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Bizionia psychrotolerans sp. nov., a psychrophilic bacterium isolated from the intestine of a sea cucumber (*Apostichopus japonicus*)

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Abstract A novel Gram-negative, non-flagellated, non-gliding, rod-shaped bacterial strain, designated PB-M7^T, was isolated from the intestine of a sea cucumber collected from Pohang, South Korea. Growth was observed at 4–30 °C (optimum, 25 °C), pH 6.0–9.0 (optimum, pH 7.0–8.0), and with 2.0–6.0 % (w/v) NaCl (optimum, 2.0 %). In a phylogenetic analysis based on 16S rRNA gene sequences, strain PB-M7^T was found to belong to the genus *Bizionia* and to be most closely related to *Bizionia echini* KMM 6177^T (99.0 % 16S rRNA gene sequence similarity), *Bizionia hallyeonensis* T-y7^T (97.9 %), *Bizionia algoritergicola* APA-1^T (97.5 %), *Bizionia argentinensis* JUB59^T (97.5 %) and *Bizionia myxarmorum* ADA-4^T (97.1 %). The predominant fatty acids of strain PB-M7^T were identified as iso-$C_{15:0}$ (22.2 %), iso-$C_{15:1}$ G (10.8 %), iso-$C_{17:0}$ 3-OH (16.7 %) and summed feature 3 ($C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$; 11.2 %). The major respiratory quinone was identified as menaquinone-6 (MK-6). The polar lipid profile of strain PB-M7^T was found to contain phosphatidylethanolamine, an unidentified phospholipid, three unidentified aminolipids and three unidentified lipids. The DNA G + C content of strain PB-M7^T was determined to be 33.4 mol% and the mean DNA–DNA relatedness values with the type strains of *B. echini*, *B. hallyeonensis*, *B. algoritergicola, B. argentinensis*, and *B. myxarmorum* were 52.9, 48.5, 46.5, 37.1 and 26.6 %, respectively. Based on the data presented, strain PB-M7^T represents a novel species of the genus *Bizionia*, for which the name *Bizionia psychrotolerans* sp. nov. is proposed. The type strain of *B. psychrotolerans* is PB-M7^T (= KCCM 43042 ^{T} = JCM 19924 ^{T}).

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Introduction

The genus *Bizionia*, a member of the family *Flavobacteriaceae* of the phylum *Bacteroidetes*, was first described by Nedashkovskaya et al. (2005), with *Bizionia paragorgiae* as the type species. Subsequently, seven additional *Bizionia* species were described: *B. algoritergicola*, *B. gelidisalsuginis*, *B. myxarmorum*, *B. saleffrena* (Bowman and Nichols 2005), *B. argentinensis* (Bercovich et al. 2008), *B. echini* (Nedashkovskaya et al. 2010) and *B. hallyeonensis* (Yoon et al. 2013). All members of the genus *Bizionia* have been isolated from various marine environmental samples, including soft coral, sea-ice brine and seawater (Bercovich et al. 2008; Bowman and Nichols 2005; Nedashkovskaya et al. 2010; Yoon et al. 2013).

During our investigation of the microbial diversity of marine animals, a novel bacterial strain, designated PB-M7^T, was isolated from the intestine of a sea cucumber obtained from Pohang in the East Sea of South Korea. Comparative 16S rRNA gene sequence analysis indicated that strain PB-M7^T is phylogenetically related to the members of the genus *Bizionia*. In this study, we determined the exact taxonomic position of strain PB-M7^T using polyphasic characterization, which included determination of chemotaxonomic and other phenotypic properties, a detailed phylogenetic analysis based on 16S rRNA gene sequences, and measurements of DNA–DNA relatedness.

Materials and methods

Isolation and culture conditions of the isolate

Strain PB-M7^T was isolated from the intestine of a sea cucumber collected from Pohang, South Korea, by the dilution plating technique on marine agar 2216 (MA; BD) with incubation at 25 °C. The isolate was cultivated routinely under the same conditions unless otherwise noted. Strain PB-M7^T was maintained at 4 °C on MA for short-term preservation or as a suspension in 20 % (w/v) glycerol at −80 °C for long-term preservation. *B. echini* KMM 6177^T, *B. hallyeonensis* T-y7^T, *B. algoritergicola* APA-1^T, *B. argentinensis* JUB59^T, *B. myxarmorum* ADA-4^T and *B. paragorgiae* KMM 6029^T were used as reference strains for phenotypic characterization, fatty acid analysis and DNA–DNA hybridization. Reference strains were obtained from the Korean Collection of Type Cultures (KCTC, Korea), Deutsche Sammlung von Mikroorganisem und Zellkulturen (DSMZ, Germany) and Centre de Ressources Biologiques de l'Institut Pasteur (CRBIP): *B. echini* KCTC 22015^T, *B. hallyeonensis* KCTC 23881^T, *B. paragorgiae* KCTC 12304^T, *B. argentinensis* DSM 19628^T, *B. algoritergicola* CIP 108533^T, and *B. myxarmorum* CIP 108535^T.

Morphological, physiological, and biochemical characteristics

Cell morphology and flagellation were observed by light microscopy (Eclipse 80i, Nikon) and transmission electron microscopy (SUPRA 55VP, Carl Zeiss), respectively. For transmission electron microscopy, strain PB-M7^T cells were fixed with 2.5 % (v/v) glutaraldehyde and dehydrated in a graded series of ethanol. The samples were examined after being dried using a critical point dryer (K850, Quorum Technologies) and sputter-coated with platinum (Q150T S, Quorum Technologies). Gram staining was performed using a bioMérieux Gram stain kit, according to the manufacturer's instructions. The optimal temperature for growth was determined using MA plates at 4, 10, 20, 25, 30, 37, 40, and 45 °C. The pH range for growth was determined using MA adjusted to different pH values with the following buffers: acetate/acetic acid (pH 5.0–6.0), Tris buffer (pH 7.0–9.0) and glycine (pH 10.0–11.0). Growth in the absence of NaCl and in the presence of 0.5, 1.0, or 2.0 % (w/v) NaCl was investigated using trypticase soy broth prepared similarly to the BD medium except that NaCl was excluded and 0.45 % (w/v) $MgCl_2{\cdot}6H_2O$ or 0.06 % (w/v) KCl was added. Growth in the presence of NaCl (concentrations ranging from 2.0 to 10.0 % [w/v], at 0.5 % intervals) was investigated using marine broth 2216 (MB; BD) medium supplemented with appropriate salt concentrations (0–8.0 %) exceeding 2.0 % NaCl of MB. Growth results were read daily over a period of 20 days with shaking incubation at 25 °C, which was determined by measuring the turbidity at

600 nm. Growth in the absence of oxygen was determined on MA plates at 25 °C for 20 days using the GasPak EZ anaerobe pouch system (BD) in an air-tight jar (AnaeroPack Rectangular Jar, Mitsubishi Gas Chemical Company Inc.). Hydrolysis of casein, starch, L-tyrosine, xanthine, and hypoxanthine was tested on MA using the substrate concentrations described by Cowan and Steel (1965) Nitrate reduction and hydrolysis of aesculin and Tween 20, 40, 60, and 80 were investigated as described previously (Lányí 1987), using artificial seawater for preparation of the media. For every liter of distilled water, the artificial seawater contained 23.6 g NaCl, 0.64 g KCl, 4.53 g $MgCl_2$·$6H_2O$, 5.94 g $MgSO_4$·$7H_2O$ and 1.3 g $CaCl_2$·$2H_2O$ (Bruns et al. 2001). Catalase and oxidase activities were determined as described by Cowan and Steel (1965). Utilization of various substrates as sole carbon source, acid production from various carbohydrates, and enzyme activities were determined using API ZYM, API 20E, API 20NE, API 50CH (bioMérieux) and GN2 Microplates (Biolog), according to the manufacturer's instructions. Susceptibility to antibiotics was tested on MA plates using antibiotic discs (Advantec) containing the following antibiotics: ampicillin (10 μg), carbenicillin (100 μg), cephalothin (30 μg), chloramphenicol (100 μg), gentamicin (30 μg), kanamycin (30 μg), lincomycin (15 μg), neomycin (30 μg), novobiocin (5 μg), penicillin G (20 U), polymyxin B (100 U), streptomycin (50 μg) and tetracycline (30 μg).

Determination of the 16S rRNA gene sequences and phylogenetic analysis

Chromosomal DNA from strain PB-M7^T was prepared using a G-spin DNA extraction kit (iNtRON Biotechnology). The bacterial 16S rRNA gene was amplified by polymerase chain reaction (PCR) using two universal primers: 9F, 5′-GAGTTTGATCCTGGCTCAG-3′; and 1512R, 5′-ACGGTTACCTTGTTACGACTTC-3′ (Yoon et al. 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described previously (Yoon et al. 2003). The 16S rRNA gene sequences of strain PB-M7^T and closely related type strains were aligned with CLUSTAL W (Thompson et al. 1994). Phylogenetic trees were constructed using the neighbour-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981) and maximum-parsimony methods (Fitch 1971) implemented in the PHYLIP package (Felsenstein 2005). A bootstrap analysis with 1,000 replications was performed to evaluate the tree topology (Felsenstein 1985).

DNA base composition and DNA–DNA hybridization

Strain PB-M7^T was cultivated for 5 days on MA plates at 25 °C to obtain the cell biomass required for DNA extraction. Measurement of DNA G + C content was performed using the method of Tamaoka and Komagata (1984) with minor modifications. DNA was hydrolyzed by nuclease P1 (Sigma) and was then analyzed using a reversed-phase high-performance liquid chromatography (HPLC) system equipped with a YMC ODS-A column (250 × 4.6 mm). The nucleotides were eluted using a mixture of 0.55 M $NH_4H_2PO_4$ (pH 4.0)/acetonitrile (40:1, v/v) at a flow rate of 1 mL min^{-1} at room temperature and detected based on the UV absorbance at 270 nm. *Escherichia coli* DNA was used as a standard.

DNA–DNA hybridization was performed by the fluorometric method of Ezaki et al. (1989) using photobiotin-labeled DNA probes and microdilution wells (MaxiSorp, FluoroNunc). Hybridization was performed with five replications for each sample. The highest and lowest values obtained for each sample were excluded and the means of the remaining three values were considered the DNA–DNA relatedness values.

Chemotaxonomic characterization

Isoprenoid quinones were extracted in a mixture of chloroform/methanol (2:1, v/v) according to the method of Komagata and Suzuki (1987). The extracted quinones were identified using a liquid chromatography mass spectrometry (LC–MS) system equipped with a diode array detector and a single quadropole mass spectrometer (HCT Ion-Trap MS; Bruker). HPLC separation was performed using a YMC Hydrosphere C18 reversed phase (ODS) column (150 × 2.0 mm). The isoprenoid quinones were eluted by a mixture of methanol/isopropanol (2:1, v/v) at a flow rate of 0.3 mL min^{-1}. Mass spectra were obtained for *m*/*z* values ranging from 50 to 1,200.

For cellular fatty acid analysis, the cell biomasses of strain PB-M7^T, *B. echini* KMM 6177^T, *B. hallyeonensis* T-y7^T, *B. algoritergicola* APA-1^T, *B. argentinensis* JUB59^T, *B. myxarmorum* ADA-4^T, and *B. paragorgiae* KMM 6029^T were harvested from MA plates after cultivation for 5 days at 25 °C. Cellular fatty acids were saponified, methylated, and extracted according to the standard MIDI protocol (version 6.2) of the Sherlock Microbial Identification System. The fatty acids were analyzed by gas chromatography (Hewlett Packard 6890) and identified using the TSBA6 database (Sasser 1990).

Polar lipids were extracted according to the procedures described by Xin et al. (2000). Extracted lipids were separated by 2-dimensional thin-layer chromatography (TLC) (Minnikin et al. 1977). The solvent systems used were chloroform/methanol/water (65:25:3.8, v/v/v) for the first dimension and chloroform/methanol/acetate/water (40:7.5:6:1.8, v/v/v/v) for the second dimension. The separated polar lipids were identified by spraying with 10 % (w/v) phosphomolybdic acid (Sigma) for detection of all lipids, ninhydrin (Sigma) for detection of aminolipids, molybdenum blue reagent (Sigma) for detection of phospholipids, α-naphtholsulfuric acid for detection of glycolipids (Minnikin et al. 1984; Tamaoka and Komagata 1984) and Dragendorff's reagent (Sigma) for detection of choline-containing lipids.

Results and discussion

Morphological, physiological, and biochemical characteristics

Strain PB-M7^T was observed to be Gram-negative, obligately aerobic, non-motile and rod-shaped. Growth was observed at 4–30 °C (optimum, 25 °C) and at pH 6.0–9.0 (optimum, pH 7.0–8.0). Strain PB-M7^T was found to grow in the presence of 2.0–6.0 % (w/v) NaCl (optimum, 2.0 %). Mg^{2+} ions were not found to be required for growth. Strain PB-M7^T was found to exhibit oxidase activity but no catalase or urease activities. Nitrate was not reduced to nitrite. H_2S was not produced. Strain PB-M7^T was found to be able to hydrolyze aesculin, casein, gelatin, tyrosine and Tween 20, 40, 60, and 80, but not starch, xanthine and hypoxanthine. Strain PB-M7^T was found fto be susceptible to carbenicillin, cephalothin, chloramphenicol, lincomycin, novobiocin, penicillin G, streptomycin, and tetracycline. Morphological, cultural, physiological and biochemical characteristics of strain PB-M7^T are given in the species description and in Table 1. Strain PB-M7^T shared many similar phenotypic features with recognized species of the genus *Bizionia*. However, the novel isolate and its closest neighbour, *B. echini*, were distinguished clearly from other members of the genus *Bizionia* by the ability to hydrolyse aesculin and susceptibility to streptomycin. However, in contrast to *B. echini*, strain PB-M7^T did not produce trypsin and utilize citrate, L-Arabinose, D-glucose, D-maltose, D-mannose and *N*-acetyl-glucosamine. Furthermore, only strain PB-M7^T showed susceptibility to tetracycline (Table 1).

Molecular-based analysis

The almost-complete 16S rRNA gene sequence obtained for strain PB-M7^T (GenBank/EMBL/DDBJ accession number KJ461691) comprises 1,449 nucleotides. In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain PB-M7^T fell within a clade comprising *Bizionia* species, clustering consistently with the type strains of *B. echini* KMM 6177^T, *B. algoritergicola* APA-1^T, and *B. hallyeonensis* T-y7^T (Fig. 1). Strain PB- M7^T shows the highest 16S rRNA gene sequence similarities with *B. echini* KMM 6177^T (99.0 %), *B. hallyeonensis* T-y7^T (97.9 %), *B. algoritergicola* APA-1^T (97.5 %), *B. argentinensis* JUB59^T (97.5 %) and *B. myxarmorum* ADA-4^T (97.1 %) and had 95.4–96.2 % similarities with the type strains of other *Bizionia* species. Mean DNA–DNA relatedness values between strain PB-M7^T and several closely related species, i.e. *B. echini* KMM 6177^T, *B. hallyeonensis* T-y7^T, *B. algoritergicola* APA-1^T, *B. argentinensis* JUB59^T and *B. myxarmorum* ADA-4^T were 52.9 ± 0.5, 48.5 ± 4.6, 46.5 ± 4.8, 37.1 ± 2.5 and 26.6 ± 4.3 %, respectively, values which are all below the 70 % cutoff recommended for the delineation of genomic species (Wayne et al. 1987).

Chemotaxonomic characteristics

The predominant isoprenoid quinone of strain PB-M7^T was identified as menaquinone-6 (MK-6), as has been observed for other *Bizionia* species. The complete cellular fatty acid profile of strain PB-

Table 1 Differential phenotypic characteristics of *B. psychrotolerans* PB-M7^T and closely related species of the genus *Bizionia*

Characteristic	1	2	3	4	5	6	7
H_2S production	–	+	–	+	–	–	+
Hydrolysis of							
Aesculin	+	+	–	–	–	–	–
Tween 80	+	+	+	+	–	+	+
Enzymatic activity of							
Arginine dihydrolase	–	–	–	+	–	+	–
Cystine arylamidase	+	+	–	–	–	–	+
Trypsine	–	+	–	–	–	–	–
Utilization of[a]							
L-Arabinose	–	+	–	–	–	–	–
Citrate	–	+	–	–	–	–	–
D-Glucose	–	+	–	+[b]	–	+[b]	–
D-Maltose	–	+	–	–	–	–	–
D-Mannose	–	+	–	+[b]	–	+[b]	–
N-acetyl- D-glucosamine	–	+	–	–	–	–	–
Susceptibility to							
Novobiocin	+	+	+	+	–	+	+
Penicillin G	+	+	–	+	+	+	+
Streptomycin	+	+	–	–	–	–	–
Tetracycline	+	–	–	–	–	–	–
DNA G + C content (mol %)[b,c]	33	34	37	45	34	43	37–38

Strains: 1, *B. psychrotolerans* PB-M7^T sp. nov.; 2, *B. echini* KMM 6177^T; 3, *B. hallyeonensis* T-y7^T; 4, *B. algoritergicola* APA-1^T; 5, *B. argentinensis* JUB59^T; 6, *B. myxarmorum* ADA-4^T; 7, *B. paragorgiae* KMM 6029^T. All data from this study unless otherwise indicated. All strains are positive for the following: hydrolysis of casein and tyrosine; activity of alkaline phosphatase, esterase (C 4), esterase lipase (C 8), leucine arylamidase, valine arylamidase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase; and susceptibility to carbenicillin, cephalothin, chloramphenicol, and lincomycin. All strains are negative for the following: indole production; hydrolysis of starch, xanthine, and hypoxanthine; activity of lysine decarboxylase, ornithine decarboxylase; acid production from carbohydrates; activity of lipase (C 14), α-chymotrypsin, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase; and susceptibility to ampicillin, gentamicin, kanamycin, and polymyxin B. +, positive; –, negative

[a] Data from API 20NE tests. Data for *B. echini*, *B. algoritergicola*, *B. argentinensis*, *B. myxarmorum*, *B. paragorgiae* were taken from Nedashkovskaya et al. (2010)

[b] Opposite results were reported by Bowman and Nichols (2005)

[c] Data for the reference stains were obtained from Bowman and Nichols (2005), Nedashkovskaya et al. (2005, 2010), Bercovich et al. (2008), and Yoon et al. (2013)

M7^T was compared with those of *B. echini* KMM 6177^T, *B. hallyeonensis* T-y7^T, *B. algoritergicola* APA-1^T, *B. argentinensis* JUB59^T, *B. myxarmorum* ADA-4^T, and *B. paragorgiae* KMM 6029^T grown and analyzed under identical conditions. The major fatty acids (>10 % of the total fatty acids) of strain PB-M7^T were identified as iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH, and summed feature 3 ($C_{16:1}$ $\omega7c$ and/or $C_{16:1}$ $\omega6c$, Table 2). The fatty acid profiles of strain PB-M7^T and the six reference strains were similar, while differences were observed in the proportions of some fatty acids (Table 2). The major polar lipids were found to consist of phosphatidylethanolamine, an unidentified phospholipid, three unidentified aminolipids and three unidentified lipids. The polar lipid profile of strain PB-M7^T was found to be similar to those of other members of the genus *Bizionia* (Bercovich et al. 2008; Yoon et al. 2013). The DNA G + C content of the strain PB-M7^T was determined to be 33.4 mol%, which is within the range reported for *Bizionia* species.

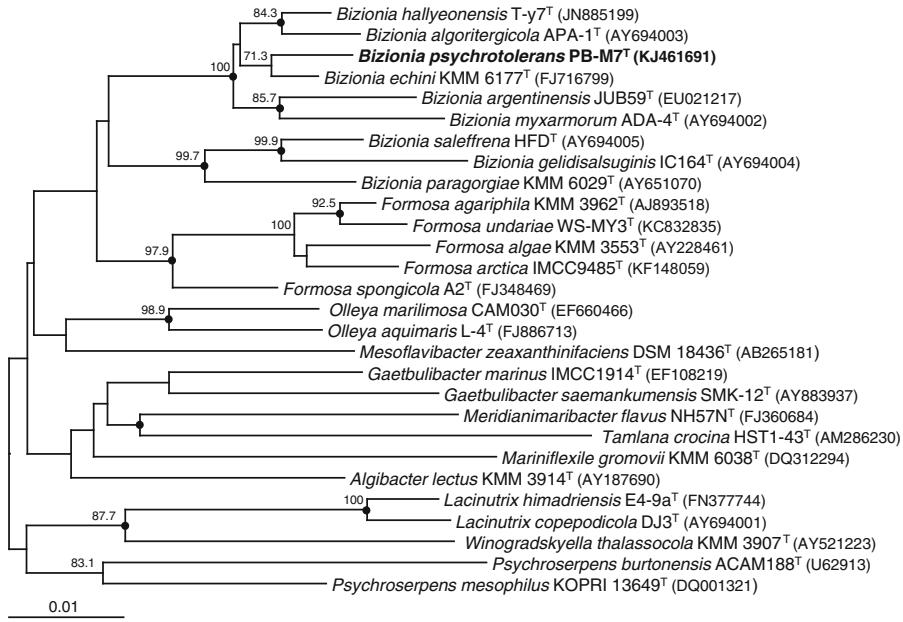

Fig. 1 Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of *B. psychrotolerans* PB-M7^T, other *Bizionia* species and representatives of some other related taxa. Only bootstrap values (expressed as percentages of 1,000 replications) > 70 % are shown at branching points. The *filled circles* indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. *Lutibacter litoralis* CL-TF09^T (GenBank accession no. AY962293) was used as an outgroup (not shown). *Bar*, 0.01 substitutions per nucleotide position

The phylogenetic properties, genetic distinctiveness and phenotypic properties showed that strain PB-M7^T can be differentiated from other *Bizionia* species. The results of the polyphasic taxonomic analysis presented in this study support the conclusion that strain PB-M7^T represents a novel species in the genus *Bizionia*, for which the name *Bizionia psychrotolerans* sp. nov. is proposed.

Description of *Bizionia psychrotolerans* sp. nov

Bizionia psychrotolerans (psy.chro.to′le.rans. Gr. adj. *psychros* cold; L. part. adj. *tolerans* tolerating; N.L. part. adj. *psychrotolerans* tolerating cold temperature).

Cells are Gram-negative, obligately aerobic, non-flagellated, non-gliding and rod-shaped, measuring approximately 0.3–0.4 μm in diameter and 1.3–3.0 μm in length after incubation on MA at 25 °C for 5 days. Colonies are circular to slightly glistening, deep yellow in colour and 0.8–1.5 mm after incubation for 10 days at 25 °C on MA. Anaerobic growth does not occur on MA. Growth occurs at 4–30 °C (optimum, 25 °C), at pH 6.0–9.0 (optimum, 7.0–8.0) and with 2–6 % NaCl (optimum, 2 %). Mg^{2+} ions are not required for growth. Positive for oxidase and negative for catalase and urease. Nitrate is not reduced to nitrite. H_2S is not produced. Aesculin, casein, gelatin, tyrosine and Tween 20, 40, 60, and 80 are hydrolyzed, but not starch, xanthine and hypoxanthine. *N*-acetyl-D-glucosamine, L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannose, sucrose, D-trehalose, acetic acid, citric acid, formic acid, succinic acid, propionic acid, L-glutamic acid, L-histidine, L-alanine, and L-proline are not utilized as carbon and energy sources for growth. Acid is not produced from D-arabinose, cellobiose, fructose, L-fucose, galactose, glucose, inositol, lactose, mannose, mannitol, maltose, melibiose, melezitose, raffinose, rhamnose, sorbitol, trehalose and DL-xylose. Enzymatic activities are positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase but negative for arginine dihydrolase, lysine decarboxylase, lipase (C14), α-chymotrypsin, trypsin, α-galactosidase, β-galactosidase,

Table 2 Cellular fatty acid compositions (%) of *B. psychrotolerans* PB-M7^T, closely related members of the genus *Bizionia* and *Biozionia paragorgiae*

Fatty acid	1	2	3	4	5	6	7
Straight-chain							
$C_{16:0}$	2.4	2.6	1.6	1.3	1.4	tr	tr
Branched							
iso-$C_{14:0}$	1.5	1.0	1.2	4.1	1.2	2.9	3.0
iso-$C_{15:0}$	22.2	15.1	13.6	10.9	6.2	15.2	10.6
iso-$C_{16:0}$	3.3	2.4	1.8	6.1	1.9	6.7	4.4
iso-$C_{15:1}$ G[a]	10.8	18.9	14.8	8.9	5.4	10.6	3.0
iso-$C_{16:1}$ H[a]	2.2	1.6	1.8	5.0	3.1	7.2	4.7
anteiso-$C_{15:0}$	4.8	tr	2.2	3.2	16.1	4.0	24.6
anteiso-$C_{15:1}$ A[a]	tr	tr	1.0	tr	2.6	1.2	1.1
anteiso-$C_{17:1}$ $\omega 9c$	1.3	–	–	–	3.7	–	3.0
Unsaturated							
$C_{15:1}$ $\omega 6c$	1.1	tr	1.2	4.0	1.1	1.7	3.0
$C_{17:1}$ $\omega 6c$	1.7	1.9	2.5	4.6	–	4.0	tr
$C_{17:1}$ $\omega 8c$	tr	tr	1.1	2.7	–	tr	tr
Hydroxy							
$C_{15:0}$ 2-OH	1.7	1.1	2.1	3.1	5.2	tr	4.4
$C_{15:0}$ 3-OH	–	1.1	1.8	2.8	–	1.6	–
$C_{16:0}$ 3-OH	2.6	tr	tr	tr	tr	tr	tr
$C_{17:0}$ 2-OH	1.2	tr	tr	1.4	10.3	tr	tr
$C_{17:0}$ 3-OH	tr	tr	tr	1.4	tr	tr	–
iso-$C_{14:0}$ 3-OH	tr	tr	tr	1.2	tr	1.2	tr
iso-$C_{15:0}$ 3-OH	4.5	6.8	5.0	3.4	3.3	5.4	4.2
iso-$C_{16:0}$ 3-OH	4.1	3.1	4.5	11.0	13.8	11.8	8.7
iso-$C_{17:0}$ 3-OH	16.7	16.6	16.1	9.31	16.1	7.4	7.7
Summed features[b]							
3	11.2	16.3	18.4	8.1	3.0	10.8	4.1
9	2.1	4.2	4.7	3.5	1.9	2.5	2.3

Strains: 1, *B. psychrotolerans* PB-M7^T sp. nov.; 2, *B. echini* KMM 6177^T; 3, *B. hallyeonensis* T-y7^T; 4, *B. algoritergicola* APA-1^T; 5, *B. argentinensis* JUB59^T; 6, *B. myxarmorum* ADA-4^T; 7, *B. paragorgiae* KMM 6029^T.All data were obtained from this study. Fatty acids that represented < 1 % of the total in all strains are omitted. Tr, Trace (<1 %); –, not detected

[a] The double bond position indicated by a capital letter is unknown

[b] Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contained $C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$; summed feature 9 contained iso-$C_{17:1}$ $\omega 9c$ and/or 10-methyl $C_{16:0}$

β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. The predominant respiratory quinone is MK-6. The major fatty acids (>10 % of the total fatty acids) are iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH and summed feature 3 ($C_{16:1}$ $\omega 7c$ and/or $C_{16:1}$ $\omega 6c$). The major polar lipids are phosphatidylethanolamine, an unidentified phospholipid, three unidentified aminolipids and three unidentified lipids. The genomic DNA G + C content of the type strain is 33.4 mol%.

The type strain, PB-M7^T (= KCCM 43042^T = JCM 19924^T), was isolated from the intestine of a sea cucumber collected from Pohang, South Korea. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain PB-M7^T is KJ461691.

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