Bizionia echini sp. nov., isolated from a sea urchin

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A bacterial strain, designated KMM 6177^T, was isolated from the sea urchin *Strongylocentrotus intermedius* and subjected to a polyphasic taxonomic investigation. The bacterium was found to be heterotrophic, aerobic, non-motile by gliding and orange-pigmented. Comparative phylogenetic analysis based on 16S rRNA gene sequencing placed the marine isolate in the genus *Bizionia*, a member of the family *Flavobacteriaceae*, with 16S rRNA gene sequence similarity of 94.9–98.6 % with recognized *Bizionia* species. Strain KMM 6177^T grew at 4–39 °C and with 1–8 % NaCl. It produced alkaline phosphatase, catalase and oxidase and hydrolysed aesculin, gelatin, DNA and Tween 20. The predominant fatty acids were iso-$C_{15:1}$, iso-$C_{15:0}$, iso-$C_{15:0}$ 3-OH, iso-$C_{17:0}$ 3-OH and a summed feature (comprising iso-$C_{15:0}$ 2-OH and/or $C_{16:1}\omega7c$). The DNA G+C content was 34.4 mol%. A combination of phylogenetic, genotypic and phenotypic data clearly indicated that strain KMM 6177^T represents a novel species in the genus *Bizionia*, for which the name *Bizionia echini* sp. nov. is proposed. The type strain is KMM 6177^T (=KCTC 22015^T=LMG 25220^T).

The genus *Bizionia*, a member of the family *Flavobacteriaceae* (Bernardet *et al.*, 2002), was created for the accommodation of heterotrophic, strictly aerobic, rod-shaped, non-motile by gliding, pigmented and Gram-negative bacteria (Nedashkovskaya *et al.*, 2005). Currently, the genus *Bizionia* comprises the representatives of six recognized species that were isolated from cold or moderately cold marine and saline lake environments (Bowman & Nichols, 2005; Nedashkovskaya *et al.*, 2005; Bercovich *et al.*, 2008).

Here we report on the isolation and characterization of an unknown bacterial strain, designated KMM 6177^T, by using a polyphasic taxonomic approach. According to a comparative phylogenetic analysis based on 16S rRNA gene sequencing, strain KMM 6177^T was placed in the genus *Bizionia* of the family *Flavobacteriaceae*, in which it represents a novel species.

Strain KMM 6177^T was isolated from a sea urchin specimen collected from Troitsa Bay of the Sea of Japan (also known as the East Sea). For strain isolation, 0.1 ml sea urchin tissue homogenate was plated on marine agar 2216 (Difco). After primary isolation and purification, strains were cultivated at 28 °C on the same medium and stored at –80 °C in marine broth (Difco) supplemented with 20 % (v/v) glycerol.

The phylogenetic position of strain KMM 6177^T was determined from its almost-complete 16S rRNA gene sequence (1448 bp). Genomic DNA extraction, PCR and sequencing of the 16S rRNA gene were performed by using previously described procedures (Cho *et al.*, 2006). The sequence obtained was aligned with those of representative members of selected genera belonging to the family *Flavobacteriaceae* by using PHYDIT version 3.1 (http://plaza.snu.ac.kr/~jchun/phydit/). Phylogenetic trees were inferred by using suitable programs of the PHYLIP package (Felsenstein, 1993). Phylogenetic distances were calculated from the Jukes–Cantor model (Jukes & Cantor, 1969), and the trees were constructed on the basis of the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1993) and maximum-parsimony (Kluge & Farris, 1969) algorithms. Bootstrap analysis was performed with 1000 resampled datasets, using the SEQBOOT and CONSENSE programs of the PHYLIP package.

Comparative phylogenetic analysis of the almost complete 16S rRNA gene sequence of strain KMM 6177^T revealed that the strain formed a distinct lineage within the genus *Bizionia*, a member of the family *Flavobacteriaceae* (Fig. 1). The closest relative of the sea urchin isolate was *Bizionia algoritergicola* APA-1^T (98.6 % sequence similarity). The

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Bizionia echini* KMM 6177^T is FJ716799.

sequence similarities of strain KMM 6177^T with the other *Bizionia* species were in the range of 94.9–97.6 %.

For determination of the DNA base composition, DNA was isolated following the method of Marmur (1961) and the DNA G+C content was determined by the thermal denaturation method (Marmur & Doty, 1962). The DNA G+C content of strain KMM 6177^T was 34.4 mol%.

Analysis of fatty acid methyl esters was carried out on cells grown on marine agar at 28 °C for 48 h in accordance with the standard protocol of the Microbial Identification System (Microbial ID). The fatty acids accounting for more than 1 % of the total were iso-$C_{15:1}$ G (21.8 %), iso-$C_{15:0}$ (17.8 %), iso-$C_{17:0}$ 3-OH (13.2 %), a summed feature comprising $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH (12.3 %), iso-$C_{15:0}$ 3-OH (5.4 %), iso-$C_{17:1}\omega9c$ (4.0 %), $C_{15:0}$ (3.8 %), iso-$C_{16:0}$ 3-OH (3.4 %), a summed feature comprising iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B (1.5 %), iso-$C_{16:1}$ (1.2 %), $C_{17:1}\omega6c$ (1.2 %), iso-$C_{16:0}$ (1.1 %) and $C_{16:0}$ (1.0 %).

Physiological and biochemical properties of strain KMM 6177^T were determined by using previously described methods (Nedashkovskaya *et al.*, 2004). API 20 E, API 20 NE, API 32 GN and API ZYM galleries (bioMérieux) were also used for studying the phenotypic features of the strain according to the manufacturer's instructions, except that the galleries were incubated at 28 °C. Susceptibility to antibiotics was tested using additional discs containing cefalexin (30 μg), cefazolin (30 μg), chloramphenicol (30 μg), doxycycline (10 μg), erythromycin (15 μg), nalidixic acid (30 μg), ofloxacin (5 μg), oxacillin (10 μg), rifampicin (5 μg) and vancomcin (30 μg).

Cells of strain KMM 6177^T were Gram-negative, aerobic, non-motile by gliding and formed circular, mucous and orange-pigmented colonies on marine agar after cultivation for 48 h at 28 °C. Other phenotypic characteristics of the strain studied are given in the species description and in Table 1. Strain KMM 6177^T shared many similar phenotypic features with recognized species of the genus *Bizionia*. It is characterized by a respiratory type of metabolism, by the absence of gliding motility, by production of catalase, oxidase and gelatinase, and by the inability to form acid from carbohydrates, to produce nitrate reductase and to hydrolyse starch (Table 1). However, the novel isolate differed from all recognized *Bizionia* species by the maximal growth temperature, by the presence of aesculin and citrate utilization and by the absence of casein hydrolysis (Table 1). In contrast to its closest neighbour, *B. algoritergicola*, strain KMM 6177^T did not produce arginine dihydrolase, hydrolyse urea or utilize L-histidine. These strains can be clearly differentiated from each other by the growth temperature range (4–39 °C for KMM 6177^T and −2 to 25 °C for *B. algoritergicola* APA-1^T). Furthermore, strain KMM 6177^T had a lower DNA G+C content in comparison with *B. algoritergicola* strain APA-1^T (34.4 vs 45 mol%). Consequently, the phenotypic distinctiveness between the novel isolate and *B. algoritergicola* strongly supports the results of 16S rRNA gene phylogenetic analysis (98.6 % sequence similarity). In this case, determination of the degree of DNA–DNA reassociation was not needed for confirmation of delineation of strain KMM 6177^T as a novel species from its nearest phylogenetic neighbour, *B. algoritergicola* APA-1^T, because DNA–DNA hybridization experiments should be carried out for strains sharing 16S rRNA gene sequence similarities higher than about 99 % according to the proposal of Stackebrandt & Ebers (2006). Differences in phenotypic and genotypic traits between strain KMM 6177^T and other species of the genus *Bizionia* are presented in Table 1.

Therefore, on the basis of the phylogenetic distances and phenotypic and genotypic data obtained, it is suggested that strain KMM 6177^T represents a novel species within the genus *Bizionia*, for which the name *Bizionia echini* sp. nov. is proposed.

Description of *Bizionia echini* sp. nov.

Bizionia echini (e.chi′ni. L. gen. n. *echini* of/from a sea urchin).

The main characteristics are as given by Nedashkovskaya *et al.* (2005) for the genus. In addition, cells are 0.4–0.5 μm wide and 1.4–3.5 μm long. On marine agar, colonies are

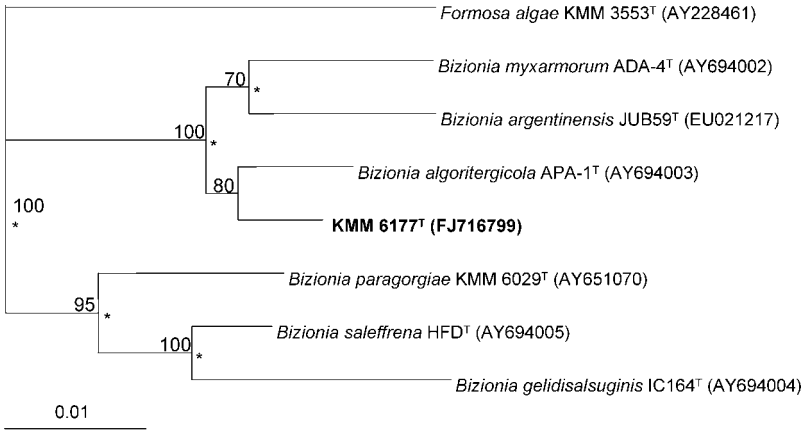

Fig. 1. Phylogenetic tree of *Bizionia* species based on the 16S rRNA gene sequences. The tree was constructed using the Jukes–Cantor model and neighbour-joining algorithm. The asterisks indicate branches that were also recovered in the maximum-likelihood and maximum-parsimony trees. The numbers at nodes indicate the levels of bootstrap support (%). Only 50 % or higher values are indicated. Scale bar, 0.01 nucleotide substitutions per position.

Table 1. Differential phenotypic characteristics of *Bizionia* species

Species: 1, KMM 6177^T; 2, *Bizionia algoritergicola*; 3, *Bizionia argentinensis*; 4, *Bizionia gelidisalsuginis*; 5, *Bizionia myxarmorum*; 6, *Bizionia paragorgiae*; 7, *Bizionia saleffrena*. Data from Bowman & Nichols (2005), Nedashkovskaya *et al.* (2005), Bercovich *et al.* (2008) and this study. All strains were positive for the following characteristics: oxidase, catalase and alkaline phosphatase activities; and hydrolysis of gelatin. All strains were negative for the following characteristics: gliding motility; nitrate reductase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase and α-fucosidase activities; flexirubin-type pigment production; hydrolysis of starch; acid production from carbohydrates; utilization of adipate; and indole production. O, Orange; Y, yellow; ND, not detected.

Characteristic	1	2	3	4	5	6	7
Colony colour	O	Y	Y–O	Y	Y	Y	Y
Arginine dihydrolase	−	+	+	+	+	−	−
H_2S production	+	+	−	ND	−	+	ND
Temperature range (°C)	4–39	−2 to 25	2–28	−2 to 29	−2 to 30	4–36	−2 to 25
Salinity range (%)	1–8	1–10	1–6	1–17	1–10	1–8	1–17
Hydrolysis of:							
Aesculin	+	−	−	−	−	−	−
Casein	−	+	+	+	+	+	+
DNA	+	+	−	−	+	−	−
Urea	−	+	−	−	+	−	+
Tween 20	+	+	ND	ND	−	−	ND
Tween 80	+	+	−	−	+	+	+
Utilization of:							
D-Glucose, D-mannose	+	+	−	−	+	−	−
Citrate	+	−	−	−	−	−	−
L-Histidine	−	+	+	+	+	−	+
Susceptibility to:							
Carbenicillin, tetracycline	+	+	ND	ND	−	+	ND
Benzylpenicillin	+	+	ND	ND	+	−	ND
Streptomycin	−	−	ND	ND	+	+	ND
Neomycin	−	−	ND	ND	+	−	ND
DNA G+C content (mol%)	34.4	45.0	34.0	39.0	43.0	37–38	40.0

circular, mucoid, orange-pigmented, shiny, with entire edges and 2–4 mm in diameter. Growth occurs with 1–8 % NaCl and at 4–39 °C. Optimal growth is observed with 2–3 % NaCl. Oxidase, catalase and alkaline phosphatase activities are present. Arginine dihydrolase, lysine and ornithine decarboxylases and tryptophan deaminase activities are absent. Decomposes aesculin, gelatin, DNA and Tweens 20, 40 and 80. Does not hydrolyse agar, casein, starch, CM-cellulose, urea or chitin. Produces no acid from L-arabinose, cellobiose, D-fructose, L-fucose, D-galactose, D-glucose, D-lactose, maltose, melibiose, raffinose, L-rhamnose, sucrose, DL-xylose, *N*-acetylglucosamine, adonitol, dulcitol, glycerol, inositol or mannitol. Utilizes mannose, but not lactose, sucrose, inositol or sorbitol. According to the API 20 NE gallery, glucose, arabinose, mannitol, *N*-acetylglucosamine, maltose, malate, gluconate, citrate and phenylacetate are also utilized. None of the API 32 GN tests indicate a positive reaction, and only two tests (citrate utilization and gelatin hydrolysis) from the API 20 E gallery are positive for the type strain. Testing by using the API ZYM gallery for the type strain indicates that esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase activities are present, but lipase (C14), α-chymotrypsin, α- and β-galactosidases, α- and β-glucosidases, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities are absent. Nitrate is not reduced. H_2S is produced but indole is not. Susceptible to ampicillin, benzylpenicillin, carbenicillin, cefalexin, chloramphenicol, erythromycin, doxycycline, lincomycin, ofloxacillin, oleandomycin, rifampicin, tetracycline and vancomycin; resistant to cefazolin, gentamicin, kanamycin, nalidixic acid, neomycin, oxacillin, polymixin and streptomycin. The predominant fatty acids of strain KMM 6177^T are iso-$C_{15:1}$ G, iso-$C_{15:0}$, iso-$C_{15:0}$ 3-OH, iso-$C_{17:0}$ 3-OH and a summed feature comprising $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH. The DNA G+C content is 34.4 mol%.

The type strain is KMM 6177^T (=KCTC 22015^T=LMG 25220^T), isolated from the sea urchin *Strongylocentrotus intermedius* collected from Troitsa Bay, Gulf of Peter the Great, the Sea of Japan (also known as the East Sea).

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