International Journal of Systematic and Evolutionary Microbiology (2008), 58, 2363–2367 DOI 10.1099/ijs.0.65599-0

Bizionia argentinensis sp. nov., isolated from surface marine water in Antarctica

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A marine bacterial strain, designated strain JUB59^T, was isolated from surface seawater in Antarctica and subsequently characterized. Cells were found to be Gram-negative, non-motile rods forming butyrous, shiny, yellowish orange colonies on marine agar. Growth occurred at 2–28 °C (optimally at 22–25 °C) but not at 30 °C; Na^+ ions were required, but 9 % NaCl (w/v) was not tolerated. Phylogenetic analysis, based on comparisons of the complete 16S rRNA gene sequence of the novel isolate with the sequences of closely related strains, showed that strain JUB59^T belonged to the family *Flavobacteriaceae*, representing a novel species of the genus *Bizionia*. The highest levels of sequence similarity were found with respect to *Bizionia myxarmorum* ADA-4^T (97.4 %) and *Bizionia algoritergicola* APA-1^T (97.1 %). However, the DNA–DNA relatedness of strain JUB59^T with respect to these two strains was low (15.9–17.3 and 19.3–22.1 %, respectively). The predominant fatty acids of strain JUB59^T were iso-15 : 1ω10*c* (18.1 %), iso-15 : 0 (17.3 %), anteiso-15 : 0 (13.9 %), iso-17 : 0 3-OH (9.2 %), 15 : 0 (6.0 %) and iso-16 : 0 3-OH (5.3 %). The main polar lipids were phosphatidylethanolamine, an aminolipid, an amino-positive phospholipid and two unidentified lipids. MK-6 was the major respiratory quinone (>90 %) and the DNA G+C content was 34 mol%. On the basis of the data obtained, strain JUB59^T represents a novel species of the genus *Bizionia*, for which the name *Bizionia argentinensis* sp. nov. is proposed. The type strain is JUB59^T (=DSM 19628^T=CCM-A-29 1259^T).

The family *Flavobacteriaceae* (Bernardet *et al.*, 1996, 2002) currently comprises more than 40 genera. Many of these genera group strains that have been recovered from a variety of marine habitats (Bernardet & Nakagawa, 2006). These genera form a distinct 'marine clade' in phylogenetic trees based on 16S rRNA gene sequences (Bowman, 2004; Bowman & Nichols, 2005; Bowman, 2006). Marine members of the family occur in tropical, temperate (Nedashkovskaya *et al.*, 2004; Jung *et al.*, 2005; Kwon *et al.*, 2006) and polar (Bowman, 2000; Bowman & Nichols, 2002; Yi *et al.*, 2005) marine environments, where they play an important role in the mineralization of organic matter, especially following algal blooms (Bowman *et al.*, 1997; Pinhassi *et al.*, 2004). The genus *Bizionia* belongs to the marine clade of the family *Flavobacteriaceae*. First described by Nedashkovskaya *et al.* (2005), the genus currently comprises five species, isolated from sea-ice brine and various marine invertebrates: *Bizionia paragorgiae* (Nedashkovskaya *et al.*, 2005), *B. saleffrena*, *B. gelidisalsuginis*, *B. algoritergicola* and *B. myxarmorum* (Bowman & Nichols, 2005). In this study, a bacterial strain isolated from seawater in Antarctica was analysed by a polyphasic taxonomic approach and was found to represent a novel member of the genus *Bizionia*.

Surface seawater was collected from Potter Cove near the Argentinean Jubany Scientific Station (62° 14′ S

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JUB59^T is EU021217.

A complete phylogenetic tree, details of the fatty acid and polar lipid compositions and micrographs of strain JUB59^T are available as supplementary material with the online version of this paper.

58° 40′ W), King George Island (Isla 25 de Mayo), South Shetland Islands, Antarctica. Aliquots (100 µl) of seawater were spread on marine agar 2216 (MA; Difco) and incubated at 10 °C. Among the colonies formed, a shiny and yellowish orange colony with entire edges and a slightly raised centre was recovered and designated strain JUB59^T. Cultivation for subsequent characterization was performed on MA at 15 °C unless stated otherwise.

Gram staining was performed using a kit according to the manufacturer's instructions (Britania). Cell morphology was observed by light microscopy (Axioscope; Zeiss) and transmission electron microscopy (model 301; Philips) using cells grown for 3 days at 15 °C in half-strength marine broth (Difco; half concentration diluted in seawater). Electron micrographs showing cell shape and size (Supplementary Figs S1 and S2) are available in IJSEM Online. The methods described by Bowman (2000) were used to look for the presence of flexirubin pigments and gliding motility. Nitrate reduction and catalase, cytochrome *c* oxidase, DNase, urease and lecithinase activities were investigated as described by MacFaddin (2000). The following characteristics were determined as described by Bowman *et al.* (1996): utilization of melibiose, lactate, salicin, acetate, propionate, L-rhamnose, L-alanine, L-histidine and L-proline; hydrolysis of tyrosine, Tween 80, casein, starch, carboxymethylcellulose, dextran, xylan, chitin and xanthine; and tolerance of bile salts and NaCl. The following characteristics were determined as described by Bowman & Nichols (2005): hydrolysis of agar; requirements for yeast extract and divalent cations (sea salts); gliding motility; growth in the absence of Na^+ ions (on marine agar at 25 °C and in anaerobic conditions); and API ID 32A, API 20E and API 20NE (bioMérieux) strip tests. Anaerobic growth was tested under an N_2/CO_2 (95 : 5) atmosphere on either MA containing 0.5 % (w/v) D-glucose or thioglycollate agar (Merck), supplemented with sea salts.

B. saleffrena HFD^T, *B. gelidisalsuginis* IC164^T, *B. algoritergicola* APA-1^T and *B. myxarmorum* ADA-4^T were used as reference strains for all of the physiological and biochemical tests shown in Table 1. All of the characteristics determined for strain JUB59^T are given in the species description, in Table 1 and also in supplementary Figs S1 and S2 (available in IJSEM Online).

The 16S rRNA gene sequence of strain JUB59^T was obtained both from the ongoing whole-genome sequence of this strain and by extracting genomic DNA using an adapted protocol of a GFX genomic blood DNA purification kit (GE Healthcare) and amplifying the 16S rRNA gene as described by Vazquez *et al.* (2005). Following BLAST analysis against the latest release of the 'Bacteria' division of GenBank, the 1519 bp sequence of strain JUB59^T was aligned with the 16S rRNA gene sequences of representative members of the family *Flavobacteriaceae* by using MUSCLE software (Edgar, 2004). The resulting alignment was edited manually and automatically refined with GBLOCK (Castresana, 2000) prior to phylogenetic analysis. The TREE-PUZZLE program (Schmidt *et al.*, 2002) was used to compute maximum-likelihood distances to determine sequence similarities. Phylogenetic trees were constructed using the neighbour-joining method with the NEIGHBOR program included in the PHYLIP package (version 3.66) (Felsenstein, 2005). Confirmation trees were also inferred using the Fitch–Margoliash and maximum-likelihood methods with the FITCH and DNAML programs, respectively (also included in the PHYLIP package). The robustness of the branches of the phylogenetic tree was assessed by taking 1000 bootstrap replicates of the dataset, which were created with SEQBOOT and analysed by using the programs PUZZLEBOOT, NEIGHBOR and CONSENSE from the PHYLIP package. Trees were drawn using TreeDyn (http://www.treedyn.org). The phylogenetic analysis based on 16S rRNA gene sequences clearly identified strain JUB59^T as belonging to the genus *Bizionia* (Fig. 1). An extended tree containing a larger number of reference sequences is available as Supplementary Fig. S3 in IJSEM Online. Sequence-similarity calculations indicated that the closest relatives of strain JUB59^T were *B. myxarmorum* ADA-4^T (97.4 %) and *B. algoritergicola* APA-1^T (97.1 %).

Chemotaxonomic (polar lipid, fatty acid and quinone compositions) and genetic (DNA G + C content and DNA–DNA hybridization) analyses were carried out by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen and Dr Brian Tindall (Braunschweig, Germany). Cell biomass for these analyses was obtained from 7-day-old cultures in half-strength marine broth 2216 at 15 °C. The Microbial Identification System (MIS) software (MIDI) was used to analyse the cellular fatty acid composition. The detailed cellular fatty acid profile of strain JUB59^T is given in Supplementary Table S1 (available in IJSEM Online). The major fatty acids of strain JUB59^T were iso-15 : 1ω10*c* (18.1 %), iso-15 : 0 (17.3 %), anteiso-15 : 0 (13.9 %), iso-17 : 0 3-OH (9.2 %), 15 : 0 (6.0 %) and iso-16 : 0 3-OH (5.3 %). Overall, this fatty acid pattern was in accordance with those of species of the genus *Bizionia*. However, the rather high proportion of iso-17 : 0 3-OH (9.2 %) was at variance with those found in *Bizionia* species, in which the proportion ranged from <0.01 % (*B. myxarmorum* ADA-4^T) to 4.3 % (*B. paragorgiae* KMM 6029^T). Also, the presence of 3.9 % 2-OH saturated fatty acids (15 : 0 2-OH and 17 : 0 2-OH) represented a remarkable feature. However, these discrepancies may result partly from different culture conditions. Polar lipids were analysed using two-dimensional TLC, resulting in the detection of phosphatidylethanolamine, an aminolipid, an amino-positive phospholipid and two unidentified lipids (see Supplementary Fig. S4 in IJSEM Online). Isoprenoid quinones were identified by using reversed-phase liquid chromatography: MK-6 was found to be the predominant (>90 %) quinone.

DNA from strain JUB59^T was hybridized with DNA from the type strains of *B. myxarmorum* and *B. algoritergicola* using the method described by De Ley *et al.* (1970) but with the modifications of Huß *et al.* (1983). Hybridization

Table 1. Differential characteristics for strain JUB59^T and recognized species of the genus *Bizionia*

Strains: 1, JUB59^T; 2, *B. saleffrena* HFD^T; 3, *B. gelidisalsuginis* IC164^T; 4, *B. algoritergicola* APA-1^T; 5, *B. myxarmorum* ADA-4^T; 6, *B. paragorgiae* KMM 6029^T. All data are from this study, except for the *B. paragorgiae* data (from Nedashkovskaya *et al.*, 2005). All of the strains are positive for the following characteristics: yeast extract requirement for growth; production of non-diffusible golden–yellow to orange non-flexirubin pigments; presence of catalase, cytochrome *c* oxidase, arginine arylamidase, leucyl glycine arylamidase, phenylalanine arylamidase, leucine arylamidase, tyrosine arylamidase, glycine arylamidase, histidine arylamidase, serine arylamidase, alanine arylamidase, glutamyl glutamate arylamidase and alkaline phosphatase activities; hydrolysis of L-tyrosine, L-arginine, casein and gelatin, and utilization of melibiose, lactate and salicin. All of the strains are negative for the following characteristics: nitrate reduction; gliding motility; growth in the absence of Na^+ ions; requirement for divalent cations (sea salts); hydrolysis of agar, starch, aesculin, chitin, carboxymethylcellulose, dextran, xylan and xanthine; acid production from carbohydrates; presence of glutamate decarboxylase, α-galactosidase, β-galactosidase, 6-phospho-β-galactosidase, α-glucosidase, β-glucosidase, α-fucosidase, *N*-acetyl-β-D-glucosaminidase and proline arylamidase activities; utilization of D-glucose, L-arabinose, D-mannose, maltose and *N*-acetyl-D-glucosamine; tolerance of 1 % (w/v) of bile salts; and growth by fermentation of glucose or under an anaerobic atmosphere (N_2/CO_2, 95 : 5) on either MA containing 0.5 % (w/v) D-glucose or thioglycollate agar (Merck), supplemented with sea salts. All of the strains are also negative in API 32 IDA tests for α-arabinosidase, β-glucuronidase and pyroglutamate arylamidase, in API 20E tests for lysine decarboxylase, ornithine decarboxylase, H_2S and indole production, citrate utilization and carbohydrate fermentation and in API 20NE tests for utilization of mannitol, potassium gluconate, malate, citrate, phenylacetate, caprate and adipate. +, Positive; (+), weak or delayed positive reaction; −, negative; ND, no data available.

Characteristic	1	2	3	4	5	6
Growth on MA at 25 °C	+	+	+	(+)	+	+
Tolerance of NaCl at:						
9 %	−	+	+	+	+	+
12 %	−	+	+	+	+	−
14 %	−	+	+	−	−	−
Arginine dihydrolase activity	−	−	+	+	+	−
Hydrolysis of:						
DNA	−	−	−	+	+	−
Urea	−	−*	−	−*	−*	−
Tween 80	−	+	+	+	+	+
Lecithinase activity	−	+	−	−	+	ND
Sole carbon and energy sources:						
Acetate	−	−	+	−	+	ND
Propionate	+	+†	−*	+†	+†	ND
L-Rhamnose	+	−*	−*	−*	−	ND
L-Alanine	+	−	+	+†	+	ND
L-Histidine	+	−‡	−*	−‡	+*	ND
L-Proline	−	+†	+†	+†	+	ND
DNA G+C content (mol%; T_m)	34	40	39	45	43	38

*A positive reaction was reported in the original description involving a different method.
†A negative reaction was reported in the original description involving a different method.
‡A weak or delayed positive reaction was reported in the original description involving a different method.

experiments were performed in duplicate. The DNA–DNA relatedness values for strain JUB59^T with respect to *B. myxarmorum* ADA-4^T (15.9–17.3 %) and *B. algoritergicola* APA-1^T (19.3–22.1 %) were well below the DNA–DNA relatedness threshold value (70 %) used for the delineation of bacterial species (Wayne *et al.*, 1987). The DNA G+C content of strain JUB59^T, determined using HPLC according to the method of Mesbah *et al.* (1989), was 34 mol%, which is lower than those of recognized species of the genus *Bizionia* (38–45 %) but similar to those of many members of the family *Flavobacteriaceae*.

The results obtained support the allocation of strain JUB59^T to the genus *Bizionia*. However, the strain could be differentiated from recognized *Bizionia* species on the basis of a number of phenotypic traits (Table 1 and Supplementary Table S1). In addition, the novel isolate was genetically distinct from recognized members of the genus, as indicated by the DNA–DNA hybridization values and DNA G+C content. Hence, strain JUB59^T represents a novel species in the genus *Bizionia*, for which the name *Bizionia argentinensis* sp. nov. is proposed.

Description of *Bizionia argentinensis* sp. nov.

Bizionia argentinensis (ar.gen.tin.en′sis. N.L. fem. adj. *argentinensis* pertaining to Argentina, the country associated with the scientific station in the vicinity of which the strain was isolated).

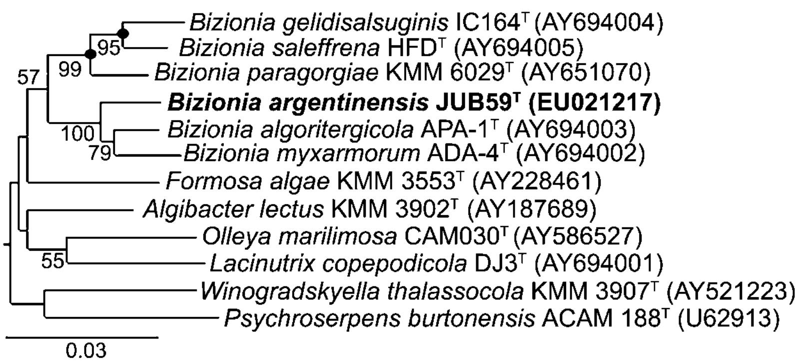

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, for strain JUB59^T, the type strains of species of the genus *Bizionia* and closely related species within the family *Flavobacteriaceae*. Bootstrap percentages (based on 1000 resampled datasets) are shown at nodes (where >50 %). Filled circles indicate that the corresponding nodes (groupings) were also recovered in the Fitch–Margoliash and maximum-likelihood trees. Bar, 0.03 substitutions per nucleotide position. A larger tree, including all type strains of the family (Supplementary Fig. S3), is available in IJSEM Online.

Cells are Gram-negative, non-motile, aerobic rods, 0.2–0.4 μm in diameter and 2–3 μm in length. Endospores are not formed. On MA, colonies have a butyrous consistency and are shiny, yellowish orange, circular (2–3 mm in diameter) with entire edges and are slightly raised in the centre. Growth occurs at 2–28 °C, but not at 30 °C (optimum, 22–25 °C). Growth occurs in the presence of 1–6 % NaCl, but not in the presence of 9 % NaCl. Na^+ ions are required for growth. Flexirubin-type pigments are not produced (negative KOH test result). Catalase, oxidase and arginine dihydrolase activities are present, but β-galactosidase activity is absent. Casein, gelatin and L-tyrosine are hydrolysed, but agar, starch, aesculin, chitin, carboxymethylcellulose, DNA, Tween 80 and urea are not hydrolysed. Nitrate is not reduced. Indole and H_2S are not produced. Propionate, L-rhamnose, L-alanine and L-histidine are utilized as sole carbon and energy sources, but acetate and L-proline are not utilized. Acid is not produced from carbohydrates. Additional phenotypic characteristics are given in Table 1. The predominant menaquinone is MK-6. The major polar lipids are phosphatidylethanolamine, an aminolipid, an amino-positive phospholipid and two unidentified lipids. Cellular fatty acids amounting to >1 % of the total fatty acids are as follows: iso-14 : 0 (2.0 %), iso-15 : 1ω10*c* (18.1 %), anteiso-15 : 1ω10*c* (3.6 %), iso-15 : 0 (17.3 %), anteiso-15 : 0 (14.0 %), 15 : 1ω6*c* (3.0 %), 15 : 0 (6.0 %), branched 16 : 1 (1.1 %), iso-16 : 0 (1.1 %), iso-15 : 0 3-OH (3.3 %), 15 : 0 2-OH (2.1 %), iso-17 : 1ω9*c* (2.6 %), 15 : 0 3-OH (2.0 %), iso-16 : 0 3-OH (5.2 %), 18 : 1ω5*c* (1.0 %), iso-17 : 0 3-OH (9.2 %), 17 : 0 2-OH (1.8 %) and summed feature 3 (comprising iso-15 : 0 2-OH and/or 16 : 1ω7*c*; 2.7 %). The DNA G+C content is 34 mol%.

The type strain, JUB59^T (=DSM 19628^T=CCM-A-29 1259^T), was isolated from surface marine water collected in Potter Cove, King George Island (Isla 25 de Mayo), South Shetland Islands, Antarctica.

Acknowledgements

The authors would like to thank Dr J. P. Bowman for providing reference strains and for his valuable help in the discussion of protocols for some biochemical tests.

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