Polaribacter atrinae sp. nov., isolated from the intestine of a comb pen shell, *Atrina pectinata*

Dong-Wook Hyun, Na-Ri Shin, Min-Soo Kim, Pil Soo Kim, Mi-Ja Jung, Joon Yong Kim, Tae Woong Whon and Jin-Woo Bae

Department of Life and Nanopharmaceutical Sciences and Department of Biology, Kyung Hee University, Seoul 130-701, Republic of Korea

Correspondence
Jin-Woo Bae
baejw@khu.ac.kr

A novel Gram-staining-negative, aerobic, non-motile, yellow-to-orange carotenoid-type-pigmented and rod-shaped bacterium, designated strain WP25^T, was isolated from the intestine of a comb pen shell, *Atrina pectinata*, which was collected from the South Sea near Yeosu in Korea. The isolate grew optimally at 20 °C, at pH 7 and with 2 % (w/v) NaCl. 16S rRNA gene sequence analysis showed that strain WP25^T belonged to the genus *Polaribacter* in the family *Flavobacteriaceae* and the highest sequence similarity was shared with the type strain of *Polaribacter sejongensis* (98.5 %). The major cellular fatty acids were iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{15:1}\omega6c$ and iso-$C_{15:0}$ 3-OH. The main respiratory quinone was menaquinone MK-6. The polar lipids of strain WP25^T were phosphatidylethanolamine, two unidentified aminolipids, an unidentified phospholipid and four unidentified lipids. The genomic DNA G+C content was 31.2 mol%. DNA–DNA hybridization experiments indicated <12.6 % genomic relatedness with closely related strains. Based on phylogenetic, phenotypic and genotypic analyses, strain WP25^T represents a novel species in the genus *Polaribacter*, for which the name *Polaribacter atrinae* sp. nov. is proposed, with the type strain WP25^T (=KACC 17473^T=JCM 19202^T).

The genus *Polaribacter* (family *Flavobacteriaceae*) was proposed by Gosink *et al.* (1998). At the time of writing, the genus *Polaribacter* contains ten species with validly published names, which were isolated from marine-related environments or polar regions: *Polaribacter irgensii* and *P. filamentus* from sea ice in Antarctica (Gosink *et al.*, 1998), *P. franzmannii*, *P. dokdonensis*, *P. gangjinensis*, *P. glomeratus* and *P. butkevichii* from seawater (Gosink *et al.*, 1998; Lee *et al.*, 2011; Nedashkovskaya *et al.*, 2005; Yoon *et al.*, 2006), *P. porphyrae* and *P. reichenbachii* from marine algae (Fukui *et al.*, 2013; Nedashkovskaya *et al.*, 2013) and *P. sejongensis* from an Antarctic soil (Kim *et al.*, 2013). Four of the ten recognized *Polaribacter* species (*P. irgensii*, *P. filamentus*, *P. franzmannii* and *P. glomeratus*) were described as psychrophilic or psychrotolerant bacteria, which cannot grow at 25 °C (Gosink *et al.*, 1998), whereas the others were described as mesophilic.

The comb pen shell *Atrina pectinata*, which belongs to the family Pinnidae, is a large, wedge- or fan-shaped, suspension-feeding bivalve (Okutani, 1997) that is found in habitats that range from muddy to sandy sediments and from tidal flats to shallow subtidal environments up to 20 m in depth (Yurimoto *et al.*, 2003). A novel *Polaribacter*-like strain, designated strain WP25^T, was isolated during an investigation of the intestinal bacterial population of a specimen of *A. pectinata* that was collected from the South Sea near Yeosu in Korea. The current study describes the taxonomic characterization of this novel bacterial strain based on a polyphasic analysis, which suggests that the isolate represents a novel species.

To isolate intestinal bacteria, detached and homogenized intestinal tissues of a comb pen shell were inoculated onto marine agar 2216 (MA; Difco) plates using the dilution-plating technique and incubated at 20, 25 and 30 °C. Strain WP25^T was isolated from a 10^{-2}-diluted sample after incubation at 25 °C for 72 h. The isolate was purified by repeated subculture and stored at −80 °C as a suspension in marine broth 2216 (MB; Difco) containing 40 % (v/v) glycerol. Physiological, biochemical, chemotaxonomic and genotypic analyses were performed according to the proposed minimal standards for the description of new taxa in the family *Flavobacteriaceae* (Bernardet *et al.*, 2002); all tests were performed at least three times.

Growth of the isolate was examined at different temperatures, pH and salinities. The isolate was incubated in MB at 0, 4, 10, 15, 20, 25, 30, 37, 45, 55 and 65 °C and at pH 4−11 (at intervals of 1.0 pH unit). The pH of the MB was adjusted with 10 mM MES (for pH 4−6), TAPS (for pH 7−8) or Na_2HPO_4 (for pH 9−11). For salinity tests, the isolate was incubated in a medium that

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain WP25^T is KC878325.

A supplementary figure is available with the online version of this paper.

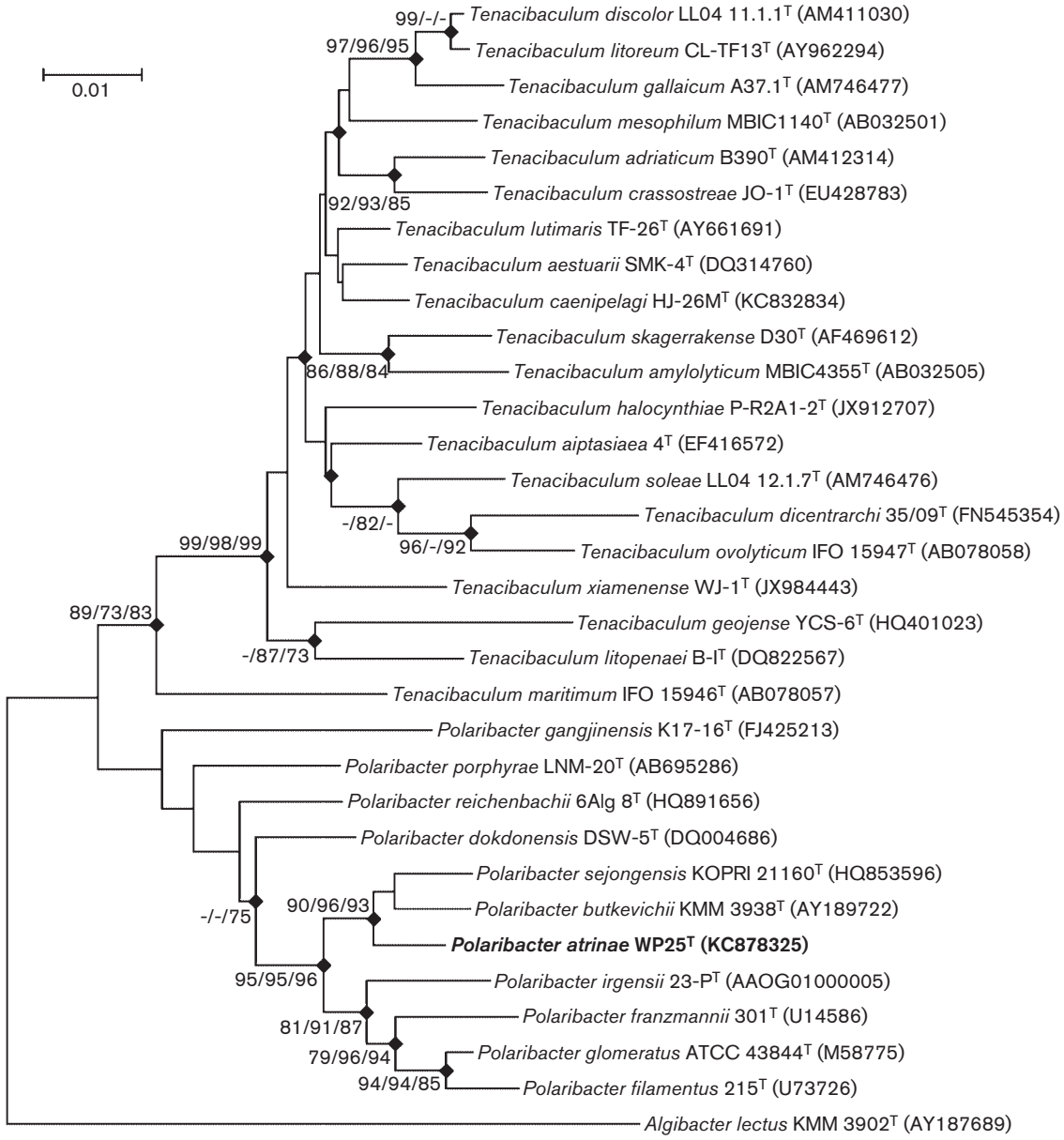

Fig. 1. Phylogenetic consensus tree based on 16S rRNA gene sequences, showing the phylogenetic position of strain WP25^T and members of closely related species, reconstructed using the neighbour-joining (NJ), maximum-parsimony (MP) and maximum-likelihood (ML) algorithms. Filled diamonds represent identical branches generated using all three algorithms. Numbers at nodes indicate bootstrap values (NJ/MP/ML) as percentages of 1000 replicates (–, <70 %). *Algibacter lectus* KMM 3902^T was used as an outgroup. Bar, 0.01 accumulated changes per nucleotide.

contained all of the constituents of MB except NaCl, which was added at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12 or 15 % (w/v). Growth under each condition was determined by measuring the OD_{600} using a spectrophotometer (Synergy MX; BioTek) after incubation for 24 h, 48 h and 7 days. Anaerobic growth was examined after 7 days of culture at 20 and 30 °C on MA plates in an anaerobic chamber filled with a $N_2/CO_2/H_2$ atmosphere (90 : 5 : 5, by vol.). Strain WP25^T grew at 4–30 °C (optimum 20 °C), pH 5–9 (optimum pH 7) and with 1–6 % NaCl (optimum 2 %). Growth was not observed under anaerobic conditions.

The isolate was incubated for 48 h at 20 °C before determining the presence of gas vesicles, cell morphology, colony appearance and Gram staining. A Gram staining kit (bioMérieux) was used according to the manufacturer's instructions. Gram staining and cell morphology were observed by light microscopy (Eclipse 50*i*; Nikon) and the presence of gas vesicles was ascertained by phase-contrast

Table 1. Differential characteristics of strain WP25^T and its closest phylogenetic relatives in the genus *Polaribacter*

Strains: 1, WP25^T; 2, *P. sejongensis* KCTC 23670^T; 3, *P. butkevichii* KCTC 12100^T; 4, *P. irgensii* KCTC 23136^T; 5, *P. reichenbachii* KCTC 23969^T. Data were obtained in this study unless indicated. +, Positive or weakly positive; −, negative. All strains were positive for the following: activities of alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phosphatase and naphthol-AS-BI-phosphohydrolase, hydrolysis of Tween 80 and acid production from aesculin. All strains were negative for the following: activities of lipase (C14), cystine arylamidase, α-chymotrypsin, β-glucuronidase, β-glucosidase, α-mannosidase, α-fucosidase, L-arginine dihydrolase, urease, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase, hydrolysis of tyrosine and chitin, production of indole and H_2S, fermentation of D-glucose, acid production from erythritol, L-arabinose, D-ribose, L-xylose, D-adonitol, methyl β-D-xyloside, L-sorbose, dulcitol, inositol, D-sorbitol, methyl α-D-mannoside, methyl α-D-glucoside, trehalose, xylitol, D-lyxose, D-tagatose, D-fucose, D- and L-arabitol, gluconate and 2-ketogluconate and utilization of citrate, Tweens 40 and 80, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, i-erythritol, L-fucose, *myo*-inositol, melibiose, methyl β-D-glucoside, D-psicose, raffinose, D-sorbitol, trehalose, xylitol, pyruvic acid methyl ester, succinic acid monomethyl ester, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-hydroxyphenylacetic acid, itaconic acid, α-ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, D-alanine, L-histidine, hydroxy-L-proline, L-leucine, L-phenylalanine, L-pyroglutamic acid, D- and L-serine, L-threonine, DL-carnitine, γ-aminobutyric acid, urocanic acid, thymidine, phenylethylamine, putrescine and 2-aminoethanol. ONPG, *o*-Nitrophenyl β-D-galactopyranoside; PNPG, *p*-nitrophenyl β-D-galactopyranoside.

Characteristic	1	2	3	4	5
Temperature range for growth (°C)	4–30	4–37[a]	4–32[b]	−1.5 to 12[c]	4–35[d]
Enzyme activities					
DNase, esterase (C4), esterase lipase (C8), α-glucosidase, β-galactosidase (ONPG, PNPG)	+	+	+	−	+
Trypsin	+	+	−	+	+
α-Galactosidase	+	+	−	−	−
β-Galactosidase (*o*-naphthyl-β-D-galactopyranosidase)	−	−	−	−	+
N-Acetyl-β-glucosaminidase	+	−	−	−	+
Lipase	+	−	−	−	−
Gelatinase	−	−	−	−	+
Hydrolysis of:					
Tween 20, starch, aesculin	+	+	+	−	+
Tween 40, Tween 60	+	+	+	−	−
Casein	+	−	+	−	−
CM-cellulose	−	+	−	−	−
Acid production from:					
D-Galactose, D-glucose, D-fructose, D-mannose, amygdalin, maltose, lactose, starch, glycogen	+	+	+	−	+
5-Ketogluconate	−	+	+	+	+
Turanose	+	+	+	−	−
L-Fucose	+	+	−	−	+
D-Xylose, L-rhamnose, D-mannitol, cellobiose, gentiobiose	−	+	+	−	+
N-acetylglucosamine, melibiose, D-arabinose	+	+	−	−	−
Glycerol	+	−	+	−	−
Sucrose, raffinose	+	−	−	−	+
Arbutin	−	+	+	−	−
Inulin, melezitose	+	−	−	−	−
Salicin	−	−	+	−	−
Utilization of:					
α-Cyclodextrin, dextrin, glycogen, cellobiose, D-galactose, α-D-glucose, lactose, maltose, D-mannose, acetic acid, α-ketobutyric acid, L-alanine, L-alanyl glycine, L-glutamic acid, glycyl L-glutamic acid, L-ornithine, uridine, DL-α-glycerol phosphate, α-D-glucose 1-phosphate, D-glucose 6-phosphate	+	+	+	−	+
D-Fructose, α-ketoglutaric acid	+	+	−	−	+
Turanose, DL-lactic acid, L-alaninamide	+	−	+	−	+
Gentiobiose, D-mannitol, L-proline	−	+	+	−	+
Glycerol	+	−	+	−	−
Sucrose	+	−	−	−	+
L-Aspartic acid, glycyl L-aspartic acid, L-rhamnose, α-hydroxybutyric acid, L-asparagine	−	+	+	−	−

Table 1. cont.

Characteristic	1	2	3	4	5
Inosine, 2,3-butanediol	+	−	−	−	−
Lactulose, D-glucuronic acid	−	−	−	−	+
Reduction of nitrates to nitrites	+	−	−	−	−
Reduction of nitrates to nitrogen	−	+	−	−	−
Acetoin production (Voges−Proskauer)	+	+	+	−	+
DNA G+C content (mol%)	31.2	30[a]*	32.4[b]	31[c]	31.6[d]
Isolation source	Comb pen shell	Soil[a]	Seawater[b]	Seawater[c]	Green alga[d]

*Data from: *a*, Kim *et al.* (2013); *b*, Nedashkovskaya *et al.* (2005); *c*, Gosink *et al.* (1998); *d*, Nedashkovskaya *et al.* (2013).

microscopy (Eclipse 50*i*; Nikon). Motility of the isolate was determined using semi-solid MA (0.4 % agar) according to the method of Tittsler & Sandholzer (1936), after incubation at 20 °C for 1 week. Gliding motility was also tested using MB according to the method of Bernardet *et al.* (2002). The cells were Gram-staining-negative rods (0.8−1.0 μm wide and 1.4−3.5 μm long) and they formed circular, convex colonies with an entire margin that were viscous and yellow−orange with a diameter of 0.8−1.2 mm on MA. Gas vesicles, motility in semi-solid agar and gliding motility were not observed.

Unless stated otherwise, tests used to characterize the isolate were conducted under optimal growth conditions. The presence of flexirubin-type pigments was detected using 20 % (w/v) KOH by flooding cell mass harvested from agar plates (Bernardet *et al.*, 2002). To determine the presence of carotenoid-type pigments, cellular pigments were extracted according to the method of Schmidt *et al.* (1994), with a modification. The extracted cellular pigments were analysed by measuring the absorption spectrum using a UV−visible spectrophotometer (Synergy MX; BioTek). Strain WP25^T was negative for flexirubin-type pigments. The absorbance spectrum of the cellular pigments extracted from strain WB5^T had a triple-peak signature, which characterizes carotenoid-type pigments (Schmidt *et al.*, 1994).

Phylogenetic analysis was performed using 16S rRNA gene sequences. PCR amplification of the 16S rRNA gene of the isolate was conducted using a PCR Premix (*i*NtRon Biotechnology) with the universal bacterial primer pair 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGYTACCTTGTTACGACTT-3′) (Lane, 1991). The 16S rRNA gene amplicon was sequenced using a BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) according to the manufacturer's instructions. Reaction mixtures were analysed using an automated DNA analyser (Applied Biosystems 3730xl DNA Analyzer). 16S rRNA gene sequence fragments were assembled using SeqMan (DNASTAR) and compared with sequences of type strains in the EzTaxon-e server (Kim *et al.*, 2012). Strain WP25^T shared 16S rRNA gene sequence similarity of 98.5 % with *P. sejongensis* KOPRI 21160^T, 98.2 % with *P. butkevichii* KMM 3938^T, 97.2 % with *P. irgensii* 23-P^T and 97.1 % with *P. reichenbachii* 6Alg 8^T. The isolate shared less than 97 % 16S rRNA gene sequence similarity with *P. glomeratus* ATCC 43844^T (96.7 %), *P. dokdonensis* DSW-5^T (96.7 %), *P. franzmannii* 301^T (96.0 %), *P. filamentus* 215^T (95.8 %) and *P. porphyrae* LNM-20^T (95.4 %). Phylogenetic consensus trees were reconstructed to determine phylogenetic relationships between the isolate and members of closely related species. The sequences were aligned using CLUSTAL W (Thompson *et al.*, 1994) and used to reconstruct phylogenetic trees with MEGA 5 (Tamura *et al.*, 2011). Phylogenetic distances were calculated using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1972) and maximum-likelihood (Felsenstein, 1981) algorithms based on 1000 bootstrap replicates. The phylogenetic trees showed that strain WP25^T formed a cluster with other members of the genus *Polaribacter* (Fig. 1). To facilitate a more comprehensive characterization of strain WP25^T, the following type strains of closely related species of the genus *Polaribacter* were obtained from the Korean Collection for Type Cultures and used as reference strains: *P. sejongensis* KCTC 23670^T, *P. butkevichii* KCTC 12100^T, *P. irgensii* KCTC 23136^T and *P. reichenbachii* KCTC 23969^T.

Enzyme activities, hydrolytic activities, acid production from various carbohydrates and utilization of sole carbon sources were tested to characterize the biochemical properties of strain WP25^T in comparison with the reference strains. Biochemical tests were performed using cell mass of the isolate and the reference strains after cultivation on MA under optimum growth conditions for 48 h, except *P. irgensii* KCTC 23136^T, which was cultivated at 4 °C for 7 days. Enzyme activities were tested using API ZYM, API 20E and API 20NE test strips (bioMérieux) according to the manufacturer's instructions. Catalase and oxidase activities were determined based on bubble production with 3 % (v/v) hydrogen peroxide solution and indophenol blue production with 1 % (w/v) tetramethyl *p*-phenylenediamine (bioMérieux), respectively. Tests for hydrolysis of various substrates used MA supplemented with each test substrate: 0.5 % soluble starch (Junsei), 5 % skimmed milk (Difco) for casein (Benson, 1994), 1 % (w/v) Tweens 20, 40, 60 and 80 (Goszczynska &

Serfontein, 1998), 0.5 g tyrosine l^{-1} and 1 % (w/v) CM-cellulose (Sigma) (Teather & Wood, 1982). Hydrolytic activity was identified by the formation of clearance zones around colonies (starch, casein, tyrosine and CM-cellulose) or turbid zones around colonies (Tweens 20, 40, 60 and 80). Citrate hydrolysis was tested using Simmons' citrate agar (Difco). DNase and lipase activity were tested using DNase test agar (Difco) and spirit blue agar (Difco) supplemented with lipase reagent, respectively (Difco). Sole carbon-source utilization was determined using GN2 MicroPlates (Biolog) with GN/GP inoculating fluid (Biolog) supplemented with 2 % (w/v) NaCl, according to the manufacturer's instructions. Acid production from various carbohydrates was tested using API 50 test strips (bioMérieux) with 50 CHB/E medium supplemented with 2 % (w/v) NaCl. The isolate was positive for catalase and oxidase, and could be distinguished from the reference strains by its utilization of various carbon sources, enzyme activities and acid production from various carbohydrates. Table 1 shows differential biochemical features of all test strains.

To determine the chemotaxonomic characteristics of strain WP25^T, its cellular fatty acid, isoprenoid quinone and polar lipid compositions were compared with those of the reference strains. Chemotaxonomic analyses were performed using cell biomass of the isolate and the reference strains of the same physiological age (stationary growth phase). The isolate and the reference strains were incubated on MA plates at 20 °C for 48 h, except *P. irgensii* KCTC 23136^T, which was incubated at 4 °C for 7 days. Cellular fatty acids were saponified, methylated and extracted according to the protocol of the Sherlock Microbial Identification System (MIDI, 1999). The cellular fatty acid profiles of the isolate and the reference strains were determined using GC (Agilent 6890 gas chromatograph) and the Microbial Identification software package (Sherlock version 6.2) with the TSBA6 database (Sasser, 1990). The major cellular fatty acids (>10 % of the total) in the isolate were iso-$C_{15:0}$ (17.0 %), anteiso-$C_{15:0}$ (13.4 %), $C_{15:1}\omega6c$ (12.0 %) and iso-$C_{15:0}$ 3-OH (11.5 %). The majority of the fatty acid components of strain WP25^T were similar to those of the reference strains, although there were quantitative differences in each predominant component. The complete fatty acid profiles of the isolate and the reference strains are shown in Table 2. Isoprenoid quinones of strain WP25^T were extracted according to the method of Collins & Jones (1981a) and identified by HPLC (Ultimate 3000; Dionex) coupled to an ion trap-mass spectrometer with an electrospray ionization probe (HCT; Bruker) according to a published protocol (Collins & Jones, 1981b). The sole respiratory quinone in strain WP25^T was menaquinone 6 (MK-6), which is the sole or major respiratory quinone in all members of the family *Flavobacteriaceae* (Bernardet, 2011). To characterize the polar lipid composition, the polar lipids of strain WP25^T and *P. reichenbachii* KCTC 23969^T were extracted according to the procedures described by Xin *et al.* (2000) and analysed by two-dimensional TLC on a silica gel 60 F_{254} plate (Merck); chloroform/methanol/water (65 : 25 : 4, by vol.) was used for the primary dimension and chloroform/methanol/acetic acid/water (80 : 12 : 15 : 4, by vol.) for the secondary dimension. The following reagents were sprayed on the glass plate to detect polar lipids (Tindall, 1990): 5 %

Table 2. Cellular fatty acid contents of strain WP25^T and the type strains of closely related species of the genus *Polaribacter*

Strains: 1, WP25^T; 2, *P. sejongensis* KCTC 23670^T; 3, *P. butkevichii* KCTC 12100^T; 4, *P. irgensii* KCTC 23136^T; 5, *P. reichenbachii* KCTC 23969^T. All data were obtained in the current study. Values are percentages of total fatty acids. TR, Trace (<0.5 %); −, not detected; TBSA, tuberculostearic acid.

Fatty acid	1	2	3	4	5
Saturated					
$C_{13:0}$	0.9	TR	0.8	TR	TR
$C_{14:0}$	0.7	0.7	1.4	1.1	1.1
$C_{16:0}$	TR	TR	0.7	TR	2.7
$C_{18:0}$	−	1.1	0.8	0.5	2.8
Unsaturated					
$C_{12:1}$ at 11–12	0.9	TR	0.7	TR	0.6
$C_{13:1}$ at 12–13	TR	TR	TR	TR	0.5
$C_{15:1}\omega6c$	12.0	11.9	9.9	9.1	10.7
$C_{16:1}\omega5c$	0.5	0.6	0.8	0.9	−
$C_{17:1}\omega6c$	3.0	1.1	1.3	1.3	3.9
$C_{18:1}\omega5c$	TR	0.7	0.5	0.8	TR
Hydroxy					
iso-$C_{14:0}$ 3-OH	−	−	0.5	TR	−
iso-$C_{15:0}$ 3-OH	11.5	13.5	12.4	22.9	14.4
$C_{15:0}$ 3-OH	1.1	0.9	0.9	TR	4.3
iso-$C_{16:0}$ 3-OH	2.2	4.8	2.9	2.1	3.3
$C_{16:0}$ 3-OH	1.3	−	0.7	1.7	2.6
iso-$C_{17:0}$ 3-OH	1.8	0.7	1.0	1.5	4.3
$C_{17:0}$ 2-OH	TR	−	−	−	TR
Branched					
iso-$C_{13:0}$	7.3	5.8	8.7	3.0	0.9
anteiso-$C_{13:0}$	0.6	TR	TR	−	−
iso-$C_{14:0}$	3.5	4.4	8.6	2.2	0.9
iso-$C_{15:1}$ G	7.2	4.9	4.0	13.2	11.9
anteiso-$C_{15:1}$ A	1.1	−	TR	0.5	−
iso-$C_{15:0}$	17.0	23.4	21.5	18.9	14.6
anteiso-$C_{15:0}$	13.4	11.0	6.1	2.2	2.4
iso-$C_{16:1}$ H	1.3	2.9	1.9	−	0.7
iso-$C_{16:0}$	0.9	1.5	1.0	TR	TR
10-Methyl $C_{18:0}$ (TBSA)	1.1	−	0.8	TR	1.9
Summed features*					
2	−	−	−	2.5	−
3	3.6	3.1	3.8	8.2	11.9
9	TR	TR	TR	0.7	0.5

*Summed features refer to groups of two or three fatty acids that could not be separated by the Microbial Identification System. Summed feature 2 comprises $C_{14:0}$ 3-OH and/or iso-$C_{16:1}$ I; summed feature 3 comprises $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; summed feature 9 comprises iso-$C_{17:1}\omega9c$ and/or 10-methyl $C_{16:0}$.

ethanolic molybdatophosphoric acid for total lipids, Zinzadze reagent for phospholipids, ninhydrin for amino-group-containing lipids and α-naphthol for glycolipids. To identify the phospholipids of the isolate, one-dimensional TLC with chloroform/methanol/acetic acid/water (50 : 6 : 6 : 1, by vol.) was performed using four standard compounds (Sigma), as follows: phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and diphosphatidylglycerol. The polar lipids of strain WP25^T were phosphatidylethanolamine, two unidentified amino lipids, an unidentified phospholipid and four unidentified lipids (Fig. S1, available in the online Supplementary Material). The polar lipid composition of the isolate was compared with those of the reference strains and other species of the genus *Polaribacter*, *P. porphyrae*, *P. dokdonensis* and *P. gangjinensis*, based on these results and those reported in previous studies (Fukui *et al.*, 2013; Kim *et al.*, 2013). Strain WP25^T and the compared strains of the genus *Polaribacter* contained phosphatidylethanolamine, an aminolipid and three unidentified lipids, but the isolate could be distinguished from the other strains based on the minor polar lipid components, such as the number of unidentified aminolipids and phospholipids.

To determine the genotypic characteristics of the isolate, the genomic DNA G+C content was measured and DNA–DNA hybridizations were performed. Genomic DNA was extracted from strain WP25^T, *P. sejongensis* KCTC 23670^T, *P. butkevichii* KCTC 12100^T, *P. irgensii* KCTC 23136^T and *P. reichenbachii* KCTC 23969^T according to the method of Rochelle *et al.* (1992). To estimate the DNA G+C content of the isolate, a fluorimetric method was applied using SYBR gold I with the CFX96 Real-Time PCR detection system (Bio-Rad) (Gonzalez & Saiz-Jimenez, 2002). Genomic DNA samples from *Bacteroides thetaiotaomicron* VPI-5482^T, *Escherichia coli* K-12, *Ruegeria pomeroyi* DSS-3^T, *Bacteroides fragilis* NCTC 9343^T and *Streptococcus parasanguinis* ATCC 15912^T were used as calibration references. The DNA G+C content of strain WP25^T was 31.2 mol%, which is in the range reported previously for members of the genus *Polaribacter*. To clarify the genetic relatedness between the isolate and the reference strains, DNA–DNA hybridization was performed using a genome-probing microarray (Bae *et al.*, 2005; Chang *et al.*, 2008). DNA–DNA relatedness was calculated based on the signal-to-noise ratio of the genomic probes (Loy *et al.*, 2005). The DNA–DNA relatedness between strain WP70^T and *P. sejongensis* KCTC 23670^T, *P. butkevichii* KCTC 12100^T, *P. irgensii* KCTC 23136^T and *P. reichenbachii* KCTC 23969^T was 7.3±1.6 % (reciprocal 3.0±0.2 %), 7.7±1.7 % (4.0±0.5 %), 6.2±0.5 % (5.4±1.1 %) and 12.6±0.8 % (5.6±0.5 %), respectively (Table 3). These values are well below the threshold of 70 %, indicating that the isolate represents a distinct species (Wayne *et al.*, 1987).

Based on our physiological, biochemical, chemotaxonomic and genotypic analyses, it is suggested that strain WP25^T represents a novel species in the genus *Polaribacter*, for which the name *Polaribacter atrinae* sp. nov. is proposed.

Description of *Polaribacter atrinae* sp. nov.

Polaribacter atrinae (a.tri′nae. N.L. gen. n. *atrinae* of *Atrina*, the systematic name of a genus of comb pen shell, referring to the isolation of the type strain from *Atrina pectinata*).

Cells are Gram-staining-negative, aerobic, rod-shaped (0.8−1.0×1.4−3.5 μm) and non-motile. Colonies are circular, convex with an entire margin, viscous and yellow−orange, with a diameter of 0.8−1.2 mm after 48 h of culture on MA at 20 °C. Yellow-to-orange carotenoid-type pigments are produced, whereas flexirubin-type pigments are not. Growth occurs at 4−30 °C (optimum 20 °C), at pH 5−9 (optimum pH 7) and with 1−6 % (w/v) NaCl (optimum 2 %). Positive for catalase, oxidase, DNase, *p*-nitrophenyl-β-D-galactopyranosidase, *o*-nitrophenyl-β-D-galactopyranosidase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, trypsin, α-galactosidase, *N*-acetyl-β-glucosaminidase, lipase (spirit blue agar), acetoin production and reduction of nitrates to nitrites. Starch, casein, aesculin and Tweens 20, 40, 60 and 80 are hydrolysed. Acid is produced from aesculin, D-galactose, D-glucose, D-fructose, D-mannose, amygdalin, maltose, lactose, starch, glycogen, turanose, L-fucose, *N*-acetylglucosamine, melibiose, D-arabinose, glycerol, sucrose, raffinose, inulin and melezitose. Utilizes α-cyclodextrin, dextrin, glycogen, cellobiose, D-galactose, α-D-glucose, lactose, maltose, D-mannose, acetic acid,

Table 3. DNA–DNA hybridization between strain WP25^T and the type strains of closely related species of the genus *Polaribacter*

Data represent means±SD of six replicates obtained in the present study (based on reciprocal analyses).

Strain	DNA–DNA hybridization (%) with:				
	1	2	3	4	5
1. Strain WP25^T	(100)	3.0±0.2	4.0±0.5	5.4±1.1	5.6±0.5
2. *P. sejongensis* KCTC 23670^T	7.3±1.6	(100)	8.8±1.8	13.0±1.0	3.8±0.4
3. *P. butkevichii* KCTC 12100^T	7.7±1.7	8.3±0.4	(100)	7.5±0.9	2.9±0.4
4. *P. irgensii* KCTC 23136^T	6.2±0.5	1.9±0.1	2.9±0.2	(100)	2.0±0.3
5. *P. reichenbachii* KCTC 23969^T	12.6±0.8	13.2±0.6	8.3±0.4	14.9±0.6	(100)

α-ketobutyric acid, L-alanine, L-alanyl glycine, L-glutamic acid, glycyl L-glutamic acid, L-ornithine, uridine, DL-α-glycerol phosphate, α-D-glucose 1-phosphate, D-glucose 6-phosphate, D-fructose, α-ketoglutaric acid, turanose, DL-lactic acid, L-alaninamide, glycerol, sucrose, inosine and 2,3-butanediol. The major fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$, $C_{15:1}\omega6c$ and iso-$C_{15:0}$ 3-OH. The sole isoprenoid quinone is MK-6. The polar lipids are phosphatidylethanolamine, two unidentified aminolipids, an unidentified phospholipid and four unidentified lipids.

The type strain, WP25^T (=KACC 17473^T=JCM 19202^T), was isolated from the intestinal tract of a comb pen shell, *Atrina pectinata*, which was collected from the South Sea near Yeosu in Korea. The DNA G+C content of the type strain is 31.2 mol%.

ACKNOWLEDGEMENTS

This work was supported by grants from the Mid-career Researcher Program through the National Research Foundation of Korea (2012-0008806) and the National Institute of Biological Resources (NIBR), funded by the Ministry of Environment (MOE) of the Republic of Korea (NIBR no. 2013-02-001). We thank Professor J. P. Euzéby (École Nationale Vétérinaire, France) for etymological advice and Mr Hae-Won Lee and Dr Seong Woon Roh (Jeju Center, Korea Basic Science Institute, Korea) for analysing the isoprenoid quinones.

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