Bizionia paragorgiae gen. nov., sp. nov., a novel member of the family *Flavobacteriaceae* isolated from the soft coral *Paragorgia arborea*

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A novel marine bacterium isolated from the soft coral *Paragorgia arborea* in the Sea of Okhotsk was studied by using a polyphasic taxonomic approach. The strain, KMM 6029^T, was strictly aerobic, heterotrophic, yellow-pigmented, non-motile by gliding, Gram-negative and oxidase-, catalase- and alkaline phosphatase-positive. From results of 16S rRNA gene sequence analysis, strain KMM 6029^T occupied a distinct lineage within the family *Flavobacteriaceae* and showed 95.5 % similarity to its closest relative, *Formosa algae*. The DNA G + C content was 37.6 mol%. Major respiratory quinone was MK-6. The predominant fatty acids were i15 : 0, a15 : 0, i15 : 1, a15 : 1, i16 : 1, i16 : 0, i16 : 0 3-OH and summed feature 3 (i15 : 0 2-OH and/or 16 : 1 ω7c). On the basis of phenotypic, chemotaxonomic, genotypic and phylogenetic characteristics the novel bacterium has been assigned to *Bizionia* gen. nov., as *Bizionia paragorgiae* gen. nov., sp. nov. The type strain is KMM 6029^T (=KCTC 12304^T = LMG 22571^T).

Members of the family *Flavobacteriaceae* are often found in various marine habitats. Many novel representatives of this group inhabiting the Sea of Japan have been isolated and described recently (Suzuki *et al.*, 2001; Nedashkovskaya *et al.*, 2003b, c, 2004a, b; Sohn *et al.*, 2004). Little is known about the diversity of flavobacteria that live in the waters of the north-western Pacific.

In the course of a study of the microbial population of the soft coral *Paragorgia arborea*, which inhabits the Sea of Okhotsk (Pacific Ocean), a novel heterotrophic, aerobic, yellow-pigmented, non-gliding, Gram-negative bacterium was isolated. By using 16S rRNA gene sequence phylogenetic analysis, the unknown strain was determined to be a member of the family *Flavobacteriaceae*, in which it forms a distinct lineage. Based on differences in molecular, fatty acid composition and phenotypic features described

in this study, we propose the description of *Bizionia paragorgiae* gen. nov., sp. nov., with KMM 6029^T (=KCTC 12304^T = LMG 22571^T) as the type strain.

Strain KMM 6029^T was isolated from the soft coral *Paragorgia arborea* collected from Makarov Bay, Iturup Island, Kurile Islands, Sea of Okhotsk, Pacific Ocean, from a depth of 120 m during September 1997. For strain isolation, 0.1 ml tissue homogenate was transferred to marine agar plates. After primary isolation and purification, strains were cultivated at 28 °C on the same medium and stored at –80 °C in marine broth supplemented with 20 % (v/v) glycerol.

Genomic DNA extraction, PCR and sequencing of the 16S rRNA gene followed previously described procedures (Kim *et al.*, 1998). The sequence data obtained were aligned with those of representatives of the family *Flavobacteriaceae* using PHYDIT version 3.2 (<http://plaza.snu.ac.kr/~jchun/phydit/>). Phylogenetic trees were inferred using appropriate programs in the PHYLIP package (Felsenstein, 1993). Phylogenetic distances were calculated from the models of Kimura (1980) and trees were constructed using neighbour-joining (Saitou & Nei, 1987), least squares (Fitch & Margoliash, 1967) and maximum-likelihood (Felsenstein, 1993) algorithms. Bootstrap analysis was performed with

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Bizionia paragorgiae* KMM 6029^T is AY651070.

Fig. 1. Phylogenetic tree based on the 16S rRNA gene sequences of strains KMM 6029^T and representative members of related genera in the family Flavobacteriaceae. The tree was generated by the neighbour-joining method (Saitou & Nei, 1987). Numbers at nodes indicate bootstrap values (%) and asterisks indicate branches that were also recovered in the maximum-likelihood tree. Scale bar represents 0.01 substitutions per nucleotide position.

1000 resampled datasets, using SEQBOOT and CONSENSE programs of the PHYLIP package.

Phylogenetic 16S rRNA gene sequence analysis revealed that strain KMM 6029^T is a member of the family Flavobacteriaceae and forms a cluster with species of the genera *Formosa*, *Gelidibacter* and *Psychroserpens* (Fig. 1). The

nearest neighbour of this strain is *Formosa algae* KMM 3553^T, with 95.5% 16S rRNA gene sequence similarity, without significant bootstrap support. Sequence similarities between KMM 6029^T and other close relatives ranged from 89.9 to 92.9%.

DNA was isolated following the method of Marmur (1961) and the G + C content of the DNA was determined by the thermal denaturation method (Marmur & Doty, 1962); the G + C content of the DNA was 37.6 mol%.

Analysis of fatty acid methyl esters was carried out according to the standard protocol of the Microbial Identification System (Microbial ID). Major cellular fatty acids were i15:0 (13.2%), a15:0 (12.3%), i15:1 (9.4%), a15:1 (5.8%), i16:1 (5.4%), i16:0 (5.8%), i16:0 3-OH (6.8%) and summed feature 3 (6.3%), comprising i15:0 2-OH and/or 16:1ω7c. It should be noted that cells of strain KMM 6029^T contained 77.1% branched fatty acids. Isoprenoid quinones were extracted from lyophilized cells and analysed as described previously (Nedashkovskaya *et al.*, 2003c). The major respiratory quinone was MK-6.

Phenotypic analysis was performed by using previously described methods (Nedashkovskaya *et al.*, 2003a, b). Gliding motility was determined as described by Bowman (2000). The physiological, biochemical and morphological characteristics of strain KMM 6029^T are listed in the species description and Table 1. The results of phenotypic analysis demonstrated many common traits between strain KMM 6029^T and *Formosa algae*. However, strain KMM 6029^T clearly differs from its closest relative by the inability to move by gliding, to form acid from carbohydrates, to hydrolyse starch and casein, and to produce hydrogen sulphide from L-cysteine (Table 1). The main phenotypic

Table 1. Differential characteristics for *Bizionia paragorgiae* gen. nov., sp. nov. and other members of the family Flavobacteriaceae

Taxa: 1, *Bizionia* gen. nov.; 2, *Formosa*; 3, *Algibacter*; 4, *Gelidibacter*; 5, *Psychroserpens*; 6, *Polaribacter*. —, Negative; +, positive; V, variable; NG, no growth. Data were taken from Reichenbach (1989), Bowman *et al.* (1997), Gosink *et al.* (1998), Macián *et al.* (2002), Ivanova *et al.* (2004), Nedashkovskaya *et al.* (2004b) and this study.

Characteristic	1	2	3	4	5	6
Metabolism*	A	A	F	A	A	A
Gliding motility	—	+	+	+	—	—
Oxidase activity	+	+	+	—	—	V
Requirement for Na ⁺ for growth	+	—	+	+	+	+
Acid formation from carbohydrates	—	+	+	+	—	+
Hydrolysis of:						
Agar	—	—	+	—	—	—
Casein	+	—	—	V	+	NG
Starch	—	+	V	+	—	+
H ₂ S production	+	—	—	—	—	—
DNA G + C content (mol%)	37.6	34–35	31–33	36–40	27–29	31–33

*A, Aerobic; F, fermentative.

characteristics that differentiate strain KMM 6029^T clearly from other relatives of the family *Flavobacteriaceae* are shown in Table 1. Significantly, *Formosa algae* KMM 3553^T is characterized by the presence of 17:0 and 19:0 cyclo fatty acids and the absence of i17:0 3-OH (Ivanova *et al.*, 2004), lending further support to the creation of a new genus for strain KMM 6029^T.

Consequently, based on the phylogenetic distinctiveness and significant differences in phenotypic features and cellular fatty acid composition, we propose that strain KMM 6029^T should be differentiated from its nearest neighbour, *Formosa algae*. Thus, the results of the polyphasic taxonomic analysis presented in this study support the placement of strain KMM 6029^T in a new genus, *Bizionia* gen. nov., as *Bizionia paragorgiae* sp. nov.

Description of *Bizionia* gen. nov.

Bizionia (Bi.zi.o'ni.a. N.L. fem. n. *Bizionia* of Bizio, named in honour of the famous Italian naturalist Bartolomeo Bizio, for his important contribution to the development of microbiology).

Rod-shaped cells, non-motile by gliding. Gram-negative. Do not form endospores. Strictly aerobic. Produce non-diffusible carotenoid pigments. Chemo-organotrophs. Cytochrome oxidase-, catalase- and alkaline phosphatase-positive. Major respiratory quinone is MK-6. Major cellular fatty acids are straight-chain unsaturated and branched-chain unsaturated fatty acids i15:0, a15:0, i15:1, a15:1, i16:1, i16:0, i16:0 3-OH and summed feature 3 (i15:0 2-OH and/or 16:1ω7c). As determined by 16S rRNA gene sequence analysis, the genus is a member of the family *Flavobacteriaceae*. The type species is *Bizionia paragorgiae*.

Description of *Bizionia paragorgiae* sp. nov.

Bizionia paragorgiae (pa.ra.gor'gi.ae. N.L. gen. n. *paragorgiae* of the generic name of the soft coral *Paragorgia arborea*, from which the type strain was isolated).

Main characteristics are as given for the genus. In addition, cells are 0.4–0.5 µm wide and 1.9–2.3 µm long. On marine agar colonies are 2–4 mm in diameter, circular, shiny with entire edges, yellow pigmented. Growth is observed at 4–36 °C. Optimal temperature for growth is 23–25 °C. Growth occurs at 1–8 % NaCl. Decomposes casein, gelatin, Tween 40 and Tween 80. Does not degrade agar, starch, DNA, urea, Tween 20, cellulose (CM-cellulose or filter paper) or chitin. Does not form acid from L-arabinose, D-cellobiose, L-fucose, D-galactose, D-glucose, D-lactose, D-maltose, D-melibiose, L-raffinose, L-rhamnose, D-sucrose, DL-xylose, citrate, adonitol, dulcitol, glycerol, inositol or mannitol. Does not utilize L-arabinose, D-glucose, D-lactose, D-mannose, D-sucrose, mannitol, inositol, sorbitol, malonate or citrate. Nitrate is not reduced. H₂S is produced. Indole and acetoin (Voges–Proskauer reaction) production are negative. Cellular fatty acids accounting for more than 1.0 % of the total fatty acid

content are i14:0 (2.5 %), i15:1 (9.4 %), a15:1 (5.8 %), i15:0 (13.2 %), a15:0 (12.3 %), 15:0 (4.3 %), 15:1ω6c (1.3 %), i16:1 (5.4 %), i16:0 (5.8 %), 16:0 (3.4 %), i15:0 3-OH (3.4 %), a17:0 (3.7 %), i17:1ω9c (1.7 %), a17:1ω9c (1.5 %), 17:1ω6c (1.9 %), i16:0 3-OH (6.8 %), i17:0 3-OH (4.3 %) and summed feature 3 (6.3 %), comprising 16:1ω7c and/or i15:0 2-OH fatty acids. The G + C content of the DNA is 37.6 mol%.

The type strain is KMM 6029^T (=KCTC 12304^T=LMG 22571^T), isolated from the soft coral *Paragorgia arborea* collected in Makarov Bay, Iturup Island, Sea of Okhotsk.

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