Ichthyenterobacterium magnum gen. nov., sp. nov., a member of the family *Flavobacteriaceae* isolated from olive flounder (*Paralichthys olivaceus*)

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A novel marine bacterium isolated from the intestine of cultured flounder (*Paralichthys olivaceus*) was studied by using a polyphasic taxonomic approach. The isolate was Gram-stain-negative, pleomorphic, aerobic, yellow and oxidase- and catalase-negative. Phylogenetic analysis of 16S rRNA gene sequences indicated that isolate Th6^T formed a distinct branch within the family *Flavobacteriaceae* and showed 96.6 % similarity to its closest relative, *Bizionia hallyeonensis* T-y7^T. The DNA G+C content was 29 mol%. The major respiratory quinone was MK-6. The predominant fatty acids were iso-$C_{15:1}$ G, iso-$C_{15:0}$, iso-$C_{15:0}$ 3-OH, iso-$C_{17:0}$ 3-OH and summed feature 3 ($C_{15:1}\omega6c$ and/or $C_{16:1}\omega7c$). On the basis of the phenotypic, chemotaxonomic and phylogenetic characteristics, the novel bacterium has been assigned to a novel species of a new genus for which the name *Ichthyenterobacterium magnum* gen. nov., sp. nov. is proposed. The type strain is Th6^T (=JCM 18636^T=KCTC 32140^T).

The family *Flavobacteriaceae* was proposed by Jooste (1985) and was included in the first edition of *Bergey's Manual of Systematic Bacteriology* (Reichenbach, 1989). The name of the family was subsequently validly published (Reichenbach, 1992) and an emended description was published (Bernardet *et al.*, 1996). At the time of writing, the bacterial family *Flavobacteriaceae* comprises 114 genera with validly published names (http://eztaxon-e.ezbiocloud.net). Members of the family *Flavobacteriaceae* are widespread and found in various habitats, such as: soil; food and dairy products; freshwater environments; marine environments; diseased freshwater and marine fish; diseased molluscs, crustaceans and sea urchins; diseased amphibians and reptiles; diseased birds; diseased dogs and cats; the eggs and digestive tracts of insects; human specimens and hospital equipment and devices (Bernardet *et al.*, 2006). Here we report on a novel strain, Th6^T, isolated from the intestine of cultured flounder (*Paralichthys olivaceus*) and the determination of its taxonomic status using a polyphasic approach.

Strain Th6^T was isolated from the intestine of cultured flounder (*Paralichthys olivaceus*) collected from a fish farm in Shandong province, PR China, in the course of screening for quorum-quenching bacteria (Tang *et al.*, 2013). The surface of the fish was cleansed with 75 % (v/v) alcohol and a section of intestine (including intestinal tissue and fluid) was sampled aseptically by dissecting the fish. Th6^T was isolated from the tissue homogenate by the plate spreading method on marine agar 2216 (MA; Becton Dickinson) at 28 °C and purified by streaking three times on MA. *Bizionia hallyeonensis* T-y7^T, *Bizionia paragorgiae* LMG 22571^T, *Bizionia echini* LMG 25220^T, *Psychroserpens burtonensis* LMG 22918^T, *Formosa algae* KCTC 12364^T, *Winogradskyella thalassocola* LMG 22492^T and *Flaviramulus basaltis* DSM 18180^T were used as reference strains. Cultures of isolate Th6^T and all the reference strains were maintained at 16 °C for short-term preservation and in sterile 0.85 % (w/v) saline solution supplemented with 15 % (v/v) glycerol at −80 °C for long-term preservation. For phenotypic analysis all strains were cultivated on MA medium at 28 °C except for *Psychroserpens burtonensis*, which was cultivated at 10 °C.

The genomic DNA of strain Th6^T was extracted and the 16S rRNA gene was obtained by PCR amplification with two universal primers (B8F: 5′-AGAGTTTGAT CCTGGCTCAG-3′ and 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 a pUCm-T vector (TaKaRa) and sequenced at BGI (Qingdao, China). The EzTaxon-e server was used for the 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 by using the CLUSTAL X program (Thompson *et al.*, 1997). Phylogenetic trees were

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of Th6^T is JX412959.

Four supplementary figures are available with the online Supplementary Material.

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.

Comparative phylogenetic analysis of the 16S rRNA gene sequence indicated that strain Th6^T had highest pairwise similarity with *B. hallyeonensis* T-y7^T (96.6 %), followed by other members of the genera: *Bizionia* (96.5–94.7 %), *Psychroserpens* (96.1–95.3 %), *Formosa* (96.1–94.3 %), *Geojedonia* (95.7 %), *Winogradskyella* (95.6–93.7 %), *Olleya* (95.4–94.7 %) and *Flaviramulus* (95.1–94.5 %). Other species belonging to the family *Flavobacteriaceae* showed less than 95.0 % 16S rRNA gene sequence similarity to isolate Th6^T. Phylogenetic analysis based on the neighbour joining (Fig. 1), maximum-likelihood and maximum-parsimony (Figs S1 and S2, available in the online Supplementary Material) algorithms of the almost-complete 16S rRNA gene (1488 bp) of isolate Th6^T revealed that the strain formed a distinct lineage within the family *Flavobacteriaceae*.

Genomic DNA of strain Th6^T was extracted according to the procedure of Moore *et al.* (1999) and the G+C content was determined according to the method of Mesbah & Whitman (1989). Gram staining and flagellum staining were investigated using standard methods (Beveridge *et al.*, 2007). Cell morphology was determined by transmission electron microscopy (JEOL; JEM-1200EX) after cells had been negatively stained with 1 % (w/v) phosphotungstic acid. 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 AaneroPack-Anaero (Mitsibishi Gas Chemical) at 28 °C for one month. The temperature range for growth was determined at 4, 10, 16, 20, 24, 28, 32, 37 and 42 °C. The salinity and pH ranges that could support growth were investigated in 96-well microplates by measuring the optical densities (at a wavelength of 590 nm) at 0, 12, 24, 48, 72, 96 and 120 h, respectively. The tolerance to salinity of the bacterium was determined by using synthetic marine ZoBell broth comprising (in modified artificial seawater, 1^{-1}) 5 g bacto peptone, 1 g yeast extract and 0.1 g $FePO_4$ (Lyman & Fleming, 1940) supplemented with 0–15 % (w/v) NaCl. The modified artificial seawater containing 5 g $MgCl_2$, 2 g $MgSO_4$, 0.5 g $CaCl_2$, 1 g KCl, 8 mg K_2HPO_4 and 22 mg H_3BO_3 in 1 liter (Smibert & Krieg, 1994). Growth in marine broth (MB; Difco) was evaluated at pH 2.0–10.0 at intervals of 1 pH unit using the following buffer systems: Na_2HPO_4/citric acid (pH 2.0–7.0), Tris/HCl (pH 8.0–9.0) and Na_2CO_3/$NaHCO_3$ (pH 9.0–10.0). The pH was verified after autoclaving MB. Various phenotypic characteristics of the isolate and reference strains were tested according to standard approaches (Tindall *et al.*, 2007) including the activities of catalase and oxidase, the hydrolysis of starch, egg yolk, casein [5 % (w/v) skimmed milk; Difco], gelatin (method 2). The degradation of chitin (0.5 %; Sigma) was examined as described by (Hsu & Lockwood, 1975); CM-cellulose (1 %, w/v) was added to MA plates to determine whether it was degraded; clear zones were formed around colonies after flooding with appropriate solutions (Teather & Wood, 1982). 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 Biotechnology) according to the manufacturer's instructions. Gliding motility was observed by the hanging-drop technique and production of flexirubin-type pigments was estimated from a colour shift following exposure to 20 % (w/v) KOH (Bernardet *et al.*, 2002). Activities of constitutive enzymes and other physiological properties were determined after growth on MA at 28 °C for 2 days using API 20E, API 20NE, API 50CH and API ZYM strips (bioMérieux) and Gram-negative MicroPlates (Biolog), according to the manufacturers' instructions, except that sterile seawater was used to prepare the inocula. Susceptibility to antibiotics was investigated on MA plates by using discs containing different antibiotics (Hangzhou Microbiology Reagent); antimicrobial susceptibility testing was performed by using the agar diffusion method using antibiotic-impregnated discs (Oxoid) as described by Buczolits *et al.* (2002).

Cells of strain Th6^T were Gram-stain-negative, aerobic, non-motile, pleomophic rods (1.5– 20 μm in length, Fig. S3). Colonies were light yellow, circular (1.0–1.5 mm in diameter), convex and slightly transparent with entire margins on MA after culturing for 2–3 days at 28 °C. Growth occurred at 16–32 °C, with an optimum growth rate at 28 °C. No growth was observed at 10 °C or 37 °C. The salinity range for growth of the isolate was 0–8 % (w/v), with an optimum growth rate at 2–3 % (w/v) NaCl. The pH range for growth was pH 6.0–8.0, with an optimum growth rate at pH 7. Other morphological, physiological and biochemical characteristics of the isolate and related strains are given in the species description and Table 1.

For cellular fatty acid analysis, the isolate and the reference strains were grown on MA at 28 °C for 2–3 days until they reached the mid-exponential phase, except for *Psychroserpens burtonensis*, which was grown at 10 °C for 10 days. 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, by vol.) for the first dimension and chloroform/methanol/acetic acid/water (80 : 12 : 15 : 4, by vol.) 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,

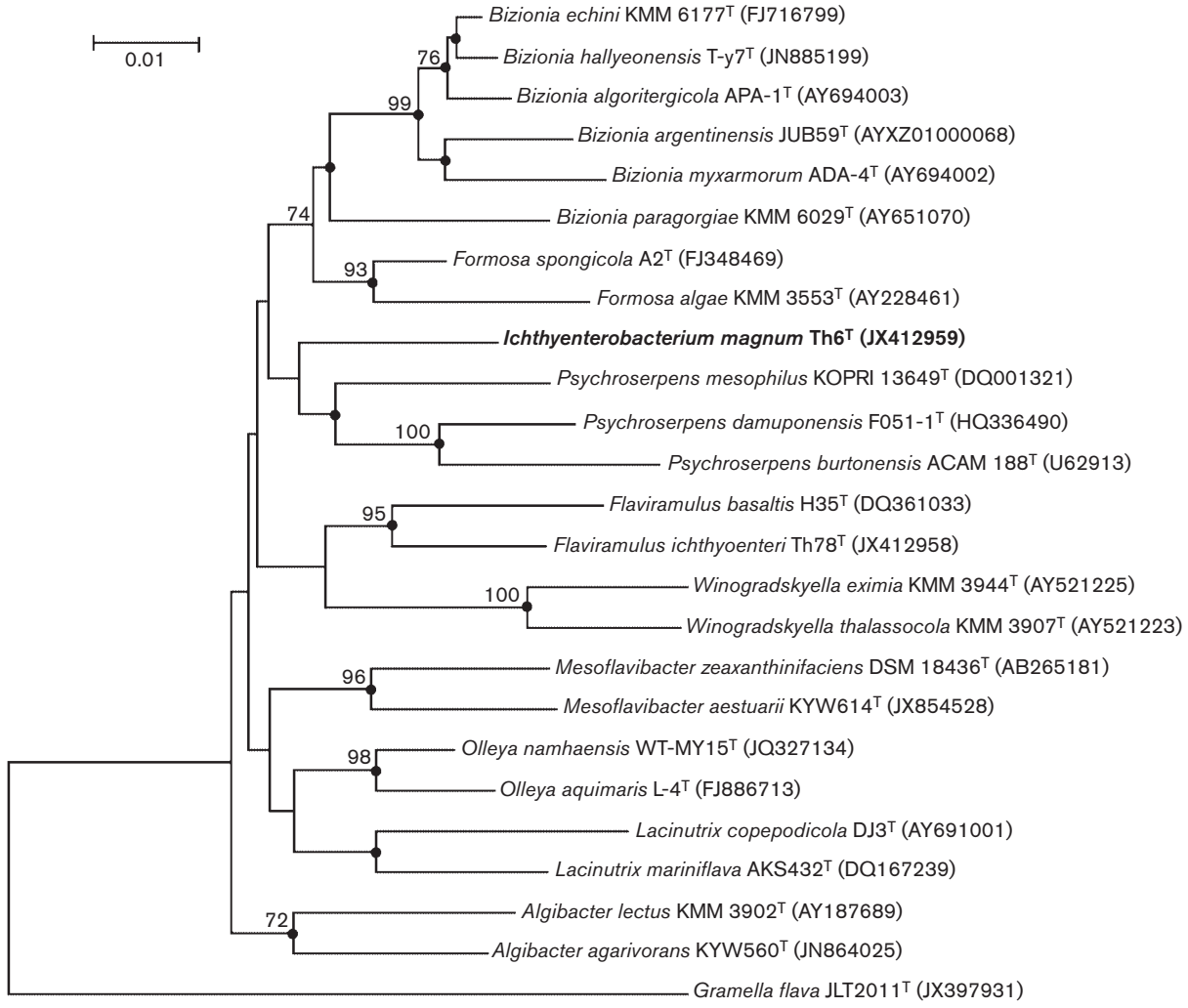

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic positions of isolate Th6^T and representative strains of species of related genera of the family *Flavobacteriaceae*. Bootstrap values (percentages) above 70 (1000 replicates) are shown at branch points. Filled circles indicate that the corresponding points were also recovered in trees generated with the maximum-parsimony and maximum-likelihood algorithms. *Gramella flava* JLT2011^T (JX397931) was used as an out-group. Bar, 0.01 substitutions per nucleotide position.

v/v), separated by TLC and identified by HPLC, as described by Xie & Yokota (2003).

The major cellular fatty acids were iso-$C_{15:0}$ (21.0 %), iso-$C_{15:1}$ G (18.4 %), iso-$C_{17:0}$ 3-OH (14.0 %), iso-$C_{15:0}$ 3-OH (13.0 %) and summed feature 3 (18.0 %). The cellular fatty acid profiles of strain Th6^T and the reference strains are listed in Table 2. The polar lipid profile of strain Th6^T comprised lysylphosphatidylglycerol, diglucosyl diacylglycerol, one unidentified glycolipid, two unidentified phospholipids and two unknown polar lipids (Fig. S4). The major respiratory quinone was MK-6. The DNA G+C content of the isolate was 29 %, which is lower than those of closely related bacteria and fell within the DNA G+C content range of members of the family *Flavobacteriaceae* (27–44 mol%). The results of the phylogenetic and phenotypic analyses demonstrated the novel generic status of isolate Th6^T. Pairwise similarity analysis revealed that the novel strain exhibited a 16S RNA gene sequence similarity value of 96.6 % to its closest relative *B. hallyeonensis* T-y7^T. Although there is no precise correlation between 16S rRNA gene sequence similarities and species delineation it is generally recognized that a divergence value $\geqslant$3 % is significant. (Stackebrandt & Goebel, 1994). The neighbour-joining tree shows that isolate Th6^T occupies a phylogenetic position within the family *Flavobacteriaceae* (Fig. 1). The topology of the phylogenetic trees generated using the maximum-parsimony and maximum-likelihood methods (Figs. S1 and S2) was different from that of the tree reconstructed using the neighbour joining method. Nevertheless, strain Th6^T formed a distinct lineage within

Table 1. Differential characteristics of strain Th6^T and other closely related members of the family *Flavobacteriaceae*

Strains: 1, *Ichthyenterobacterium magnum* gen. nov., sp. nov. Th6^T; 2, *B. hallyeonensis* KCTC 23881^T (Yoon *et al.*, 2013); 3, *B. echini* LMG 22520^T (Nedashkovskaya *et al.*, 2010); 4, *B. paragorgiae* LMG 22571^T (Nedashkovskaya *et al.*, 2005b); 5, *W. thalassocola* LMG 22492^T (Nedashkovskaya *et al.*, 2005a); 6, *Psychroserpens burtonensis* LMG 22918^T (Bowman *et al.*, 1997); 7, *Formosa algae* KCTC 12364^T (Ivanova *et al.*, 2004); 8, *Flaviramulus basaltis* DSM 18180^T (Einen and Øvreås, 2006). All data are from this study, except for the DNA G+C contents of the reference strains, which were from the original species description. +, Positive reaction; −, negative reaction; w, weakly positive reaction; LPG, lysylphosphatidylglycerol; G, diglucosyl diacylglycerol; PE, phosphatidylethanolamine; AL, unidentified aminolipids; G1, unidentified glycolipid; L, unidentified polarlipids; P, unidentified phospholipids.

Characteristic	1	2	3	4	5	6	7	8
Cell size	1.5–20 μm	0.8–4.5 μm	1.4–3.5 μm	1.9–2.3 μm	4–7.3 μm	2–6 μm	0.8–1.8 μm	1–3 μm
Cell shape	Pleomorphic rod	Regular rod	Regular rod	Regular rod	Regular rod	Pleomorphic rod	Regular rod	Pleomorphic rod
Gliding motility	−	−	−	−	+	−	+	+
Temperature range for growth	16–32	4–35	4–39	4–36	4–33	0–18	5–35	2–30
Salinity range for growth (%, w/v)	0–8	0–9	1–8	1–8	2–3	2–3	0–6	2–5
Growth under microaerophilic conditions	−	−	−	−	−	−	+	−
Presence of flexirubin-type pigments	−	+	+	−	−	−	−	−
Oxidase	−	+	+	+	+	−	−	−
Catalase	−	+	+	+	+	+	+	+
Hydrolysis of:								
Casein	−	+	−	+	−	+	w	+
Agar	+	−	−	+	+	+	−	+
Gelatin	+	−	+	+	+	w	w	+
Tween 20	+	+	−	+	−	−	+	+
Tween 40	+	−	+	+	+	+	+	+
Tween 80	+	+	+	+	−	w	−	+
Enzyme activities (API ZYM)								
Valine arylamidase	w	+	w	+	+	+	+	−
α-Chymotrypsin	−	−	−	−	−	−	+	+
API 20E/20NE								
Glucose	+	−	−	−	+	−	+	−
Arabinose	−	−	+	−	−	−	−	+
Nitrate reduction	+	−	−	−	−	−	+	−
Aesculin	+	−	+	−	−	−	+	−
Polar lipids	LPG, G, G1, 2P, 2L	PE, 2AL, 2L	PG, PE, G, 2L	PE, 2AL, 2L	PE, 3AL, 2L	PE, 2AL, 2L	PE, 7AL, 1L	PE*, AL, 3L
DNA G+C content (% mol)	29	37.1	37.6	34.4	34.6	27–29	34	31.4

*Reported by Zhang *et al.* (2013)

Table 2. Cellular fatty acid composition of isolate Th6^T and strains of some closely related species

Strains: 1, *Ichthyenterobacterium magnum* gen. nov., sp. nov. Th6^T; 2, *Bizionia hallyeonensis* KCTC 23881^T; 3, *Bizionia echini* LMG 22520^T; 4, *Bizionia paragorgiae* LMG 22571^T; 5, *Winogradskyella thalassocola* LMG 22492^T; 6, *Psychroserpens burtonensis* LMG 22918^T; 7, *Formosa algae* KCTC 12364^T; 8, *Flaviramulus basaltis* DSM 18180^T. All data are from this study. The culture conditions and analytical procedures used were the same for all strains, except for *Psychroserpens burtonensis* LMG 22918^T, which was grown at 10 °C for 10 days. TR, Trace amounts (<1 %); −, not detected. Values represent percentages of the total fatty acids. Fatty acids amounting to <1 % of the total fatty acids in all strains are not shown. Fatty acids accounting for more than 5 % of the total are highlighted in bold.

Fatty acid	1	2	3	4	5	6	7	8
Straight chain								
$C_{16:0}$	2.1	4.8	4.7	**5.9**	2.6	1.0	**7.5**	3.7
$C_{18:0}$	−	4.0	2.0	3.0	TR	−	3.9	2.4
Branched								
iso-C $_{14:0}$	TR	1.3	1.0	TR	−	4.3	TR	1.4
iso-$C_{15:1}$ G	**18.4**	**14.0**	**20.0**	**11.0**	**15.0**	**12.2**	**11.0**	**12.0**
iso-$C_{15:0}$	**21.0**	**17.1**	**18.2**	**21.0**	**10.0**	**11.3**	**16.1**	**21.0**
anteiso-$C_{15:1}$ A	TR	TR	TR	TR	2.2	8.3	1.0	TR
anteiso-$C_{15:0}$	TR	2.0	TR	4.0	4.0	**14.3**	6.1	4.7
iso-$C_{16:0}$	1.3	4.3	3.3	**7.0**	2.0	3.0	3.0	1.1
iso-$C_{16:1}$ H	−	3.0	−	**5.3**	4.0	−	2.0	TR
iso-$C_{16:1}$ G	TR	TR	2.6	TR	TR	**5.2**	−	1.0
Hydroxy								
$C_{14:0}$ 3-OH	TR	TR	TR	−	−	1.0	−	TR
$C_{15:0}$ 2-OH	1.0	1.0	TR	TR	2.0	4.4	2.0	1.7
$C_{15:0}$ 3-OH	TR	2.0	2.0	TR	1.6	1.4	2.3	3.3
iso-$C_{15:0}$ 3-OH	**13.0**	4.0	5.0	5.0	**10.3**	4.0	**7.2**	**13.3**
$C_{16:0}$ 3-OH	1.3	2.0	1.20	−	2.0	−	−	2.0
iso-$C_{16:0}$ 3−OH	3.0	**5.4**	4.0	**9.0**	**17.0**	**13.0**	**7.0**	**6.0**
$C_{17:0}$ 2-OH	TR	−	−	−	−	3.0	−	TR
iso-$C_{17:0}$ 3-OH	**14.0**	**11.3**	**11.0**	**7.3**	**7.0**	4.0	**6.0**	**13.0**
Unsaturated								
$C_{15:1}\omega6c$	−	2.0	1.4	TR	**6.0**	2.0	**6.0**	1.4
$C_{17:1}\omega6c$	TR	2.7	1.8	1.5	1.1	TR	2.3	TR
$C_{18:1}\omega6c$	−	TR	TR	1.4	TR	TR	2.0	1.2
Summed features*								
3	**18.0**	**13.5**	**14.0**	**6.0**	**7.4**	2.1	**7.0**	**6.4**
9	2.0	3.5	4.4	2.5	−	−	2.0	−

*Summed features are groups of two or three fatty acids that are treated together for the purposes 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 $C_{16:1}\omega6c$ and/or $C_{16:1}\omega7c$. Summed feature 9 comprised 10-methyl $C_{16:0}$ and/or iso- $C_{17:1}\omega9c$.

the clade containing species of the genera *Bizionia*, *Formosa*, *Psychroserpens*, *Flaviramulus* and *Winogradskyella*. The phylogenetic position of strain Th6^T was strongly supported by its phenotypic characteristics. Strain Th6 was clearly distinguishable from members of the genera *Bizionia*, *Formosa*, *Psychroserpens*, *Flaviramulus* and *Winogradskyella* due to its pleomorphic nature, temperature range over which it grows, DNA G+C content and polar lipid profile. Similarly, degradation of agar and gelatin, catalase and oxidase activities, glucose and aesculin degradation, nitrate reduction, acid production from trehalose, glycogen and gentiobiose and the fatty acid composition, particularly the abundance of iso-$C_{15:0}$ 3-OH, iso-$C_{17:0}$ 3-OH and summed feature 3, support it being assigned to a novel generic position.

Based on this phylogenetic distinctiveness and the significant differences in phenotypic features, we propose that isolate Th6^T should be classified as representative of a novel genus and species, for which the name *Ichthyenterobacterium magnum* gen. nov., sp. nov. is proposed.

Description of *Ichthyenterobacterium* gen. nov.

Ichthyenterobacterium (Ich.thy.o.en′te.ri.bac.te′ri.um. Gr. n. *ichthys* fish; Gr. n. *enteron* gut; L. neut. n. *bacterium* a small rod; N.L. neut. n. *Ichthyenterobacterium* a rod from fish gut).

Cells are Gram-stain-negative, aerobic and oxidase- and catalase-negative. Gliding motility is not observed. Sea salts are required for growth. The major polar lipids are lysylphosphatidylglycerol, diglucosyl diacylglycerol, two unidentified phospholipids, one unidentified glycolipid and two unidentified polar lipids. As determined by 16S rRNA gene sequence analysis, the genus is a member of the family *Flavobacteriaceae*. The type species is *Ichthyenterobacterium magnum*.

Description of *Ichthyenterobacterium magnum* sp. nov.

Ichthyenterobacterium magnum (mag′num. L. n. *magnus* big, referring to cell size).

Displays the following characteristics in addition to those given for the genus. Colonies on MA are light yellow, convex, transparent, glistening and circular with entire margins. Cell morphology varies with the age of the culture. In the exponential phase, cells are curved rods, 0.5 to 0.6 μm in diameter and 1.5 to 5 μm in length, and form compact microcolonies. In the stationary phase, cells are pleomorphic rods of vibroid and spiral-shape and up to 20 μm in length; helical shapes also occur. Flexirubin-type pigment is absent (KOH test-negative). Growth occurs at 16–32 °C (optimum, 28 °C). The pH range for growth is pH 6.0–8.0, with an optimum growth rate at pH 7.0. Positive for Tween 20, Tween 40, Tween 80, starch, agar, gelatin and DNA degradation; negative for hydrolysis of casein, cellulose (CM-cellulose), chitin and egg yolk. In API 20E and 20NE strips, positive for reduction of nitrate to nitrite, glucose fermentation, hydrolysis of aesculin and urease.

In API ZYM strips, alkaline phosphatase, esterase lipase, leucine arylamidase and naphthol-AS-BI-phosphohydrolase

are present. Activities of esterase, lipase and valine arylamidase are weakly positive. Cystine arylamidase, trypsin, α-chymotrypsin acid phosphatase, α-galactosidase, β-glucosidase, β-glucuronidase, α-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are absent. In API 50 CH strips, acid is produced from aesculin, cellobiose, maltose, lactose, starch, glycogen and potassium 5-ketogluconate, but not from other substrates. The following carbon sources are utilized in the Biolog GN2 Micro Plate system: hydroxy-L-proline, L-ornithine, L-glutamic acid, glycyl-L-aspartic acid and glycyl-L-glutamic acid. Susceptible to neomycin, cephalosporin, erythromycin, rifampicin, streptomycin, nalidixic acid, kanamycin, ampicillin, gentamicin, tetracycline and lincomycin. The major respiratory quinone is MK-6 and the major cellular fatty acids are iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH, iso-$C_{15:0}$ 3-OH and summed feature 3.

The type strain, Th6^T (=JCM 18636^T=KCTC 32140^T), was isolated from the intestine of flounder fish. The genomic DNA G+C content of the type strain is 29 mol%.

Acknowledgements

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References

Bernardet, J. F. & Nakagawa, Y. (2006). An introduction to the family *Flavobacteriaceae*. In *The Prokaryotes*, pp. 455–480. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer & E. Stackebrandt. New York: Springer.

Bernardet, J.-F., Segers, P., Vancanneyt, M., Berthe, F., Kersters, K. & Vandamme, P. (1996). Cutting a Gordian knot: emended classification and description of the genus *Flavobacterium*, emended description of the family *Flavobacteriaceae*, and proposal of *Flavobacterium hydatis* nom. nov. (basonym, *Cytophaga aquatilis* Strohl and Tait 1978). *Int J Syst Bacteriol* **46**, 128–148.

Bernardet, J.-F., Nakagawa, Y., Holmes, B. & Subcommittee on the taxonomy of Flavobacterium and Cytophaga-like bacteria of the International Committee on Systematics of Prokaryotes (2002). Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* **52**, 1049–1070.

Beveridge, T., Lawrence, J. & Murray, R. (2007). Sampling and staining for light microscopy. *In Methods for General and Molecular Microbiology*, 19–33. Edited by C. A. Reddy, T. J. Beveridge, J. A. Breznak, G. Marzluf, T. M. Schmidt & L. R. Snyder. Washington, DC: American Society for Microbiology.

Bowman, J. P., McCammon, S. A., Brown, J. L., Nichols, P. D. & McMeekin, T. A. (1997). *Psychroserpens burtonensis* gen. nov., sp. nov., and *Gelidibacter algens* gen. nov., sp. nov., psychrophilic bacteria isolated from antarctic lacustrine and sea ice habitats. *Int J Syst Bacteriol* **47**, 670–677.

Buczolits, S., Denner, E. B. M., Vybiral, D., Wieser, M., Kämpfer, P. & Busse, H.-J. (2002). Classification of three airborne bacteria and proposal of *Hymenobacter aerophilus* sp. nov. *Int J Syst Evol Microbiol* **52**, 445–456.

Collins, M. D. & Shah, H. N. (1984). Fatty acid, menaquinone and polar lipid composition of *Rothia dentosacariosa*. *Arch Microbiol* **137**, 247–249.

Einen, J. & Øvreås, L. (2006). *Flaviramulus basaltis* gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae* isolated from seafloor basalt. *Int J Syst Evol Microbiol* **56**, 2455–2461.

Gonzalez, C., Gutierrez, C. & Ramirez, C. (1978). *Halobacterium vallismortis* sp. nov. An amylolytic and carbohydrate-metabolizing, extremely halophilic bacterium. *Can J Microbiol* **24**, 710–715.

Hsu, S. C. & Lockwood, J. L. (1975). Powdered chitin agar as a selective medium for enumeration of actinomycetes in water and soil. *Appl Microbiol* **29**, 422–426.

Ivanova, E. P., Alexeeva, Y. V., Flavier, S., Wright, J. P., Zhukova, N. V., Gorshkova, N. M., Mikhailov, V. V., Nicolau, D. V. & Christen, R. (2004). *Formosa algae* gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae*. *Int J Syst Evol Microbiol* **54**, 705–711.

Jooste, P. J. (1985). ***The taxonomy and significance of Flavobacterium–Cytophaga strains from dairy sources*. PhD thesis, University of the Orange Free State, Bloemfontein, South Africa.**

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H. & other authors (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.

Komagata, K. & Suzuki, K. (1987). Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.

Lyman, J. & Fleming, R. H. (1940). Composition of seawater. *J Mar Res* **3**, 134–146.

Mesbah, M. & Whitman, W. B. (1989). Measurement of deoxyguanosine/thymidine ratios in complex mixtures by high-performance liquid chromatography for determination of the mole percentage guanine + cytosine of DNA. *J Chromatogr A* **479**, 297–306.

Minnikin, D. E., O'Donnell, A. G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A. & Parlett, J. H. (1984). An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* **2**, 233–241.

Moore, E. R. B., Arnscheidt, A., Krüger, A., Strömpl, C. & Mau, M. (1999). Simplified protocols for the preparation of genomic DNA from bacterial cultures. In *Molecular Microbial Ecology Manual 1.6.1*, pp. 1–15. Edited by A. D. L. Akkermans, J. D. van Elsas & F. J. de Bruijn. Dordrecht: Kluwer.

Nedashkovskaya, O. I., Kim, S. B., Han, S. K., Snauwaert, C., Vancanneyt, M., Swings, J., Kim, K. O., Lysenko, A. M., Rohde, M. & other authors (2005a). *Winogradskyella thalassocola* gen. nov., sp. nov., *Winogradskyella epiphytica* sp. nov. and *Winogradskyella eximia* sp. nov., marine bacteria of the family *Flavobacteriaceae*. *Int J Syst Evol Microbiol* **55**, 49–55.

Nedashkovskaya, O. I., Kim, S. B., Lysenko, A. M., Frolova, G. M., Mikhailov, V. V. & Bae, K. S. (2005b). *Bizionia paragorgiae* gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae* isolated from the soft coral *Paragorgia arborea*. *Int J Syst Evol Microbiol* **55**, 375–378.

Nedashkovskaya, O. I., Vancanneyt, M. & Kim, S. B. (2010). *Bizionia echini* sp. nov., isolated from a sea urchin. *Int J Syst Evol Microbiol* **60**, 928–931.

Reichenbach, H. (1989). The order *Cytophagales* Leadbetter 1974. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, pp. 2011–2013. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. Baltimore, MD: Williams & Wilkins.

Reichenbach, H. (1992). *Flavobacteriaceae* fam. nov. In *Validation of the Publication of New Names and New Combinations Previously Effectively Published Outside the IJSB*, List no. 41. *Int J Syst Bacteriol* **42**, 327–329.

Sasser, M. (1990). *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.

Smibert, R. M. & Krieg, N. R. (1994). Phenotypic characterization. In *Methods for General and Molecular Bacteriology*, pp. 607–654. Edited by P. Gerherdt, R. G. E. Murray, W. A. Wood & N. R. Krieg. Washington, DC: American Society for mMicrobiology.

Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.

Tang, K., Zhang, Y., Yu, M., Shi, X., Coenye, T., Bossier, P. & Zhang, X.-H. (2013). Evaluation of a new high-throughput method for identifying quorum quenching bacteria. *Sci Rep* **3**, 2935.

Teather, R. M. & Wood, P. J. (1982). Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. *Appl Environ Microbiol* **43**, 777–780.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Tindall, B., Sikorski, J., Smibert, R. & Krieg, N. (2007). Phenotypic characterization and the principles of comparative systematics.*In Methods for General and Molecular Microbiology*, pp. 330–393. Edited by C. A. Reddy, T. J. Beveridge, J. A. Breznak, G. Marzluf, T. M. Schmidt & L. R. Snyder Washington, DC, American Society for Microbiology.

Weisburg, W. G., Barns, S. M., Pelletier, D. A. & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* **173**, 697–703.

Xie, C.-H. & Yokota, A. (2003). Phylogenetic analyses of *Lampropedia hyalina* based on the 16S rRNA gene sequence. *J Gen Appl Microbiol* **49**, 345–349.

Yoon, J. H., Kang, C. H., Jung, Y. T. & Kang, S. J. (2013). *Bizionia hallyeonensis* sp. nov., isolated from seawater in an oyster farm. *Int J Syst Evol Microbiol* **63**, 685–690.

Zhang, Y., Tang, K., Shi, X. & Zhang, X. H. (2013). Flaviramulus ichthyoenteri sp. nov., an N-acylhomoserine lactone-degrading bacterium isolated from the intestine of a flounder (Paralichthys olivaceus), and emended descriptions of the genus Flaviramulus and Flaviramulus basaltis. *Int J Syst Evol Microbiol* **63**, 4477–4483.