

Bizionia fulviae sp. nov., isolated from the gut of an egg cockle, *Fulvia mutica*

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A novel Gram-staining-negative, non-spore-forming, non-flagellated, non-motile, aerobic, saffron-coloured, rod-shaped bacterium that did not produce flexirubin-type pigments was designated strain EM7^T and was distinct from other members of the genus *Bizionia* by produce carotenoid-type pigments and being able to grow independently of NaCl. Strain EM7^T was isolated from the intestinal tract of an egg cockle, *Fulvia mutica*, which had been collected from the West Sea in Korea. Phylogenetic analysis based on the 16S rRNA gene sequence showed that strain EM7^T belonged to the genus *Bizionia*, and showed sequence similarity to *Bizionia paragorgiae* KMM 6029^T (97.9 %) and *Bizionia saleffrena* HFD^T (97.73 %). Growth occurred on marine agar 2216 at 0–25 °C (optimum, 20 °C) and at pH 6–9 (optimum, pH 7). Growth occurred in the presence of 0–10 % (w/v) NaCl (optimum, 2 %, w/v, NaCl). The major cellular fatty acids were anteiso-C₁₅:₀, iso-C₁₅:₀, iso-C₁₅:₁ G, summed feature 3 (C₁₆:₁ω7c and/or C₁₆:₁ω6c), iso-C₁₇:₀ 3-OH and iso-C₁₆:₀ 3-OH. The major respiratory quinone was menaquinone MK-6. The polar lipids of strain EM7^T comprised phosphatidylethanolamine, three unidentified aminolipids, an unidentified aminophospholipid and two unidentified lipids. The genomic DNA G + C content was 34.8 mol%. *Bizionia paragorgiae* KMM 6029^T and *Bizionia saleffrena* HFD^T to *Bizionia paragorgiae* KCTC 12304^T and *Bizionia saleffrena* CIP 108534^T, respectively. Thus, it is proposed that the isolate represents a novel species, *Bizionia fulviae* sp. nov., with strain EM7^T (=KACC 18255^T=JCM 30417^T) as the type strain.

To date, the family *Flavobacteriaceae* comprises more than 100 genera. Many genera within the family *Flavobacteriaceae* have been isolated from a variety of marine habitats (Bernardet & Nakagawa, 2006). Phylogenetic studies based on dissimilarities between 16S rRNA gene sequences revealed that these genera cluster as a distinct clade, defined as a ‘marine clade’, within the family *Flavobacteriaceae* (Bowman, 2004, 2006; Bowman & Nichols, 2005). Bacteria belonging to the marine clade are closely associated with red tides, algal blooms and the mineralization of organic matter in marine environments (Bowman *et al.*, 1997; Pinhassi *et al.*, 2004). These findings may help to elucidate the ecological role and physiological potential of the novel strain in the microbial community. Furthermore, identify-

ing and characterizing the novel strain demonstrates its interactions within communities.

The genus *Bizionia* belongs to the marine clade within the family *Flavobacteriaceae* and was first described by Nedashkovskaya *et al.* (2005); *Bizionia paragorgiae* is the type species. At the time of writing, the genus *Bizionia* comprises representatives of nine different species: *B. paragorgiae* (Nedashkovskaya *et al.*, 2005), *Bizionia saleffrena*, *Bizionia gelidisalsuginis*, *Bizionia algorithergicola*, *Bizionia myxarmorum* (Bowman & Nichols, 2005), *Bizionia argentinensis* (Bercovich *et al.*, 2008), *Bizionia echini* (Nedashkovskaya *et al.*, 2010), *Bizionia hallyeonensis* (Yoon *et al.*, 2013) and *Bizionia psychrotolerans* (Song *et al.*, 2014). All of these species were isolated from marine invertebrates and cold marine environments such as Antarctic surface seawater and Antarctic coastal areas. Members of the genus *Bizionia* are Gram-staining-negative, yellow–orange, rod-shaped, non-spore-forming, non-flagellated, non-gliding and do not produce flexirubin-type pigments; yeast extract and Na⁺ are required for growth. Here, we analyse a bacterial strain, EM7^T, isolated from the intestinal tract of an egg

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain EM7^T is KJ801961.

Two supplementary figures and one supplementary table are available with the online Supplementary Material.

cockle to verify its taxonomic position using a polyphasic study approach. The results showed that EM7^T is a representative of a novel species belonging to the genus *Bizionia*.

Strain EM7^T was isolated from the intestinal tract of an egg cockle, *Fulvia mutica*, which was collected from the West Sea in Korea. Homogenized intestinal tract tissue was diluted with filtered PBS buffer (Bioneer) and inoculated onto marine agar 2216 (MA; Difco) at 15 °C or 25 °C using the standard dilution-plating method. The bacteria were isolated at 15 °C. A pure culture from a single colony was obtained after repeated cultivation. All tests were conducted in triplicate. The isolate was cryopreserved at -80 °C as a suspension in marine broth 2216 (MB; Difco) containing 40 % (v/v) glycerol.

To confirm the taxonomic position of the isolate, the 16S rRNA gene was amplified by colony PCR using a PCR pre-mix (iNtRon Biotechnology) containing two universal bacteria-specific primers: Bac 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and Bac 1492R (5'-GGYTACCTTGTTACGACTT-3') (Lane, 1991). The 16S rRNA gene amplicon was sequenced using a BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems). The sequences of the 16S rRNA gene fragments were assembled using the SeqMan program (DNASTAR). The assembled 16S rRNA gene sequence (1440 nt) was compared with that of other strains using the EzTaxon-e server (Kim *et al.*, 2012). The results showed that strain EM7^T shared 97.9 % sequence similarity with *Bizionia paragorgiae* KMM 6029^T and 97.7 % sequence similarity with *Bizionia saleffrena* HFD^T. For phylogenetic analysis, the 16S rRNA gene sequences of the novel strain were aligned with those of closely related species using the multiple sequence alignment program CLUSTAL W (Thompson *et al.*, 1997). A phylogenetic consensus tree was reconstructed in MEGA version 5 (Tamura *et al.*, 2011) using three algorithms based on 1000 randomly chosen bootstrap replications: neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981). The phylogenetic tree revealed that strain EM7^T clustered with other closely related species belonging to the genus *Bizionia* (Fig. 1). To perform a polyphasic study to further characterize strain EM7^T, *Bizionia paragorgiae* KCTC 12304^T (=KMM 6029^T) and *Bizionia saleffrena* CIP 108534^T (=HFD^T) were obtained from the Korean Collection for Type Cultures (KCTC) and the Collection of Institut Pasteur (CIP), respectively.

Gram-staining, cell morphology, and the general appearance of the colonies were examined after incubation at 20 °C for 48 h. Gram-staining was performed using a Gram stain kit (bioMérieux) according to the manufacturer's instructions. Cell morphology was observed under a light microscope (ECLIPSE 50i; Nikon) and cell size was measured by using ProgRes CapturePro version 2.8.8. To observe detailed cellular morphology, a field-emission electron microscope (SUPRA VP55; Carl Zeiss) was used (Fig. S1, available in the online Supplementary

Material). A cellular motility test was performed in MB medium containing 0.4 % agar (Tittsler & Sandholzer, 1936). Growth under anaerobic conditions was investigated by incubating the bacteria for 7 days in an anaerobic chamber containing N₂, H₂ and CO₂ (90 % : 5 % : 5 %). The growth of strain EM7^T was examined under various conditions, including different temperatures, salinity and pH, to identify the optimal growth conditions. The growth temperature test was performed in MB at 0, 4, 10, 15, 25, 30, 37, 45, 55 and 65 °C. For the growth salinity test, the isolate was incubated in a medium that comprised all of the components of MB except NaCl, which was then supplemented at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 15 % (w/v) NaCl. The optimum pH for growth was tested by culturing bacteria in MB supplemented with one of three buffer solutions: 10 mM (w/v) MES (Sigma-Aldrich) for pH 4, 5 and 6; 10 mM (w/v) TAPS (Sigma-Aldrich) for pH 7, 8 and 9; and 10 mM (w/v) Na₂HPO₄ (Sigma-Aldrich) for pH 10 and 11. All pH values were adjusted by adding 2 M (v/v) HCl or 1 M (w/v) KOH. The turbidity of each individual culture was assessed by measuring the OD at 600 nm in a spectrophotometer (Synergy MX; Bio Tek) according to the method of Hyun *et al.* (2015). Cells of strain EM7^T were Gram-staining-negative, rod-shaped (0.8–0.9 µm wide and 3.6–3.8 µm long) and non-motile. The cells did not grow under anaerobic conditions. The results of the growth tests indicated that the isolate grew optimally at 20 °C in the presence of 2 % (w/v) NaCl at pH 7. The range of conditions for growth were 0–25 °C, pH 6–9, in the presence of 0–10 % (w/v) NaCl.

The isolate was grown in the absence of light to detect flexirubin and carotenoid-type pigments under optimal conditions for 48 h. The presence of flexirubin-type pigments was examined by exposing cells to 20 % (w/v) KOH, which causes a colour shift from yellow to red, purple or brown (Bernardet *et al.*, 2002). The results showed that the isolate did not produce flexirubin-type pigments. To test for the presence of carotenoid-type pigments, cellular pigments were extracted according to the method of Schmidt *et al.* (1994), with minor modifications, and analysed by measuring the absorption spectrum using a scanning UV/visible spectrophotometer (Synergy MX; BioTek). A three-peak signature (peaks at 420 nm, 450 nm and 480 nm) confirmed that strain EM7^T contained carotenoid-type pigments.

Catalase activity was checked by observing bubble production in a 3 % (v/v) hydrogen peroxide solution. Oxidase activity was confirmed by observing colour changes after exposure to 1 % (w/v) tetramethyl-*p*-phenylenediamine (bioMérieux). Carbohydrate fermentation, enzyme activity and utilization of various carbon sources were also tested to identify the biochemical characteristics of strain EM7^T. These characteristics were then compared with those of the reference strains. Utilization of individual carbon sources was tested under optimal growth conditions using GN2 MicroPlates (Biolog) and GN/GP inoculating fluid (Biolog), according to the manufacturer's instructions. Enzyme activities and assimilation patterns were

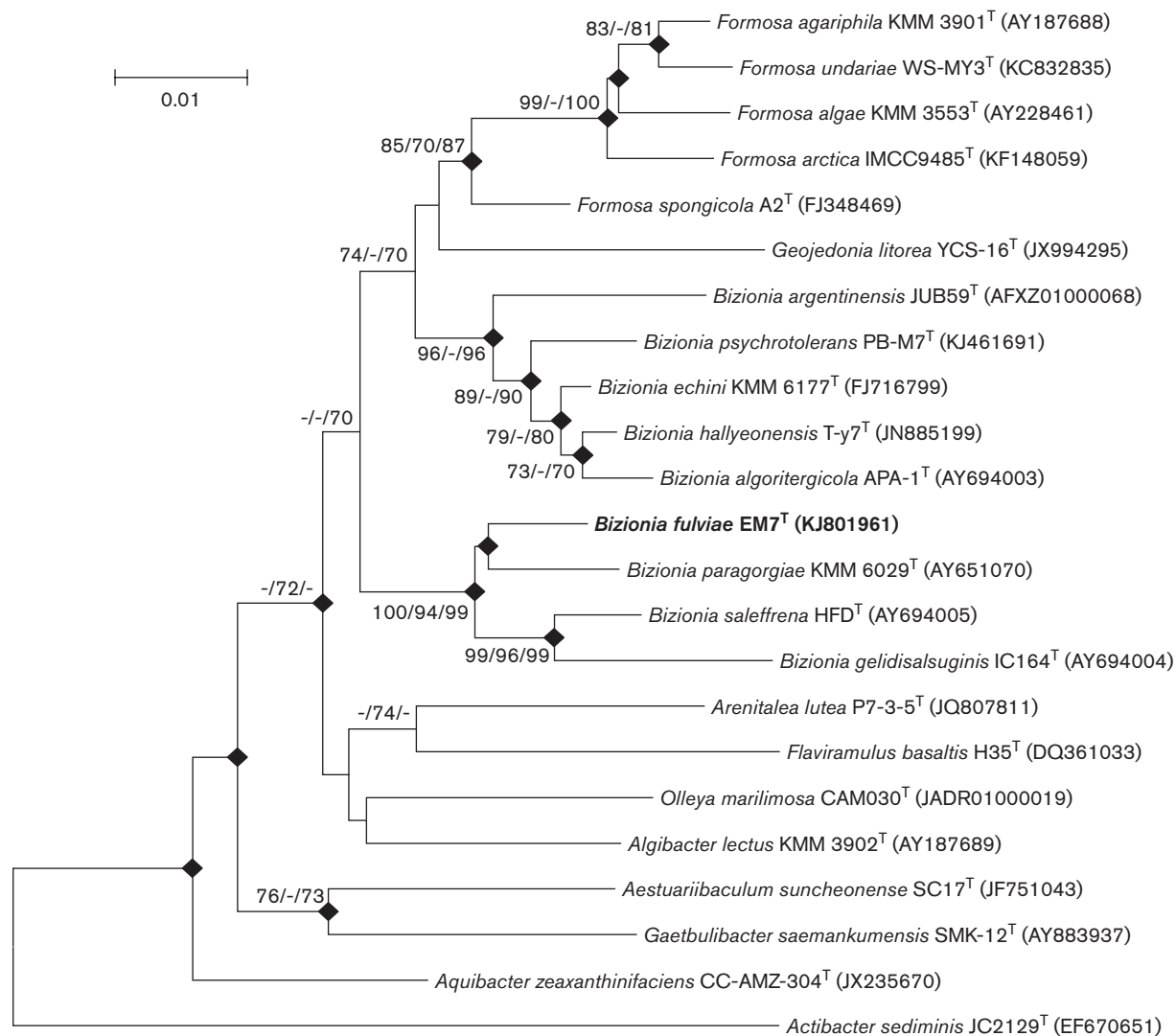


Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences generated using the neighbour-joining (NJ), maximum-parsimony (MP) and maximum-likelihood (ML) algorithms. The tree shows the locations of strain EM7^T and related species. Diamonds indicate communal branches in the phylogenetic trees generated using the three algorithms (NJ, MP and ML). Bootstrap values shown at nodes are expressed as a percentage of 1000 replicates. Values <70 % are not shown. *Actibacter sediminis* JC2129^T was used as an out-group. Bar indicates 0.01 substitutions per nucleotide.

characterized using API ZYM and API 20NE test strips (bioMérieux), with a minor modification to the manufacturer's instructions (distilled water supplemented with 2 % (w/v) NaCl was used as the inoculating fluid). Strain EM7^T was catalase-positive and oxidase-positive. The biochemical characteristics of strain EM7^T and its closest relatives are presented in Table 1.

Cellular fatty acids, isoprenoid quinone and polar lipids were examined to identify the chemotaxonomic characteristics of strain EM7^T. Strain EM7^T, *Bizonia paragorgiae* KCTC 12304^T and *Bizonia saeffrena* CIP 108534^T were cultured on MA plates at 20 °C for 48 h. The fatty acids were then extracted according to the method of the Sherlock Microbial

Identification System (MIDI, 1999) and analysed by GC using an Agilent 6890 gas chromatograph (Agilent Technologies). Extracted fatty acids were identified using the Microbial Identification software package (Sherlock, version 6.2) and the TSBA6 database (Sasser, 1990). The major cellular fatty acids (>5 % of total fatty acids) identified in strain EM7^T were anteiso-C_{15:0} (18.29 %), iso-C_{15:0} (11.13 %), iso-C_{15:1} G (8.83 %), summed feature 3 (C_{16:1}ω7c and/or C_{16:1}ω6c, 8.13 %), iso-C_{17:0} 3-OH (7.24 %) and iso-C_{16:0} 3-OH (5.56 %). The cellular fatty acid profiles of strain EM7^T and the reference strains are presented in Table 2. Polar lipids were extracted from strain EM7^T as described by Xin *et al.* (2000) and separated by

Table 1. Characteristics of strain EM7^T and type strains of the most closely related species

Strains: 1, EM7^T; 2, *Bizionia paragorgiae* KCTC 12304^T; 3, *Bizionia saleffrena* CIP 108534^T; 4, *Bizionia gelidisalsuginis* IC164^T. All data are derived from the current study unless indicated. All strains required yeast extract for growth. Data reflecting carbon source assimilation were obtained using API 20NE test strips and a GN2 MicroPlate, and enzyme activity was examined using API ZYM and API 20NE test strips except for strain *Bizionia gelidisalsuginis* IC164^T. All strains didn't showed anaerobic condition, were positive for catalase, oxidase and alkaline phosphatase activities, and assimilated Tween 80, acetic acid, propionic acid and L-leucine. Strain EM7^T, *Bizionia paragorgiae* KCTC 12304^T and *Bizionia saleffrena* CIP 108534^T were positive for esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities, and assimilated Tween 40, succinic acid monomethyl ester, α -ketobutyric acid, α -ketovaleric acid, L-alanylglycine, L-aspartic acid and glycyl L-glutamic acid; these characteristics were not determined for *Bizionia gelidisalsuginis* IC164^T. +, Positive; -, negative; ND, no data reported.

Characteristic	1	2	3	4 ^{a,b*}
Temperature conditions for growth (°C)				
Range	0–25	4–36 ^c	1–25 ^a	2–29
Optimum	20	23–25 ^c	22 ^a	20
pH conditions for growth (pH)				
Range	6–9	ND ^c	ND ^a	ND
Optimum	7	7 ^c	7 ^a	7.6
NaCl concentrations for growth (% w/v)				
Range	0–10	1–8 ^c	1.1–17.4 ^a	1.1–17.4
Optimum	2	2 ^c	5.8 ^a	5.8
Enzyme activity				
Cystine arylamidase	–	+	+	ND
Arginine dihydrolase	–	–	–	+
Hydrolysis of gelatin	–	+	+	+
Fermentation of D-glucose	+	–	–	+
Utilization of:				
Pyruvic acid methyl ester	+	–	+	ND
β -Hydroxybutyric acid	–	+	–	ND
γ -Hydroxybutyric acid	–	+	–	ND
L-Alaninamide	+	–	+	ND
L-Alanine	+	–	+	+
L-Asparagine	–	–	+	ND
L-Glutamic acid	+	+	+	–
Glycyl L-aspartic acid	+	–	+	ND
L-Histidine	–	+	–	+
L-Ornithine	+	+	+	–
L-Proline	+	+	+	–
L-Serine	+	+	+	–
L-Threonine	+	+	–	ND
Urocanic acid	–	+	–	ND
Uridine	–	–	+	ND
2,3-Butanediol	–	+	–	ND
Glycerol	–	+	–	ND
DNA G+C content (mol%)	34.8	37.6 ^c	40 ^a	39
Isolation source	Egg cockle	Soft coral ^c	Sea-ice brines ^a	Sea-ice brine

*Data from: a, Bowman & Nichols (2005); b, Nedashkovskaya *et al.* (2010); c, Nedashkovskaya *et al.* (2005).

two-dimensional TLC on a silica gel 60 F₂₅₄ plate (Merck). Spots were visualized by spraying with four different detection spray reagents as described by Tindall (1990), with minor modifications: 5 % (v/v) ethanolic molybdophosphoric acid (Sigma-Aldrich) for total lipids, ninhydrin spray reagent (Sigma-Aldrich) for amino-group-containing lipids, molybdenum blue spray reagent (Sigma-Aldrich) for phosphorous-group-containing lipids and α -naphthol

reagent (Komagata & Suzuki, 1987; Minnikin *et al.*, 1977) for visualizing glycolipids. Phospholipids were identified by one-dimensional TLC with standard compound (Sigma). Strain EM7^T contained phosphatidylethanolamine, three unidentified aminolipids, an unidentified aminophospholipid and two unidentified lipids (Fig. S2). Isoprenoid quinone was extracted from strain EM7^T and from the reference strains using chloroform/methanol (2 : 1, v/v) according to

the method of Collins & Jones (1981a). One-dimensional TLC on a silica gel 60 F₂₅₄ plate (Merck) in hexane/benzene/chloroform (5 : 2 : 1, by vol.) was performed to purify the isoprenoid quinones extracted from strain EM7^T and the reference strains (Bowman & Nichols, 2005; Nedashkovskaya *et al.*, 2005). The purified isoprenoid quinones were dissolved in acetone and examined by reverse-phase HPLC (Collins & Jones, 1981b) on a Thermo ODS HYPERSIL (250 × 4.6 mm) column. The respiratory quinone isolated from strain EM7^T was menaquinone MK-6, which is the major or the sole respiratory quinone among members of the genus *Bizionia* as well as the family *Flavobacteriaceae* (Bernardet, 2011). Taken together, the chemotaxonomic indicators (fatty acid composition, polar lipid composition and quinone type) suggested that strain EM7^T was a representative of a novel species belonging to the genus *Bizionia*.

Genomic DNA was extracted from strain EM7^T, *Bizionia paragorgiae* KCTC 12304^T and *Bizionia saleffrena* CIP 108534^T, as described by Rochelle *et al.* (1992), and purified using an UltraClean kit (MO BIO) according to the manufacturer's instructions. The DNA G + C content was determined using a fluorometric method based on the CFX96 Real-Time PCR Detection System (Bio-Rad) and quantified using SYBR Gold (Gonzalez & Saiz-Jimenez, 2002). Genomic DNA from *Bacteroides thetaiotaomicron* VPI 5482^T, *Escherichia coli* K-12, *Ruegeria pomeroyi* DSS-3^T, *Bacteroides fragilis* NCTC 9343^T and *Streptococcus parasanguinis* ATCC 15912^T was used for calibration. The DNA G + C content of members of the genus *Bizionia* is 33–45 mol% (Bercovich *et al.*, 2008; Bowman & Nichols, 2005; Nedashkovskaya *et al.*, 2010; Song *et al.*, 2014; Yoon *et al.*, 2013). The DNA G + C content of strain EM7^T was 34.8 mol%. DNA–DNA hybridization (DDH) was performed using a genome probing microarray (Bae & Park, 2006; Bae *et al.*, 2005; Chang *et al.*, 2008a, b) to clarify the genetic relatedness between strain EM7^T and the type strains *Bizionia paragorgiae* KCTC 12304^T and *Bizionia saleffrena* CIP 108534^T. Microarray analysis was performed using Array Sifter Express version 1.3. The degree of DDH relatedness was calculated from the signal-to-noise ratio for the genomic probes (Loy *et al.*, 2005). The DDH relatedness values for strains EM7^T and *Bizionia paragorgiae* KCTC 12304^T, and for EM7^T and *Bizionia saleffrena* CIP 108534^T (which shows >97 % 16S rRNA gene sequence similarity with the new isolate), were 14 ± 1.2 % (13 ± 3.9 % reciprocal) and 17 ± 1.3 % (17 ± 1.4 % reciprocal), respectively (Table S1). The finding that the DDH values were below the species classification threshold of 70 % indicates that the isolate is novel at the species level (Wayne *et al.*, 1987).

The results of the biochemical, chemotaxonomic, phylogenetic and genotypic analyses performed herein suggest that strain EM7^T is a representative of a novel species belonging to the genus *Bizionia*, for which the name *Bizionia fulviae* sp. nov. is proposed.

Table 2. Cellular fatty acid contents of strain EM7^T and type strains of the most closely related species

Strains: 1, EM7^T; 2, *Bizionia paragorgiae* KCTC 12304^T; 3, *Bizionia saleffrena* CIP 108534^T; 4, *Bizionia gelidalsuginis* IC164^T. Values are expressed as a percentage of total fatty acids. TR, Trace level (<1.0 %); –, not detected.

Fatty acid	1	2	3	4*
Saturated acids				
C ₁₅ : 0	–	–	–	2.3
C ₁₆ : 0	1.86	1.33	1.69	1.6
C ₁₈ : 0	–	–	–	1.1
Unsaturated acids				
C ₁₅ : 0	–	–	–	1.2
C ₁₅ : 1 ω 6c	2.06	1.09	1.85	–
C ₁₇ : 1 ω 8c	TR	TR	1.03	–
C ₁₇ : 1 ω 6c	3.06	2.17	2.89	TR
iso-C ₁₅ : 1 ω 10c	TR	TR	TR	2.1
anteiso-C ₁₅ : 1 ω 10c	–	TR	–	7.0
anteiso-C ₁₇ : 1 ω 9c	3.02	1.72	3.34	–
Branched acids				
iso-C ₁₄ : 0	1.19	1.46	1.12	1.4
iso-C ₁₅ : 0	11.13	21.57	6.15	3.1
iso-C ₁₅ : 1 G	8.83	11.11	4.84	TR
iso-C ₁₆ : 0	4.31	5.48	3.67	1.7
iso-C ₁₆ : 1 H	3.82	3.74	3.71	–
iso-C ₁₇ : 0	–	–	–	15.1
anteiso-C ₁₇ : 1	TR	–	–	2.0
anteiso-C ₁₅ : 0	18.29	6.63	17.39	13.9
anteiso-C ₁₅ : 1 A	4.75	1.68	4.63	–
anteiso-C ₁₅ : 0 3-OH	–	–	–	22.9
anteiso-C ₁₇ : 0 3-OH	TR	TR	–	2.9
iso-C ₁₅ : 0 3-OH	3.04	5.39	3.27	6.2
iso-C ₁₆ : 0 3-OH	5.56	6.58	8.13	8.5
iso-C ₁₇ : 0 3-OH	7.24	10.36	6.3	TR
C ₁₅ : 0 2-OH	2.03	1.24	2.65	–
C ₁₇ : 0 2-OH	4.02	2.43	7.12	–
C ₁₅ : 0 3-OH	–	–	–	1.2
Summed features†				
3	8.13	7.49	12.36	3.9
9	2.52	3.14	2.32	–

*Data from Bowman & Nichols (2005).

†Summed features could not be separated by the Microbial Identification System. Summed feature 3 comprises C₁₆ : 1 ω 7c and/or C₁₆ : 1 ω 6c; summed feature 9 comprises iso-C₁₇ : 1 ω 9c and/or C₁₆ : 0 10-methyl.

Description of *Bizionia fulviae* sp. nov.

Bizionia fulviae (ful'vi.ae. N.L. gen. n. *fulviae* of *Fulvia*).

Cells are aerobic, Gram-staining-negative, non-spore-forming, non-flagellated, rod-shaped (0.8–0.9 µm × 3.6–3.8 µm) and do not produce flexirubin-type pigments. Yeast extract is required for growth. Positive for catalase and oxidase activities. In contrast to other members of the genus *Bizionia*,

produces carotenoid-type pigments and grows independently of NaCl. Colonies are circular, raised, convex with an intact margin, saffron-coloured and 0.7–1.0 mm in diameter after 48 h of incubation on MA at 20 °C under aerobic conditions. Growth occurs at 0–25 °C (optimum, 20 °C), at pH 6–9 (optimum, pH 7) and with 0–10 % (w/v) NaCl (optimum, 2 %). Yellow–orange carotenoid-type pigments are produced, but flexirubin-type pigments are not. Positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities and ferments D-glucose. Utilizes Tween 40, Tween 80, pyruvic acid methyl ester, succinic acid monomethyl ester, acetic acid, α -ketobutyric acid, α -ketovaleric acid, propionic acid, L-alaninamide, L-alanine, L-alanylglycine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-leucine, L-ornithine, L-proline, L-serine and L-threonine. The major cellular fatty acids are anteiso-C_{15:0}, iso-C_{15:0}, iso-C_{15:1} G, summed feature 3 (C_{16:1} ω 7c and/or C_{16:1} ω 6c), iso-C_{17:0} 3-OH and iso-C_{16:0} 3-OH. The major respiratory quinone is MK-6. The polar lipids comprise phosphatidylethanolamine, three unidentified aminolipids, an unidentified phosphoaminolipid and two unidentified lipids.

The type strain, EM7^T (=KACC 18255^T=JCM 30417^T), was isolated from the intestinal tract of an egg cockle, *Fulvia mutica*, which had been collected from the West Sea in Korea. The genomic DNA G+C content of the type strain is 34.8 mol%.

Acknowledgements

This work was supported by grants from the National Research Foundation of Korea through the Mid-career Researcher Program (2011-0028854) and the National Institute of Biological Resources funded by the Ministry of Environment (MOE) of Korea (NIBR no. 2013-02-001).

References

- Bae, J. W. & Park, Y. H. (2006). Homogeneous versus heterogeneous probes for microbial ecological microarrays. *Trends Biotechnol* **24**, 318–323.
- Bae, J. W., Rhee, S. K., Nam, Y. D. & Park, Y. H. (2005). Generation of subspecies level-specific microbial diagnostic microarrays using genes amplified from subtractive suppression hybridization as microarray probes. *Nucleic Acids Res* **33**, e113.
- Bercovich, A., Vazquez, S. C., Yankilevich, P., Coria, S. H., Foti, M., Hernández, E., Vidal, A., Ruberto, L., Melo, C. & other authors (2008). *Bizionia argentinensis* sp. nov., isolated from surface marine water in Antarctica. *Int J Syst Evol Microbiol* **58**, 2363–2367.
- Bernardet, J.-F. (2011). Family I. *Flavobacteriaceae* Reichenbach 1992. In *Bergey's Manual of Systematic Bacteriology*, vol. 4, 2nd edn., pp. 106–111. Edited by N. R. Krieg, W. Ludwig, W. B. Whitman, B. P. Hedlund, B. J. Paster, J. T. Staley, N. Ward, D. Brown & A. Parte. New York: Springer.
- Bernardet, J.-F. & Nakagawa, Y. (2006). An introduction to the family *Flavobacteriaceae*. In *The Prokaryotes*, vol. 7, 3rd edn., pp. 455–480.
- Edited by M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer & E. Stackebrandt. New York: Springer.
- 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.
- Bowman, J. P. (2004). Psychrophilic prokaryote structural-functional relationships, biogeography and evolution within marine sediment. *Cell Mol Biol (Noisy-le-grand)* **50**, 503–515.
- Bowman, J. P. (2006). The marine clade of the family *Flavobacteriaceae*: the genera *Aequorivita*, *Arenibacter*, *Cellulophaga*, *Croceibacter*, *Formosa*, *Gelidibacter*, *Gillisia*, *Maribacter*, *Mesonina*, *Muricauda*, *Polaribacter*, *Psychroflexus*, *Psychroserpens*, *Robiginitalea*, *Salegentibacter*, *Tenacibaculum*, *Ulvibacter*, *Vitellibacter* and *Zobellia*. In *The Prokaryotes: a Handbook on the Biology of Bacteria*, vol. 7, 3rd edn., pp. 677–694. Edited by M. Dworkin, S. Falkow, E. Rosenberg, K.-H. Schleifer & E. Stackebrandt. New York: Springer.
- Bowman, J. P. & Nichols, D. S. (2005). Novel members of the family *Flavobacteriaceae* from Antarctic maritime habitats including *Subsaximicrobium wynnwilliamsii* gen. nov., sp. nov., *Subsaximicrobium saxinquilinus* sp. nov., *Subsaximicrobium broadyi* gen. nov., sp. nov., *Lacinutrix copepodicola* gen. nov., sp. nov., and novel species of the genera *Bizionia*, *Gelidibacter* and *Gillisia*. *Int J Syst Evol Microbiol* **55**, 1471–1486.
- Bowman, J. P., McCammon, S. A., Brown, M. V., Nichols, D. S. & McMeekin, T. A. (1997). Diversity and association of psychrophilic bacteria in Antarctic sea ice. *Appl Environ Microbiol* **63**, 3068–3078.
- Chang, H. W., Nam, Y. D., Jung, M. Y., Kim, K. H., Roh, S. W., Kim, M. S., Jeon, C. O., Yoon, J. H. & Bae, J. W. (2008a). Statistical superiority of genome-probing microarrays as genomic DNA-DNA hybridization in revealing the bacterial phylogenetic relationship compared to conventional methods. *J Microbiol Methods* **75**, 523–530.
- Chang, H. W., Sung, Y., Kim, K. H., Nam, Y. D., Roh, S. W., Kim, M. S., Jeon, C. O. & Bae, J. W. (2008b). Development of microbial genome-probing microarrays using digital multiple displacement amplification of uncultivated microbial single cells. *Environ Sci Technol* **42**, 6058–6064.
- Collins, M. D. & Jones, D. (1981a). Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implication. *Microbiol Rev* **45**, 316–354.
- Collins, M. D. & Jones, D. (1981b). A note on the separation of natural mixtures of bacterial ubiquinones using reverse-phase partition thin-layer chromatography and high performance liquid chromatography. *J Appl Bacteriol* **51**, 129–134.
- Felsenstein, J. (1981). Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* **17**, 368–376.
- Gonzalez, J. M. & Saiz-Jimenez, C. (2002). A fluorimetric method for the estimation of G+C mol% content in microorganisms by thermal denaturation temperature. *Environ Microbiol* **4**, 770–773.
- Hyun, D. W., Kim, J. Y., Kim, M. S., Shin, N. R., Kim, H. S., Lee, J. Y. & Bae, J. W. (2015). *Actibacter haliotis* sp. nov., isolated from the gut of an abalone, *Haliotis discus hannai*, and emended description of the genus *Actibacter*. *Int J Syst Evol Microbiol* **65**, 49–55.
- 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.
- Kluge, A. G. & Farris, F. S. (1969). Quantitative phyletics and the evolution of anurans. *Syst Zool* **18**, 1–32.

- Komagata, K. & Suzuki, K.-I. (1987).** Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–207.
- Lane, D. J. (1991).** 16S/23S rRNA sequencing. In *Nucleic acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.
- Loy, A., Schulz, C., Lücker, S., Schöpfer-Wendels, A., Stoecker, K., Baranyi, C., Lehner, A. & Wagner, M. (2005).** 16S rRNA gene-based oligonucleotide microarray for environmental monitoring of the betaproteobacterial order *Rhodocyclales*. *Appl Environ Microbiol* **71**, 1373–1386.
- MIDI (1999).** *Sherlock Microbial Identification System Operating Manual, version 3.0*. Newark, DE: MIDI, Inc.
- Minnikin, D., Patel, P., Alshamaony, L. & Goodfellow, M. (1977).** Polar lipid composition in the classification of *Nocardia* and related bacteria. *Int J Syst Bacteriol* **27**, 104–117.
- Nedashkovskaya, O. I., Kim, S. B., Lysenko, A. M., Frolova, G. M., Mikhailov, V. V. & Bae, K. S. (2005).** *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.
- Pinhassi, J., Sala, M. M., Havskum, H., Peters, F., Guadayol, O., Malits, A. & Marrasé, C. (2004).** Changes in bacterioplankton composition under different phytoplankton regimens. *Appl Environ Microbiol* **70**, 6753–6766.
- Rochelle, P. A., Fry, J. C., Parkes, R. J. & Weightman, A. J. (1992).** DNA extraction for 16S rRNA gene analysis to determine genetic diversity in deep sediment communities. *FEMS Microbiol Lett* **100**, 59–65.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sasser, M. (1990).** *Identification of bacteria by gas chromatography of cellular fatty acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc.
- Schmidt, K., Connor, A. & Britton, G. (1994).** Analysis of pigments: carotenoids and related polyenes. In *Chemical Methods in Prokaryotic Systematics*, pp. 403–461. Edited by M. Goodfellow & A. G. O'Donnell. New York: Wiley.
- Song, E. J., Lee, M. H., Seo, M. J., Yim, K. J., Hyun, D. W., Bae, J. W., Park, S. L., Roh, S. W. & Nam, Y. D. (2014).** *Bizionia psychrotolerans* sp. nov., a psychrophilic bacterium isolated from the intestine of a sea cucumber (*Apostichopus japonicus*). *Antonie van Leeuwenhoek* **106**, 837–844.
- 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.
- 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. J. (1990).** Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.
- Tittsler, R. P. & Sandholzer, L. A. (1936).** The use of semi-solid agar for the detection of bacterial motility. *J Bacteriol* **31**, 575–580.
- Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987).** International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Xin, H., Itoh, T., Zhou, P., Suzuki, K., Kamekura, M. & Nakase, T. (2000).** *Natrinema versiforme* sp. nov., an extremely halophilic archaeon from Aibi salt lake, Xinjiang, China. *Int J Syst Evol Microbiol* **50**, 1297–1303.
- 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.