Bizionia sediminis sp. nov., isolated from coastal sediment

Hui Zhang,1 Ming-Jing Shi,1 Hai-Feng Xia,1 Guan-Jun Chen1,2 and Zong-Jun Du1,2,*

Abstract

A Gram-stain-negative, aerobic, non-gliding, rod-shaped and orange-coloured bacterium, designated strain P131T, was isolated from marine sediment of the coast of Weihai, China, and subjected to a polyphasic study. Strain P131T was found to grow optimally at 28–30 °C, at pH 7.0–7.5 and in the presence of 2–3 % (w/v) NaCl. In a phylogenetic analysis based on 16S rRNA gene sequences, strain P131T was found to belong to the genus Bizionia and exhibited 94.6–97.0 % 16S rRNA gene sequence similarity with recognized Bizionia species. The dominant cellular fatty acids of strain P131T were identified as iso-C15:0, iso-C15:0 3-OH and iso-C17:0 3-OH. The predominant polar lipids were phosphatidylethanolamine, phospholipid, two aminolipids and two unidentified lipids. The predominant respiratory quinone was menaquinone MK-6 and the DNA G+C content was 36.7 mol%. On the basis of the phylogenetic and phenotypic evidence presented, strain P131T represents a novel species of the genus Bizionia, for which the name Bizionia sediminis sp. nov. is proposed. The type strain is P131T (=KCTC 42587T=MC011H00124T).

The genus Bizionia, belonging to the family Flavobacteriaceae, was proposed by Nagashkovskaya et al. [1] with the description of one novel species, Bizionia paragorgiae. At the time of writing, the genus Bizionia comprises 11 species: B. paragorgiae [1], B. algortergicola, B. gelidalsuginis, B. myxarmorun and B. salefrenae [2], B. argentinensis [3], B. echini [4], B. halycoenssens [5], B. psychrotolerans [6], B. arctica [7] and B. fulviae [8]. Members of the genus Bizionia have been isolated from various marine environmental samples, including soft coral, sea-ice brine, sea cucumber, sea urchin, seawater and egg cockle [1–8]. In this study, we describe a new bacterial strain, designated P131T, isolated from a marine sediment sample collected from the coast of Weihai, China (36°54′ 32.6′′ N 122° 15′ 16.2′′ E). Comparative analysis of 16S rRNA gene sequences indicated that strain P131T represented a novel species of the genus Bizionia.

Strain P131T was isolated from a sediment sample by plating 1:10 serial dilutions of the sample on 2216E agar (Hopebio) for cultivation at 28 °C for 4 days. After primary isolation and repeated streaking on 2216E agar at 28 °C, a pure culture was obtained and preserved at −80 °C in sterile 1 % (w/v) saline supplemented with 15 % (v/v) glycerol. B. echini KCTC 22015T and B. argentinensis DSM 19628T were obtained from the Korean Collection for Type Cultures (KCTC) and Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ), respectively, and used as reference strains for physiological tests and chemotaxonomic characterizations (except analysis of polar lipids).

Colony morphology of strain P131T was observed on 2216E agar after 2 days of incubation at 28 °C. Gram staining was performed according to Smibert and Krieg [9]. Cell morphology and size were observed by light microscopy (Ci-L; Nikon) after incubation on 2216E agar for 2 days at 28 °C. Gliding motility was examined both on half-strength 2216E liquid medium (Hopebio) solidified with 0.3 % agar and in a hanging-drop preparation according to the methods of Bernardet et al. [10]. The temperature range for growth was studied on 2216E agar at 4, 15, 20, 25, 28, 30, 33, 37, 42 and 45 °C. The pH range for growth was determined in 2216E liquid medium at pH 5.5–9.5 (in increments of 0.5 pH units) by using MES (pH 5.5 and 6.0), PIPES (pH 6.5 and 7.0), HEPES (pH 7.5 and 8.0), Tricine (pH 8.5) and CAPSO (pH 9.0 and 9.5) (Sangon) buffers at concentrations of 20 mM. The tolerance of NaCl for growth was tested on modified marine ZoBell agar comprising (per litre): 1 g yeast extract, 5 g peptone and 18 g agar, made with artificial seawater (consisting of, per litre: MgSO4 3.2 g, MgCl2 2.2 g, CaCl2 1.2 g, KCl 0.7 g, NaHCO3 0.2 g) at NaCl concentrations of 0–15 % (w/v, at intervals of 1 %). Tests for reduction of nitrate and hydrolysis of agar, starch, CM-cellulose, alginate and Tween 40, 40, 60 and 80 were performed as described by Dong and Cai [11]. Oxidase activity was tested by using the bioMérieux oxidase reagent kit and catalase activity was

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Three supplementary figures and one supplementary table are available with the online Supplementary Material.
detected by bubble production after applying 3 % (v/v) hydrogen peroxide solution on bacterial colonies. Anaerobic growth was determined after incubation in an anaerobic chamber on 2216E agar with or without 0.1 % (v/v) NaN₃ for 2 weeks at 28 °C. Since strain P131ᵀ showed poor growth on Mueller-Hinton agar, susceptibility to antibiotics was determined on 2216E agar incubated at 28 °C for 7 days by using antibiotic-impregnated discs containing various antibiotics (Binhe), according to the disc diffusion method described by Du et al. [12] and according to procedures outlined by the Clinical and Laboratory Standards Institute (CLSI) [13]. Acid production from carbohydrates was determined by using the API 50CHB fermentation kit (bio-Mérieux). The substrate oxidation profile was tested with GEN III microplates (Biolog). Additional physiological and biochemical characteristics were examined with API 20E and API ZYM kits (bio-Mérieux) according to the manufacturer’s instructions, except the NaCl concentration of all suspension media was adjusted to 3 % (w/v).

Fatty acids, respiratory quinone and polar lipids were determined from cells cultured in 2216E liquid medium at 28 °C for 2 days (end of the late-exponential phase), except fatty acids of B. argentinensis DSM 19628ᵀ were determined from cells cultured at 25 °C for 2 days. The analysis of fatty acid compositions was determined by using the instructions of the Microbial Identification (Microbial ID) System. The respiratory quinone was extracted and purified according to a method described previously [14] and analysed by HPLC [15]. Polar lipids were analysed by the Identification Service of the DSMZ. Genomic DNA was extracted and purified using the bacterial genomic DNA Mini kit (TaKaRa) following the manufacturer’s protocol. The G+C content of the DNA was determined by HPLC as described by Mesbah et al. [16] and ADNA (Takara) was used as a standard.

The 16S rRNA gene was amplified by PCR with two universal primers, 27F and 1492R [17]. The purified PCR product was ligated into the pGM-T vector (Tiangen) and cloned according to the manufacturer’s instructions. Sequencing reactions was performed by Shanghai Sunny Biotechnology. The 16S rRNA gene sequence (1450 bp) of strain P131ᵀ was submitted to GenBank. The EzTaxon server was used to obtain the sequences of reference type strains (http://www.ezbiocloud.net/identify) [18]. Multiple alignments of the sequences were performed using CLUSTAL X (version 1.81) [19] and alignments were then manually adjusted using BioEdit version 7.0 [20]. A phylogenetic tree of strain P131ᵀ was reconstructed using the neighbour-joining method implemented in the computer program MEGA version 6.06 [21]. The maximum-likelihood [22] and maximum-parsimony [23] methods, as implemented in MEGA 6.06, were also used to reconstruct phylogenetic trees to determine the phylogenetic placement of the novel isolate. Statistical reliability was assessed from 1000 bootstrap replicates.

Phylogenetic analysis based on 16S rRNA gene sequences clearly showed that strain P131ᵀ was a member of the family Flavobacteriaceae and belonged to the genus Bizonia (Fig. 1). The most closely related species was B. echini (97.0 % sequence similarity to the type strain). The maximum-parsimony and maximum-likelihood phylogenetic trees also identified strain P131ᵀ as belonging to the genus Bizonia (Figs S1 and S2, available in the online Supplementary Material). The organism shared 94.6–96.7 % 16S rRNA gene sequence similarity with the type strains of other Bizonia species. The phylogenetic tree revealed that strain P131ᵀ was placed within the genus Bizonia and formed a lineage with the other species.

Colonies of strain P131ᵀ had entire edges, were orange and slightly sticky after cultivation on 2216E agar for 2 days at 28 °C. Cells were rod-shaped, aerobic and non-gliding. Strain P131ᵀ was resistant to (μg per disc) tobramycin (10), tetracycline (30), norfloxacin (10), neomycin (30), gentamycin (10), nalidixic acid (30), ofloxacin (5), sulfamethoxazole (10), kanamycin (30) and streptomycin (10), but sensitive to erythromycin (15), ampicillin (10), penicillin G (10), chloramphenicol (30), rifampicin (5), cefotaxime sodium (30), acetylspiramycin (30) and clindamycin (30). The polar lipid profile of strain P131ᵀ included phosphatidylethanolamine, unidentified aminolipids, aminophospholipid, phospholipid and other lipids (Fig. S3). Strain P131ᵀ and B. argentinensis DSM 19628ᵀ shared similar polar lipid profiles with phosphatidylethanolamine, unidentified aminolipids, aminophospholipid and lipids; however, some quantitative differences were observed and one unidentified lipid was present in the polar lipid profile of strain P131ᵀ, but was absent in that of B. argentinensis DSM 19628ᵀ [3]. The main cellular fatty acids (>5 %) were iso-C₁₅ : 0 (40.7 %), iso-C₁₅ : 0 G (10.4 %), iso-C₁₇ : 0 3-OH (10.0 %) and iso-C₁₇ : 1 ω9c (6.1 %). The fatty acid profiles of strain P131ᵀ and the reference strains were similar, but differences were observed in the proportions of some fatty acids (Table S1). The predominant respiratory quinone of strain P131ᵀ was MK-6, which is consistent with members of the genus Bizonia. The genomic DNA G+C content of the new isolate was 36.7 mol%, which is within the range of values for members of the genus Bizonia (33.0–45.0 mol%).

Strain P131ᵀ produced alkaline phosphatase and catalase, and hydrolysed Tweens 20 and 40. Strain P131ᵀ was unable to perform gliding motility, and could not reduce nitrate, hydrolyse starch or produce acid from carbohydrates. The predominant respiratory quinone (MK-6) was consistent with the genus Bizonia. Despite these common chemotaxonomic traits, the new isolate differed sufficiently from the reference strains on the basis of phenotypic, genotypic and phylogenetic evidence. Strain P131ᵀ could be distinguished from its reference strains by the inability to produce oxidase, by the ability to grow without NaCl and by the maximal growth temperature (45 °C for P131ᵀ, 39 °C for B. echini KCTC 22015ᵀ and 28 °C for B. argentinensis DSM 19628ᵀ). Furthermore, strain P131ᵀ could be distinguished from B. echini KCTC 22015ᵀ by the inability to hydrolyse Tween 80 and produce esterase (C4). In addition, strain
P131T could be distinguished from *B. argentinensis* DSM 19628T by the ability to tolerate higher concentrations of NaCl and an inability to utilize L-histidine.

Morphological, cultural, physiological and biochemical properties of strain P131T are given in the species description and in Table 1. Altogether morphological, physiological and phylogenetic data from this study clearly distinguished strain P131T from members of the most closely related genus *Bizionia*. We conclude that strain P131T represents a novel species in the genus *Bizionia*, for which the name *Bizionia sediminis* sp. nov. is proposed.

**DESCRIPTION OF *BIZIONIA SEDIMINIS* SP. NOV.**

*Bidionia sediminis* (se.di’mi.nis. L. gen. n. *sediminis* of sediment).

Cells are Gram-stain-negative, aerobic and approximately 0.4–0.6 µm wide and 1.0–2.0 µm long. Colonies on 2216E agar are circular, entire, smooth, orange and about 2–4 mm in diameter after incubation for 2 days at 28°C. Growth occurs at 4–45°C (optimum 28–30°C), at pH 5.5–9.0 (optimum 7.0–7.5) and in the presence of 0–10% (w/v) NaCl (optimum 2–3%). Tweens 20, 40 and 60 and gelatin can be hydrolysed, but starch, agar, alginate, Tween 80 and CM-cellulose cannot. Oxidase-negative, but catalase-positive. Nitrate is not reduced to nitrite. O-Nitrophenyl β-D-galactopyranoside, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, Simmon’s citrate utilization, H₂S production, urease, tryptophan deaminase and indole production are negative; Voges-Proskauer reaction is positive. Alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-Bl-phosphohydrolase activities are present, but esterase (C4), lipase (C14), cystine arylamidase, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase, N-acetyl-β-glucosaminidase and β-fucosidase activities are absent. Acid is not produced from carbohydrates without oxygen. The following substrates are oxidized: d-mannose, d-sorbitol, d-mannitol, d-arabitol, d-glucose 6-phosphate, glycyld L-proline, L-arginine, L-serine, L-aspartic acid, L-glutamic acid, d-gluconic acid, acetic acid, citric acid, L-lactic acid and stachyose. The predominant respiratory quinone is MK-6 and the major fatty acids are iso-C₁₅:₀.
iso-C\textsubscript{15:0} G, iso-C\textsubscript{17:0} 3-OH and iso-C\textsubscript{17:1}ω9c. The predominant polar lipids are phosphatidylethanolamine, phosphatidylglycerol, two aminolipids and two unidentified lipids.

The type strain, P131\textsuperscript{T} (=KCTC 42587\textsuperscript{T}=MCCC 1H00124\textsuperscript{T}), was isolated from marine sediment of the coast of Weihai, China. The DNA G+C content of the type strain is 36.7 mol\%.

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### Conflicts of interest
The authors declare that they have no conflicts of interest.

### References

### Table 1. Differential phenotypic characteristics between Bizonia species

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