Ponticaulis koreensis gen. nov., sp. nov., a new member of the family *Hyphomonadaceae* isolated from seawater

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An obligately halophilic, stalked bacterium, designated strain GSW-23^T, was isolated from seawater that had been collected on the coast of Jeju, Korea. Cells of the strain were characteristically Gram-negative, aerobic, chemo-organotrophic, non-budding, motile rods or vibrioids that possessed prosthecae and holdfasts. Multiplication occurred by means of binary fission. The major ubiquinone was Q-10. The dominant cellular fatty acids were summed feature 7 (one or more of C_{18:1}ω9c, C_{18:1}ω12t and C_{18:1}ω7c; 37.1 %), C_{16:0} (25.5 %) and C_{18:0} (14.1 %). The DNA G + C content was 53.3 mol%. 16S rRNA gene sequence analyses showed that the organism was related to members of the family *Hyphomonadaceae* and formed a distinct clade between members of the genus *Hyphomonas* and *Hirschia baltica* DSM 5838^T. Strain GSW-23^T was most closely related to the genus *Hyphomonas* (92.5–93.9 % sequence similarity), but differed from members of the genus by reproduction by binary fission, some physiological properties (gelatin liquefaction and tolerance of 6 % NaCl) and chemotaxonomic features (major fatty acids, major quinones and DNA G + C content). The other genera of the family can be readily differentiated from the isolate by a battery of cultural, physiological and chemotaxonomic characteristics. On the basis of the phenotypic and phylogenetic data presented here, strain GSW-23^T represents a novel genus and species in the family *Hyphomonadaceae*, for which the name *Ponticaulis koreensis* gen. nov., sp. nov. is proposed. The type strain of *Ponticaulis koreensis* is strain GSW-23^T (=KCTC 22146^T =DSM 19734^T =JCM 14975^T).

The family *Hyphomonadaceae* was recently defined by Lee *et al.* (2005) and encompasses Gram-negative, prosthecate bacteria that have mainly been isolated from marine environments. The family currently includes *Hyphomonas* (Pongratz, 1957), *Hirschia* (Schlesner *et al.*, 1990), *Maricaulis* (Abraham *et al.*, 1999), *Oceanicaulis* (Strömpl *et al.*, 2003) and *Robiginotomaculum* (Lee *et al.*, 2007). In this study, we describe the isolation, classification and identification of a stalked bacterium from beach seawater by using a polyphasic approach.

Strain GSW-23^T was isolated from seawater collected from Kymnyeong Beach on the island of Jeju, Republic of Korea. For the isolation of marine bacteria, the seawater sample was filtered through a membrane filter (0.45 µm pore size) and was then transferred directly onto marine agar (MA; Difco). Following incubation at 30 °C for 8 days, a colony on plates was selectively taken and subcultured on MA. The

pure culture was stored at –20 and –80 °C in glycerol suspensions supplemented with 60 % (v/v) natural seawater and 20 % (v/v) distilled water. For phenotypic comparison, *Hirschia baltica* DSM 5838^T, *Hyphomonas polymorpha* DSM 2665^T, *Maricaulis maris* DSM 4734^T and *Oceanicaulis alexandrii* DSM 11625^T were grown on MA for 5 days at 30 °C.

The isolation of chromosomal DNA and the amplification of the 16S rRNA gene sequence by PCR were carried out as described elsewhere (Lee, 2007). The PCR product was purified from unincorporated primer oligonucleotides using the Wizard PCR Preps DNA Purification System (Promega). The PCR products were sequenced using an ABI PRISM BigDye Terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (3730xl; Applied Biosystems). A partial 16S rRNA gene sequence (1328 bp) of strain GSW-23^T was subjected to a preliminary BLAST search against the GenBank database, revealing that strain GSW-23^T was related to members of the family *Hyphomonadaceae* (class *Alphaproteobacteria*). CLUSTAL_X software (Thompson *et al.*, 1997) was used to align the corresponding sequences. A phylogenetic tree was constructed with the neighbour-joining method (Saitou & Nei,

The GenBank/EMBL/DDBJ accession number of the 16S rRNA gene sequence of strain GSW-23^T is FM202497.

A supplementary table showing the cellular fatty acid compositions of strain GSW-23^T and related species is available with the online version of this paper.

1987) and the model of Jukes & Cantor (1969). A bootstrap analysis (Felsenstein, 1985) was performed with 1000 resamplings for assessing the reliability of tree topology.

The neighbour-joining tree (Fig. 1) showed that strain GSW-23^T was related to members of the family *Hyphomonadaceae* and formed a distinct cluster between members of the genus *Hyphomonas* and *Hirschia baltica* DSM 5838^T, with a high bootstrap value of 89%. 16S rRNA gene sequence similarity values between strain GSW-23^T and members of the family indicated that members of the genus *Hyphomonas* (92.5–93.9%) were the closest relatives. Other members of the family, including *Hirschia baltica* DSM 5838^T (90.6%), *Robiginitomaculum antarcticum* IMCC3195^T (89.7%), *Maricaulis* species (89.0–89.8%) and *Oceanicaulis alexandrii* CC116-3 (88.1%), were more distantly related to strain GSW-23^T.

For chemotaxonomic analyses, biomass was obtained from cultures grown in marine broth 2216 (MB; Difco) for 3 days at 30 °C in a shaking incubator. Ubiquinones were extracted by the method of Minnikin *et al.* (1984) and identified by HPLC as described by Kroppenstedt (1985). The Sherlock Microbial Identification System version 6 (MIDI) was used to analyse cellular fatty acids according to the manufacturer's instructions. The extraction and preparation of fatty acid methyl esters were performed using cells grown on MA for 5 days at 30 °C. The base

composition of the DNA was determined by HPLC as described by Mesbah *et al.* (1989).

The cellular fatty acid composition of strain GSW-23^T was represented by considerable amounts of saturated and unsaturated acids (Table 1), with minor proportions of branched and hydroxy acids. The major cellular fatty acids were summed feature 7 (consisting of C_{18:1}ω9c, C_{18:1}ω12t and/or C_{18:1}ω7c; 37.1%), C_{16:0} (25.5%) and C_{18:0} (14.1%). The major isoprenoid quinone of strain GSW-23^T was Q-10 and the DNA G + C content was 53.3 mol%, revealing a value intermediate to those of related genera in the family *Hyphomonadaceae*. The fatty acid compositions of strain GSW-23^T and the type strains of the type species of related genera of the family *Hyphomonadaceae* are given in Supplementary Table S1 (available in IJSEM Online).

Cell morphology was examined by light microscopy of cultures grown on MA at 30 °C for 5 days. Cell suspensions in sterile distilled water were stained using the bioMérieux Gram stain kit according to the manufacturer's directions. Cell motility was observed with an Olympus light microscope equipped with phase-contrast optics (×400 magnification). The presence of flagella was checked with a transmission electron microscope (1200EXII; JEOL). Colony morphology and pigmentation were observed after 5 days of cultivation at 30 °C on MA. The cells of strain GSW-23^T were non-budding, motile rods or vibrioids and possessed prosthecae and holdfasts. The elongated cells reproduce by binary fission. A polar flagellum was observed by electron microscopy. A detailed depiction of the typical cellular morphology of strain GSW-23^T is given in Fig. 2.

Unless otherwise indicated, phenotypic characteristics were examined on MA as the basal medium. The temperature for growth was tested at 4, 10, 20, 30, 37 and 42 °C. The pH range for growth was determined on MA with the pH adjusted to pH 4.1–12.1 (at intervals of 1.0 pH unit) using 10 M NaOH and 6 M HCl. Tolerance of various NaCl concentrations was determined in MB supplemented with 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10% NaCl (w/v). The OD₆₀₀ of the cell suspension was measured spectrophotometrically at the beginning of the experiment and every day for 5 days. Catalase and oxidase activities were determined using 3% (v/v) H₂O₂ and 1% (w/v) N,N,N',N'-tetramethyl-*p*-phenylenediamine dihydrochloride solutions, respectively. Growth under anaerobic conditions was tested by using the GasPak EZ anaerobe pouch system (BD). Degradation tests were carried out on MA supplemented with 0.5% colloidal chitin, 0.5% CM-cellulose, 0.4% elastin, 0.4% hypoxanthine and 0.4% xanthine. Protease, amylase and tyrosinase activities were determined as described previously (Lee, 2007). Hydrolysis of DNA was tested on MA supplemented with DNase test agar (Difco). The ability to oxidize various substrates as sole carbon sources was tested using GN2 MicroPlates (Biolog) according to the manufacturer's instructions. Other physiological and biochemical properties were tested using API 20NE and API ZYM strips (bioMérieux).

Fig. 1. Neighbour-joining tree constructed with 16S rRNA gene sequences, showing the phylogenetic position of strain GSW-23^T within the family *Hyphomonadaceae*. Evolutionary distances were computed using the method of Jukes & Cantor (1969). The sequence of *Flavobacterium aquatile* ATCC 11947^T (GenBank accession no. M62797; not shown) was used as an outgroup. Asterisks indicate nodes that were also recovered in maximum-likelihood and maximum-parsimony analyses. Bootstrap values (>50%) based on 1000 resamplings are shown at branch nodes. Bar, 0.05 substitutions per nucleotide position.

Table 1. Differential characteristics of strain GSW-23^T and related genera of the family Hyphomonadaceae

Genera: 1, *Ponticaulis* gen. nov. (strain GSW-23^T); 2, *Hirschia* (Schlesner *et al.*, 1990); 3, *Hyphomonas* (Pongratz, 1957; Moore *et al.*, 1984; Weiner *et al.*, 1985, 2000); 4, *Maricaulis* (Abraham *et al.*, 1999, 2002); 5, *Oceanicaulis* (Strömpl *et al.*, 2003); 6, *Robiginitomaculum* (Lee *et al.*, 2007). Members of all genera possess prosthecae. +, Positive; –, negative; v, variable; tr, trace amount (<1 % of total); ND, no data available/not detected.

Characteristic	1	2	3	4	5	6
Cell morphology						
Shape*	R, V	O, R	O, R	R, V	R, V	R, V
Budding	–	+	+	–	–	–
Flagella	+	+	+	+†	+	–
Colony pigmentation‡	NC	Y	NC, G, RB	NC	NC	RO
Oxidase	+	ND	+	ND	+	–
Nitrate reduction	–	–	v	+	v	+
Gelatin hydrolysis	+	+	–	–	+	–
Optimum temperature (°C)	30–37	22–28	20–37	20–40	30	20
Tolerance of 6 % NaCl§	+	–	–	–	+	–
Major fatty acids (%)§						
C _{16:0}	26	25	6	16	3	–
C _{17:0}	4	2	22	8	ND	14
C _{18:0}	14	3	4	5	14	ND
C _{17:1ω6c}	tr	tr	19	3	7	8
C _{17:1ω8c}	ND	1	19	6	1	21
i-C _{17:1ω9c}	ND	ND	ND	12	ND	ND
C _{17:0} cyclo	ND	ND	ND	ND	17	ND
Summed feature 7	37	44	2	29	40	42
Major quinone(s)	Q-10	Q-10	Q-9, Q-10, Q-11, Q-12	Q-10	ND	Q-10
DNA G + C content (mol%)	53.3	45.6	57–62	62.5–65.2	61.8	60.3

*O, Ovoid; R, rod; v, vibrioid.

†Data for *Maricaulis marinus*.

‡NC, Colourless; G, grey; RB, reddish brown; RO, rusty orange; Y, yellow.

§Data for *Hirschia baltica* DSM 5838^T, *Hyphomonas polymorpha* DSM 2665^T, *Maricaulis maris* DSM 4734^T and *Oceanicaulis alexandrii* DSM 11625^T were obtained in this study.

||Summed features represent groups of two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 7 consisted of one or more of C_{18:1ω9c}, C_{18:1ω12t} and C_{18:1ω7c}.

according to the manufacturer's instructions. The results of physiological and biochemical tests are shown in Table 1 and in the species description.

Members of the family Hyphomonadaceae can be divided into two groups according to their life cycle. One group consists of the genera *Hyphomonas* and *Hirschia* and reproduces by means of budding, while the other group contains the genera *Maricaulis*, *Oceanicaulis* and *Robiginitomaculum* and reproduces by binary fission. Strain GSW-23^T was similar to the latter group but differed from its members by the optimum temperature range for growth and in some chemotaxonomic characters (i.e. DNA G + C contents and cellular fatty acid profiles) and also exhibited 16S rRNA gene sequence similarity of less than 90 % to members of the group. As well as the genera *Hyphomonas* and *Hirschia* differing from strain GSW-23^T in their means of reproduction, the genus *Hyphomonas* differed from strain GSW-23^T in some physiological properties (gelatin liquefaction and tolerance of 6 % NaCl) and chemotaxonomic features (major fatty

acids, major quinones and DNA G + C contents). The genus *Hirschia* can be distinguished from strain GSW-23^T by its colony pigmentation, different optimum temperature range for growth, lack of tolerance of 6 % NaCl and not having C_{18:0} as a predominant fatty acid. The DNA G + C content of the type strain of the type species of the genus *Hirschia* was 45.6 mol%, which is considerably lower than that of strain GSW-23^T (53.3 mol%). The differential features of strain GSW-23^T and related genera of the family Hyphomonadaceae are given in Table 1.

Based on the data from phenotypic and phylogenetic analyses, strain GSW-23^T represents a novel genus and species in the family Hyphomonadaceae, for which the name *Ponticaulis koreensis* gen. nov., sp. nov. is proposed.

Description of *Ponticaulis* gen. nov.

Ponticaulis (Pon.ti.cau'lis. L. neut. n. *pontus* the sea; L. masc. n. *caulis* a stalk, referring to a prostheca; N.L. masc. n. *Ponticaulis* stalk from the sea).

Fig. 2. Transmission electron micrograph of a cell of strain GSW-23^T possessing a prostheca (arrow) and a holdfast (triangle). Bar, 1 μm.

Cells are strictly aerobic, Gram-negative, non-spore-forming, non-budding, obligately halophilic rods or vibrioids that are motile by means of a polar flagellum. Multiplication occurs by binary fission. Some cells possess a long prostheca (0.2 μm in diameter) and a holdfast. The major isoprenoid quinone is Q-10. The major cellular fatty acids are summed feature 7 (C_{18:1}ω9c, C_{18:1}ω12t and/or C_{18:1}ω7c), C_{16:0} and C_{18:0}. The genus belongs phylogenetically to the family *Hyphomonadaceae* in the class *Alphaproteobacteria*. The type species is *Ponticaulis koreensis*.

Description of *Ponticaulis koreensis* sp. nov.

Ponticaulis koreensis (ko.re.en'sis. N.L. masc. adj. *koreensis* pertaining to Korea, where the type strain was isolated).

Exhibits the following properties in addition to those described in the genus. Cells are 3.1–6.6 μm long and 0.4–0.5 μm wide. Colonies are colourless, circular, smooth, convex and 0.3–0.5 mm in diameter after 5 days of incubation. Growth occurs at 10–42 °C (optimum 30–37 °C), at pH 6.1–10.1 (optimum pH 7.1) and with up to 6 % (w/v) NaCl. Hydrolyses DNA but not casein or starch. Degrades DL-tyrosine but not elastin, chitin, cellulose, hypoxanthine or xanthine. Positive for gelatin hydrolysis but negative for nitrate reduction, indole production, glucose fermentation, arginine dihydrolase, urease, aesculin degradation and β-galactosidase (API 20NE). In API ZYM tests, positive for alkaline phosphatase, esterase (C4) (weak), esterase lipase (C8) (weak), leucine arylamidase, trypsin, α-chymotrypsin, acid phosphatase and naphthol-AS-BI-phosphohydrolase, but negative for lipase (C14), valine arylamidase, cystine arylamidase, α- and β-galacto-

sidase, β-glucuronidase, α- and β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. In GN2 MicroPlates, metabolizes Tweens 40 and 80, raffinose, methyl pyruvate, monomethyl succinate, β- and γ-hydroxybutyric acid, α-ketoglutaric acid, succinic acid, bromosuccinic acid, L-alaninamide, L-alanine, L-alanyl glycine, L-aspartic acid, L-glutamic acid, glycyl L-glutamic acid, L-proline and L-pyroglutamic acid as sole carbon and energy sources, but not α-cyclodextrin, dextrin, glycogen, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, cellobiose, i-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, myo-inositol, α-lactose, lactulose, maltose, D-mannitol, D-mannose, melibiose, methyl β-D-glucoside, D-psicose, L-rhamnose, D-sorbitol, sucrose, trehalose, turanose, xylitol, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketobutyric acid, α-ketovaleric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinamic acid, glucuronamide, D-alanine, L-asparagine, glycyl L-aspartic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, D- or L-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, α-DL-glycerol phosphate, α-D-glucose 1-phosphate or D-glucose 6-phosphate. The DNA G + C content of the type strain is 53.3 mol%.

The type strain, GSW-23^T (=KCTC 22146^T =DSM 19734^T =JCM 14975^T), was isolated from seawater taken from Kymnyeong Beach on the island of Jeju, Republic of Korea.

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