2010, from a fish farm in Shandong Province, China. The surface of the fish was cleaned with 75 % alcohol and the fish was then dissected and a piece of intestinal tissue was sampled aseptically for the isolation of intestinal bacteria. Strain Th68<sup>T</sup> was

isolated from the tissue homogenate by the plate dilution method on marine agar 2216 (MA; Becton Dickinson) at 28 °C and purified by streaking three times on MA. *Winogradskyella thalassocola* LMG 22492<sup>T</sup>, *Algibacter pectinivorans* JCM 17107<sup>T</sup>, *Flaviramulus basaltis* DSM 18180<sup>T</sup>, *Gaetbulibacter saemankumensis* KCTC 12379<sup>T</sup>, *Bizionia paragorgiae* LMG 22571<sup>T</sup>, *Formosa algae* KCTC 12364<sup>T</sup> and *Flavivirga jejuensis* JCM 17113<sup>T</sup> were used as reference strains. For short-term preservation cultures of strain Th68<sup>T</sup> and the reference strains were maintained at 16 °C and for long-term cultures were preserved in sterile 0.85 % (w/v) saline supplemented with 15 % (v/v) glycerol at –80 °C.

The genomic DNA of strain Th68<sup>T</sup> was extracted and the 16S rRNA gene was obtained by PCR amplification with two universal primers (B8F, 5'-AGAGTTTGATCCTG-GCTCAG-3'; B1510, 5'-GGTTACCTTGTTACGACTT-3') (Weisburg *et al.*, 1991). For cloning and sequencing of the 16S rRNA gene, the PCR product was purified using a TIANGel Midi Purification kit (TIANGEN Biotech), ligated into the pUCm-T vector (TaKaRa) and sequenced at the Beijing Genomics Institute. The EzTaxon-e server was used for identification of phylogenetic neighbours and calculation of pairwise 16S rRNA gene sequence similarities (<http://eztaxon-e.ezbiocloud.net/>; Kim *et al.*, 2012). The 16S rRNA gene sequences of related strains were downloaded from the NCBI database and aligned using the

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Flavirhabdus iliipiscaria* Th68<sup>T</sup> is JX412960.

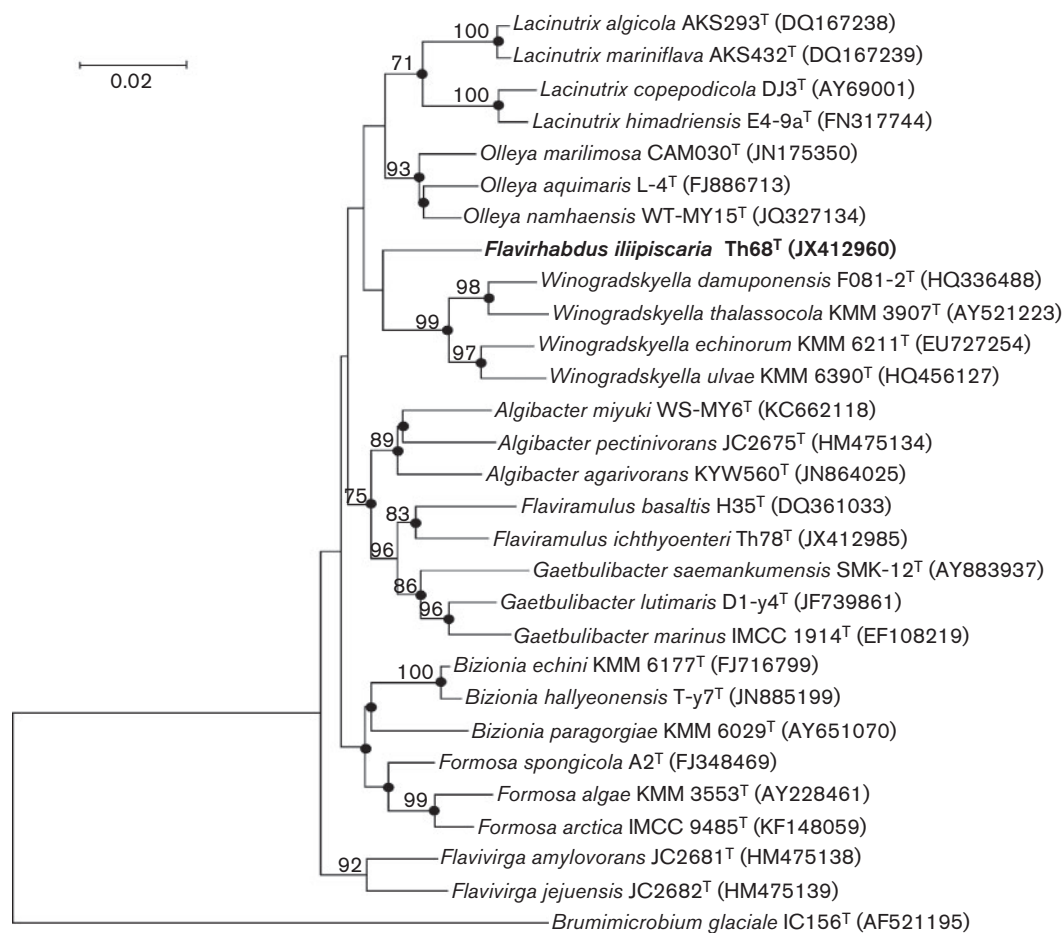
Four supplementary figures are available with the online Supplementary Material.

CLUSTAL X program (Thompson *et al.*, 1997). Phylogenetic trees were reconstructed using the neighbour-joining, maximum-likelihood and maximum-parsimony methods with Kimura two-state parameter model analyses (Kimura, 1980) implemented in the program MEGA version 5 (Tamura *et al.*, 2011). In each case, bootstrap values were calculated based on 1000 replicates.

Pairwise alignment according to the nearly complete 16S rRNA gene sequence (1441 bp) of strain Th68<sup>T</sup> showed 96.1% sequence similarity to the type strain of *Pontirhabdus pectinivorans* (Yi *et al.*, 2011), which was later reclassified as *Algibacter pectinivorans* by Park *et al.* (2013), followed by members of the genera *Olleya* (96.1–95.3%), *Flavivirga* (96.1–95.1%), *Lacinutrix* (96.0–95.0%), *Formosa* (96.0–94.1%), *Arenitalea* (95.4%), *Winogradskyella* (95.4–93.4%), *Bizionia* (95.1–94.1%), *Flaviramulus* (95.0%) and *Gaetbulibacter* (95.0–94.4%). In the neighbour-joining (Fig. 1), maximum-likelihood and maximum-parsimony trees (Figs S1

and S2, available in the online Supplementary Material), the isolate formed a distinct phylogenetic branch within the family *Flavobacteriaceae*. Thus, based on the 16S rRNA gene sequence analysis, strain Th68<sup>T</sup> appeared to represent a novel species of a new genus in the family *Flavobacteriaceae*.

Gram-staining and flagellum staining were investigated using standard methods (Beveridge *et al.*, 2007). Cell morphology was determined by transmission electron microscopy (JEM-1200EX; JEOL) after cells had been negatively stained with 1% (w/v) phosphotungstic acid. Gliding motility was observed by the hanging-drop technique and production of flexirubin-type pigments was estimated by a colour shift following exposure to 20% (w/v) KOH (Bernardet *et al.*, 2002). To test for anaerobic growth, bacterial strains were cultured on MA with resazurin as an indicator of anaerobic conditions in an anaerobic jar filled with nitrogen and a packet of AneroPack-Anaero (Mitsubishi Gas Chemical) at 28 °C for 1 month. Salinity



**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain Th68<sup>T</sup> and representatives of some other related members of the family *Flavobacteriaceae*. Percentage bootstrap values above 70 (1000 replicates) are shown at branch nodes. Filled circles indicate that the corresponding nodes were also recovered in trees generated with the maximum-parsimony and maximum-likelihood algorithms. *Brumimicrobium glaciale* IC156<sup>T</sup> (GenBank accession no. AF521195) was used as an outgroup. Bar, 0.02 substitutions per nucleotide position.

and pH range supporting growth were investigated in 96-well microplates by measuring the optical densities (wavelength 590 nm). Growth in synthetic ZoBell broth was tested in the presence of 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 10, 12, 15 and 20 % (w/v) NaCl or sea salts (Sigma) to determine salinity tolerance of the bacterium. The temperature range for growth was evaluated at 0, 4, 10, 16, 28, 30, 37 and 42 °C on MA and at pH 2.0–11.0 in marine broth 2216 (MB; Becton Dickinson) using the following buffer systems: Na<sub>2</sub>HPO<sub>4</sub>/citric acid (pH 2.0–7.0), Tris/HCl (pH 8.0–9.0) and Na<sub>2</sub>CO<sub>3</sub>/NaHCO<sub>3</sub> (pH 10.0–11.0). Various phenotypic characteristics of the isolate and the reference strains were tested according to standard approaches (Tindall *et al.*, 2007), including catalase and oxidase, and hydrolysis of starch, egg yolk, casein (5 % skimmed milk; Difco) and gelatin (method 2). Degradation of chitin (0.5 %; Sigma) was detected as described by Hsu & Lockwood (1975). Cellulose degradation was observed by formation of clear zones around colonies on MA plates supplemented with CM-cellulose (1 %, w/v) after flooding with appropriate solutions (Teather & Wood, 1982) and degradation of Tweens 20, 40 and 80 was determined as described by Gonzalez *et al.* (1978). DNase activity was examined by using DNase agar (Qingdao Hope Bio-technology) according to the manufacturer's instructions. Activities of constitutive enzymes, the fermentation/oxidation profile, acid production, and substrate utilization as sole carbon and energy source were determined using API 20E, API 20NE, API 50CH, API ZYM strips (bioMérieux) and the GN2 MicroPlate kit (Biolog) according to the manufacturers' instructions except that sterile seawater was used to prepare the inoculum. A transmission electron micrograph of a cell of strain Th68<sup>T</sup> is shown in Fig. S3. Susceptibility to antibiotics was tested on MA plates as described by Buczolits *et al.* (2002) by using antibiotic discs containing (µg per disc): neomycin (30), cephalosporin (14), erythromycin (13), rifampicin (5), streptomycin (10), nalidixic acid (30), kanamycin (30), ampicillin (10), gentamicin (10), tetracycline (30) and lincomycin (20).

Cells of strain Th68<sup>T</sup> were Gram-stain-negative, aerobic, non-motile rods (0.2–0.4 µm in width, 1.0–1.6 µm in length). Colonies on MA were orange, convex, circular and 1.0–1.5 mm in diameter after culturing for 2–3 days at 28 °C. Growth occurred at 16–30 °C (optimum 28 °C). No growth was observed at 10 or 37 °C. The salinity range for growth of strain Th68<sup>T</sup> was 2–6 % (w/v) (optimum 3 %) and the pH range was 6.0–8.0 (optimum pH 7.0). Cells were oxidase-positive and catalase-negative; flexirubin-type pigments were produced. Other morphological, physiological and biochemical characteristics of the isolate and related strains are given in the species description and in Table 1.

For cellular fatty acid analysis, the isolate and reference strains were grown on MA at 28 °C for 2–3 days until they reached the mid-exponential phase. Fatty acid methyl esters were prepared and analysed according to the standard protocol of MIDI (Sherlock Microbial Identification System,

version 6.0), and identified by the TSBA6.0 database of the Microbial Identification System (Sasser, 1990). For analysis of respiratory quinones and polar lipids, cells were harvested from MB after incubation at 28 °C for 72 h and freeze-dried. Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984), and separated by two-dimensional TLC on silica gel 60 F254 plates (Merck) using chloroform/methanol/water (65:25:4, v/v) for the first dimension and chloroform/methanol/acetic acid/water (80:12:15:4, v/v) for the second dimension (Collins & Shah, 1984). The identification of individual lipid spots was performed by spraying with the appropriate detection reagents (Komagata & Suzuki, 1987). The respiratory quinones were extracted with chloroform/methanol (2:1, v/v), separated by TLC and identified by HPLC as described by Xie & Yokota (2003). DNA was extracted according to the procedure of Moore *et al.* (1999) and the G+C content was determined according to Mesbah & Whitman (1989).

The cellular fatty acid profiles of the new isolate and the reference strains are listed in Table 2. The dominant fatty acids of strain Th68<sup>T</sup> were iso-C<sub>15:0</sub> (32.1 %), iso-C<sub>15:1</sub> G (29.1 %), iso-C<sub>17:0</sub> 3-OH (9.0 %) and iso-C<sub>15:0</sub> 3-OH (8.0 %). Phosphatidylethanolamine (PE), two unknown aminolipids (AL1, AL2) and two unknown polar lipids (L1, L2) were the major polar lipids (Fig. S4). The major respiratory quinone was menaquinone 6 (MK-6). The DNA G+C content of the isolate was 33.0 mol%, which fell within the range of members of the family *Flavobacteriaceae* (27–44 mol%).

Phylogenetic analyses based on 16S rRNA gene sequences showed that strain Th68<sup>T</sup> represented a distinct phyletic line that reflected a new genus status. The phenotypic characteristics of strain Th68<sup>T</sup> were different from those of related genera within the family *Flavobacteriaceae*, in particular the presence of flexirubin-type pigments, indole production, nitrate reduction, production of arginine dihydrolase and fermentation of arabinose. As compared with other members of *Flavobacteriaceae*, strain Th68<sup>T</sup> had a very narrow temperature range (16–30 °C) for growth. The polar lipid pattern of strain Th68<sup>T</sup> was different from those of *Winogradskyella*, *Algibacter*, *Flavivirga*, *Formosa*, *Flaviramulus* and *Gaetbulibacter*, but not *Bizionia*. However, strain Th68<sup>T</sup> was clearly distinguishable from the genus *Bizionia* based on the catalase test and hydrolysis of casein, agar, Tween 40 and Tween 80. There were also notable differences between the fatty acid profiles of strain Th68<sup>T</sup> and phylogenetically related genera, particularly in the proportion of iso-C<sub>15:0</sub> (32.1 %) and iso-C<sub>15:1</sub> G (29.1 %). The DNA G+C content of strain Th68<sup>T</sup> was lower than those of all the closely related bacteria except *Flavivirga* (Yi *et al.*, 2012) which had a lower DNA G+C content than strain Th68<sup>T</sup>. Based on phylogenetic analysis as well as phenotypic and biochemical data, strain Th68<sup>T</sup> should be classified as a member of a novel species in a new genus, for which the name *Flavirhabdus iliipiscaria* gen. nov., sp. nov. is proposed.

**Table 1.** Differential characteristics between strain Th68<sup>T</sup> and other members of the family *Flavobacteriaceae*

Strains: 1, Th68<sup>T</sup>; 2, *Winogradskyella thalassocola* LMG 22492<sup>T</sup> (Nedashkovskaya *et al.*, 2005); 3, *Algibacter pectinivorans* JCM 17107<sup>T</sup> (Yi *et al.*, 2011); 4, *Flaviramulus basaltis* DSM 18180<sup>T</sup> (Einen & Øvreås, 2006); 5, *Gaetbulibacter saemankumensis* KCTC 12379<sup>T</sup> (Jung *et al.*, 2005); 6, *Bizionia paragorgiae* LMG 22571<sup>T</sup> (Nedashkovskaya *et al.*, 2004); 7, *Formosa algae* KCTC 12364<sup>T</sup> (Ivanova *et al.*, 2004); 8, *Flavivirga jejuensis* JCM 17113<sup>T</sup> (Yi *et al.*, 2012). All data were from this study except DNA G+C content of the reference strains, which were from the original references. o, Orange; y, yellow; l, light; +, positive reaction; −, negative reaction; w, weak positive reaction.

Characteristic	1	2	3	4	5	6	7	8
Colony colour	o	o	y	y	y	o	l-y	l-o
Temperature range for growth (°C)	16–30	4–33	4–35	−2 to 30	13–40	4–36	4–35	4–30
Salinity range (%)	2–6	2–3	2–3	2–5	0.5–6	1–8	0–6	2–5
Cell size (µm)	1.0–1.6	1.9–2.3	1.0–2.4	1–3	3–4.5	1.9–2.3	0.81.8	3.3–7.5
Gliding motility	−	+	+	+	+	−	+	+
Presence of flexirubin-type pigments	+	−	−	−	−	−	−	−
Oxidase	+	+	+	−	+	+	−	−
Catalase	−	+	−	+	+	+	+	−
Hydrolysis of:								
Agar	−	+	+	+	−	+	−	−
Gelatin	+	+	−	+	−	+	w	+
Starch	−	−	−	+	+	−	−	+
Casein	−	+	−	+	−	+	−	−
Asculin	−	−	+	+	+	−	+	+
DNA	−	−	−	+	−	−	−	+
Tween 20	+	+	+	+	−	+	−	−
Tween 40	−	+	−	+	−	+	+	−
Tween 80	−	+	+	+	−	+	−	+
Enzyme activities (API ZYM)								
Valine arylamidase	+	+	−	−	+	+	+	+
α-Chymotrypsin	−	w	−	+	+	w	+	−
Naphthol-AS-BI-phosphohydrolase	−	+	+	−	−	+	+	w
N-Acetyl-β-glucosaminidase	−	−	−	+	−	−	−	+
API 20E results								
Arginine dihydrolase	+	−	−	−	−	−	−	−
Tryptophan degradation	+	−	−	−	−	−	−	−
Arabinose	+	−	−	+	−	−	−	−
Nitrate reduction	+	−	−	−	+	−	+	+
Polar lipids*	PE, 2AL, 2L	PE, 2AL, 2L	PE, 2AL, 1GL, 2PL, 4L	†PE, AL, 3L	PE, 2AL, 4L	PE, 2AL, 2L	PE, 7AL, 1L	PE, 1AL, 4L
DNA G+C content (mol%)	33.0	37.6	38.6	31.4	34.7–34.9	37.6	34.4	27.0

\*PE, phosphatidylethanolamine; AL, unidentified aminolipids; L, unidentified polar lipids; PL, unidentified phospholipids.

†Reported by Zhang *et al.* (2013).

### Emended description of the genus *Flavivirga* Yi *et al.* (2012)

The description of the genus is as given by Yi *et al.* (2012) with the following amendments. Cells are catalase-negative. Starch can be degraded by cells. The major polar lipids are phosphatidylethanolamine, one unidentified aminolipid and four unidentified polar lipids.

### Emended description of the genus *Algibacter* Yi *et al.* (2011)

The description of the genus is as given by Yi *et al.* (2011) with the following amendments. Cells are catalase-negative. The major polar lipids are phosphatidylethanolamine, two unidentified aminolipids, one glycolipid, two phospholipids and four unidentified polar lipids.

**Table 2.** Cellular fatty acid composition (%) of strain Th68<sup>T</sup> and closely related species

Strains: 1, Th68<sup>T</sup>; 2, *Winogradskyella thalassocola* LMG 22492<sup>T</sup>; 3, *Algabacter pectinivorans* JCM 17107<sup>T</sup>; 4, *Flaviramulus basaltis* DSM 18180<sup>T</sup>; 5, *Gaetbulibacter saemankumensis* KCTC 12379<sup>T</sup>; 6, *Bizionia paragorgiae* LMG 22571<sup>T</sup>; 7, *Formosa algae* KCTC 12364<sup>T</sup>; 8, *Flavivirga jejuensis* JCM 17113<sup>T</sup>. All data were taken from this study. TR, Trace amount (<1%); –, not detected. Fatty acids amounting to <1% of the total fatty acids in all strains are not shown. Fatty acids representing more than 5% are highlighted in bold.

Fatty acid	1	2	3	4	5	6	7	8
<b>Straight chain</b>								
C <sub>16:0</sub>	2.4	3.0	<b>5.4</b>	4.1	4.3	<b>6.0</b>	<b>7.4</b>	3.0
C <sub>18:0</sub>	TR	–	2.0	2.4	1.4	3.0	4.0	TR
<b>Branched</b>								
iso-C <sub>13:0</sub>	3.0	–	2.0	TR	2.0	TR	–	3.3
iso-C <sub>14:0</sub>	2.0	–	2.0	1.4	TR	3.0	TR	2.3
iso-C <sub>15:0</sub>	<b>32.1</b>	<b>10.0</b>	<b>10.0</b>	<b>14.3</b>	<b>21.0</b>	<b>21.0</b>	<b>16.1</b>	<b>25.4</b>
iso-C <sub>15:1</sub> G	<b>29.1</b>	<b>15.0</b>	<b>14.0</b>	<b>16.0</b>	<b>12.0</b>	<b>11.0</b>	<b>11.0</b>	<b>11.0</b>
anteiso-C <sub>15:1</sub> A	TR	2.2	1.4	1.3	TR	–	1.0	TR
anteiso-C <sub>15:0</sub>	2.0	4.0	<b>6.0</b>	5.0	<b>8.3</b>	3.4	<b>6.1</b>	1.4
iso-C <sub>16:0</sub>	2.0	2.0	0.41	TR	TR	<b>7.0</b>	3.0	2.0
iso-C <sub>16:1</sub> H	3.0	4.0	TR	–	–	<b>5.3</b>	2.0	TR
iso-C <sub>16:1</sub> G	TR	TR	TR	–	–	–	–	2.0
<b>Hydroxy</b>								
C <sub>15:0</sub> 2-OH	TR	2.0	2.1	2.0	2.0	TR	2.0	TR
C <sub>15:0</sub> 3-OH	TR	2.0	3.1	3.0	1.3	TR	2.3	1.2
iso-C <sub>15:0</sub> 3-OH	<b>8.0</b>	<b>10.2</b>	<b>15.0</b>	<b>15.0</b>	<b>8.1</b>	5.0	<b>7.2</b>	<b>13.0</b>
C <sub>16:0</sub> 3-OH	TR	2.0	4.0	2.0	1.3	TR	–	2.1
iso-C <sub>16:0</sub> 3-OH	4.0	<b>17.0</b>	<b>7.0</b>	<b>6.0</b>	3.0	<b>9.0</b>	<b>7.0</b>	4.1
C <sub>17:0</sub> 2-OH	TR	–	1.3	1.2	2.1	TR	–	TR
iso-C <sub>17:0</sub> 3-OH	<b>9.0</b>	<b>7.0</b>	<b>12.0</b>	<b>13.0</b>	<b>14.3</b>	<b>7.3</b>	<b>6.0</b>	<b>16.0</b>
<b>Unsaturated</b>								
C <sub>15:1</sub> ω6c	2.0	<b>6.0</b>	2.0	1.1	TR	–	6.0	1.1
C <sub>17:1</sub> ω6c	–	1.1	–	TR	1.1	3.0	–	–
C <sub>18:1</sub> ω6c	TR	–	TR	1.1	–	1.4	2.0	1.0
<b>Summed feature 3*</b>	3.4	<b>7.4</b>	<b>6.0</b>	<b>7.0</b>	<b>12.3</b>	<b>6.0</b>	<b>6.5</b>	3.3
<b>Summed feature 9*</b>	–	–	–	–	–	2.1	2.0	1.2

\*Summed features are groups of two or three fatty acids that are treated together for the purpose of evaluation in the MIDI system and include both peaks with discrete ECLs as well as those where the ECLs are not reported separately. Summed feature 3 comprised C<sub>16:1</sub>ω6c and/or C<sub>16:1</sub>ω7c; summed feature 9 comprised 10-methyl C<sub>16:0</sub> and/or iso-C<sub>17:1</sub>ω9c.

**Emended description of the genus *Formosa* Ivanova et al. (2004)**

The description of the genus is as given by Ivanova et al. (2004) with the following amendments. Tween 20 is not utilized by cells. The major polar lipids are phosphatidylethanolamine, seven aminolipids and one unidentified polar lipid.

**Description of *Flavirhabdus* gen. nov.**

*Flavirhabdus* (Fla.vi. rhab'dus. L. adj. *flavus* yellow; Gr. fem. n. *rhabdos* rod, stick, wand; N.L. fem. n. *Flavirhabdus* a yellow rod).

Cells are Gram-stain-negative, aerobic, oxidase-positive and catalase-negative. Cells are rods with rounded ends and without gliding motility. Spores are not formed. Cells produce flexirubin-type pigments and require sea salts for

growth. The major isoprenoid quinone is menaquinone 6 (MK-6). The major polar lipids are phosphatidylethanolamine, two unidentified aminolipids and two unidentified polar lipids. Phylogenetically, it is a member of the family *Flavobacteriaceae* of the class *Flavobacteria* of the phylum *Bacteroidetes*. The type species is *Flavirhabdus iliipiscaria*.

**Description of *Flavirhabdus iliipiscaria* sp. nov.**

*Flavirhabdus iliipiscaria* (i.li.i.pis.ca'ri.a. L. n. *ilium* entrails, intestine; L. adj. *piscarius* of or belonging to fish; N.L. fem. adj. *iliipiscaria* belonging to intestines of fish).

Has the following characteristics in addition to those given for the genus. Cells are 0.2–0.4 μm in width and 1.0–1.6 μm in length. Colonies on MA are orange, convex, circular and 1.0–1.5 mm in diameter after culturing for 2–3 days at 28 °C. Growth occurs at 16–30 °C (optimum



28 °C), with 2–6% sea salts (optimum 3%) and at pH 6.0–8.0 (optimum 7.0). Hydrolyses gelatin, Tween 20 and casein, but not DNA, egg yolk, cellulose, chitin, starch, Tween 40 or Tween 80. In API 20NE strips, positive for reduction of nitrates to nitrites, indole production, glucose fermentation, hydrolysis of gelatin and aesculin, and arabinose assimilation. In API 20E strips, positive for tryptophan, gelatin and urea degradation, indole production, and fermentation of arabinose and mannitol, but negative for all other characteristics. In API ZYM strips, positive for alkaline phosphatase, valine arylamidase and trypsin, but negative for esterase (C4), leucine arylamidase, cysteine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, esterase lipase (C8), lipase,  $\alpha$ -chymotrypsin,  $\alpha$ -galactosidase,  $\beta$ -galactosidase,  $\beta$ -glucuronidase,  $\beta$ -glucosidase, *N*-acetyl- $\beta$ -glucosaminidase,  $\alpha$ -mannosidase and  $\alpha$ -fucosidase activities. In the API 50CH system, acid is produced from glycerol, L-arabinose, D-ribose, D-xylose, D-glucose, D-fructose, D-mannitol, *N*-acetylglucosamine, maltose, sucrose, trehalose, raffinose, starch, glycogen and gentiobiose but not from any of the other substrates. In the Biolog GN2 Micro Plate system, only L-asparagine can be used. The dominant fatty acids are iso-C<sub>15:0</sub>, iso-C<sub>15:1</sub> G, iso-C<sub>17:0</sub> 3-OH and iso-C<sub>15:0</sub> 3-OH. Susceptible to neomycin, cephalosporin, erythromycin and rifampicin; resistant to streptomycin, nalidixic acid, kanamycin, ampicillin, gentamicin, tetracycline and lincomycin.

The type strain, Th68<sup>T</sup> (=JCM 18637<sup>T</sup>=KCTC 32141<sup>T</sup>), was isolated from the intestine of cultured flounder in Shandong Province, China. The DNA G + C content of the type strain is 33.0 mol%.

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## Reference

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