Polaribacter sejongensis sp. nov., isolated from Antarctic soil, and emended descriptions of the genus *Polaribacter*, *Polaribacter butkevichii* and *Polaribacter irgensii*

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A Gram-staining-negative, catalase- and oxidase-positive, non-motile bacterium, designated strain KOPRI 21160^T, was isolated from Antarctic soil. Based on 16S rRNA gene sequence analysis, strain KOPRI 21160^T was found to belong to the genus *Polaribacter*. Sequence similarity between strain KOPRI 21160^T and the type strains of species of the genus *Polaribacter* was 94.2–98.3 %. The nearest phylogenetic neighbours of strain KOPRI 21160^T were *Polaribacter butkevichii* KCTC 12100^T (98.3 % similarity) and *Polaribacter irgensii* KCTC 23136^T (97.5 %). DNA–DNA relatedness was 50.6 %, between strain KOPRI 21160^T and *P. butkevichii* KCTC 12100^T, and 45.2 % between strain KOPRI 21160^T and *P. irgensii* KCTC 23136^T. Strain KOPRI 21160^T grew at 4–37 °C and at pH 7.0–8.5. It could hydrolyse DNA, starch and Tweens 20, 40, 60 and 80. Menaquinone-6 (MK-6) was the only respiratory quinone, and iso-$C_{15:0}$, iso-$C_{15:0}$ 3-OH and $C_{15:1}\omega6c$ were the major cellular fatty acids. The major polar lipids were phosphatidylethanolamine, two unidentified aminolipids and one unidentified lipid. The DNA G+C content was 30.0 mol%. Based on data from our polyphasic study, the organism is considered to represent a novel species of the genus *Polaribacter*, for which we propose the name *Polaribacter sejongensis* sp. nov. The type strain is KOPRI 21160^T (=KCTC 23670^T=JCM 18092^T). Emended descriptions of the genus *Polaribacter*, *Polaribacter butkevichii* Nedashkovskaya *et al.* 2005 and *Polaribacter irgensii* Gosink *et al.* 1998 are also proposed.

The genus *Polaribacter* (family *Flavobacteriaceae*, phylum *Bacteroidetes*) was established by Gosink *et al.* (1998) for four Gram-staining-negative, psychrophilic, gas vacuolate marine bacteria. At the time of writing, the genus *Polaribacter* contains the following species: *Polaribacter butkevichii* (Nedashkovskaya *et al.*, 2005), *P. dokdonensis* (Yoon *et al.*, 2006), *P. glomeratus* (Gosink *et al.*, 1998), *P. irgensii* (Gosink *et al.*, 1998), *P. filamentus* (type species; Gosink *et al.*, 1998), *P. franzmannii* (Gosink *et al.*, 1998), *P. gangjinensis* (Lee *et al.*, 2011) and *P. porphyrae* (Fukui *et al.*, 2013). Emended descriptions of the genus *Polaribacter* and two species of the genus *Polaribacter* have recently been proposed (Fukui *et al.*, 2013). The four species originally described by Gosink *et al.* (1998) are psychrophilic organisms isolated from polar marine environments, but the four species subsequently described are mesophilic.

In the course of investigations on culturable bacteria in Antarctic soil, a number of bacterial strains were isolated. One of these isolates, designated strain KOPRI 21160^T, was allocated to the genus *Polaribacter* on the basis of 16S rRNA gene sequence analysis. In this study, we used a polyphasic approach to determine the exact taxonomic position of strain KOPRI 21160^T.

Strain KOPRI 21160^T was isolated from a seashore soil sample collected near the King Sejong Station on King George Island (62° 13′ 21″ S 58° 47′ 17″ W), following inoculation on marine agar 2216 (MA; BD) and incubation at 20 °C. The isolate was routinely cultured under the same conditions and stored as a suspension in an aqueous glycerol solution (20 %, w/v) at −70 °C for long-term

Abbreviations: CMC, carboxymethyl cellulose; FAME, fatty acid methyl ester.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain KOPRI 21160^T is HQ853596.

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

preservation. Unless otherwise mentioned, *P. butkevichii* KCTC 12100^T and *P. irgensii* KCTC 23136^T were used as the reference strains. For physiological tests, the isolate and the reference strains were cultured on MA or in marine broth 2216 (MB; BD) at 4–25 °C and pH 7.0.

The genomic DNA from strain KOPRI 21160^T, *P. butkevichii* KCTC 12100^T and *P. irgensii* KCTC 23136^T was extracted according to Sambrook & Russell (2001). The 16S rRNA gene of strain KOPRI 21160^T was amplified by PCR using the universal primers 27F and 1492R (Lane, 1991) and analysed as described by Kim *et al.* (2012a). The closest relatives of the new isolate were determined by database searches, and their sequences were retrieved from the EzTaxon-e (http://eztaxon-e.ezbiocloud.net/) server (Kim *et al.*, 2012b) and GenBank using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple alignments of the sequences were carried out using CLUSTAL X (Thompson *et al.*, 1997).

The nearly complete 16S rRNA gene sequence of strain KOPRI 21160^T, comprising 1382 nt, was determined. Based on 16S rRNA gene sequence similarity, strain KOPRI 21160^T was closely related to species of the genus *Polaribacter*, with similarity values of 94.2–98.3 %. The nearest phylogenetic neighbours of strain KOPRI 21160^T were *P. butkevichii* KCTC 12100^T (98.3 % sequence similarity) and *P. irgensii* KCTC 23136^T (97.5 %). The next closest relatives were members of the genera *Tenacibaculum*, *Aquimarina* and *Lutibacter*.

Neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) phylogenetic trees were reconstructed using MEGA5 software (Tamura *et al.*, 2011), based on an alignment with a length of 1372 nt. Kimura's two-parameter method was used for calculation of phylogenetic distances (Kimura, 1980). Confidence limits for phylogenetic trees were estimated from bootstrap analysis (Felsenstein, 1985) using 1000 replicates.

Strain KOPRI 21160^T fell within the cluster comprising the species of the genus *Polaribacter* in the neighbour-joining, maximum-parsimony and maximum-likelihood phylogenetic trees (Fig. 1). In the neighbour-joining phylogenetic tree, strain KOPRI 21160^T clustered with *P. butkevichii* KCTC 12100^T and *P. irgensii* KCTC 23136^T.

DNA–DNA relatedness between strain KOPRI 21160^T, *P. butkevichii* KCTC 12100^T and *P. irgensii* KCTC 23136^T, was assessed by DNA–DNA hybridization experiments using the fluorometric method in microdilution wells (Ezaki *et al.*, 1989). Hybridization was carried out in triplicate at 37 °C for 6 h in the presence of 50 % formamide. Strain KOPRI 21160^T shared 50.6 % DNA–DNA relatedness with *P. butkevichii* KCTC 12100^T and 45.2 % with *P. irgensii* KCTC 23136^T; the results of the reciprocal experiments were 26.6 % and 22.4 %, respectively.

For G+C mol% analysis, the genomic DNA of strain KOPRI 21160^T was hydrolysed with nuclease P1 and dephosphorylated with alkaline phosphatase, and the mixture of nucleosides was analysed by HPLC equipped with a reversed-phase column (Mesbah *et al.*, 1989). The DNA of *Escherichia coli* KCTC 2441^T was used as a reference. The DNA G+C content of strain KOPRI 21160^T was 30.0 mol%, a value within the range reported for species of the genus *Polaribacter* (28.6–34.6 mol%; Gosink *et al.*, 1998; Nedashkovskaya *et al.*, 2005; Yoon *et al.*, 2006; Lee *et al.*, 2011; Fukui *et al.*, 2013).

Except for Gram staining, cell morphology and gliding motility, all phenotypic characteristics listed below were tested on strain KOPRI 21160^T and the two reference strains. Gram staining was tested using a Gram stain kit (BD). Oxidase activity was colorimetrically determined using Oxidase Reagent (bioMérieux), and catalase activity was determined by bubble production in a 3 % (v/v) hydrogen peroxide solution. Cell morphology was observed by light (Eclipse 80i; Nikon), transmission electron (model CM20; Philips) and scanning electron (model S4300N; Hitachi) microscopy, after 3 days of cultivation at 25 °C on MA. Gliding motility was tested with the hanging drop method (Bernardet *et al.*, 2002). The growth temperature range was determined on MA after incubation at 4, 10, 15, 20, 25, 30, 37, 40 and 45 °C. The initial pH for growth was determined in MB, the pH of which had been adjusted after sterilization to pH 4.0–10.0 (in 0.5 pH-unit increments), using filter-sterilized buffers (0.1 M sodium citrate/citric acid, 0.2 M sodium phosphate and 0.1 M sodium carbonate/bicarbonate). The salt concentration for growth was determined in MB prepared according to the composition of the BD medium without NaCl and subsequently supplemented with 0–5 % (w/v) NaCl (in 0.5 % increments). Growth in liquid media was measured by change in OD_{600} over a period of 3–6 days using a DU 730 UV/Vis Scanning Spectrophotometer (Beckman Coulter). Growth under anaerobic conditions was observed on MA incubated at 25 °C for 7 days in a GasPak EZ Anaerobe Pouch System (BD). Presence of flexirubin-type pigments was assessed as described by Bernardet *et al.* (2002) using 20 % KOH. Hydrolysis of casein, carboxymethyl cellulose (CMC) and starch was assessed after 5 days of culture on MA containing 3 % (w/v) skimmed milk (BD), 0.5 % (w/v) CMC (Sigma) and 0.2 % (w/v) soluble starch (BD), respectively. Hydrolysis of CMC and starch was visualized by presence of clear haloes around the colonies after staining with 1 % Congo red solution or Lugol's iodine solution (Teather & Wood, 1982), respectively. Hydrolysis of Tweens 20, 40, 60 and 80 was assessed according to Chakrabarty *et al.* (1970) and hydrolysis of DNA was tested on DNase agar (BD). Additional enzyme activities and biochemical characteristics of the isolate and reference strains were tested using the API 20 E, API 20 NE and API ZYM strips (bioMérieux) according to the manufacturer's instructions. API 20 E and API 20 NE strips for strain KOPRI 21160^T and *P. butkevichii* KCTC 12100^T were incubated at 25 °C for 3 days, while the strips for *P. irgensii* KCTC 23136^T were incubated at 4 °C for

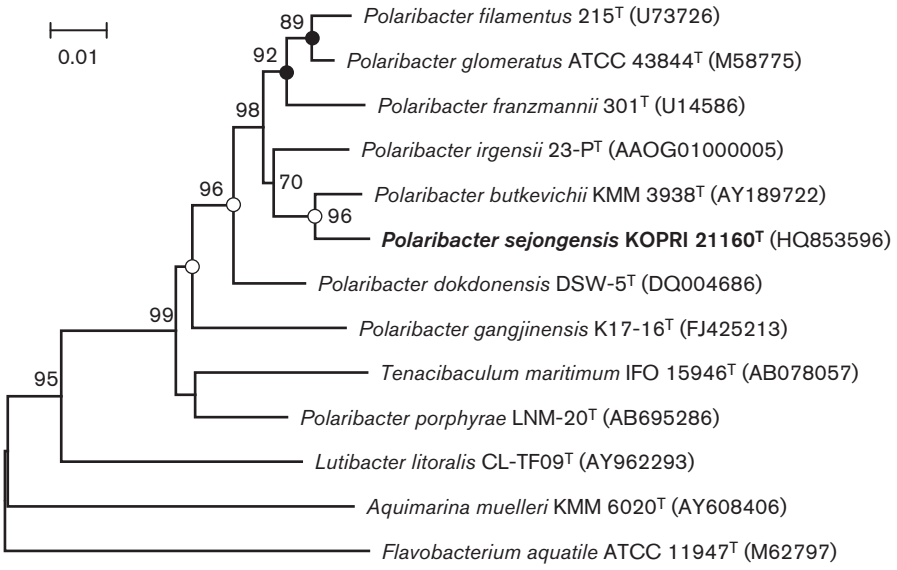

Fig. 1. Phylogenetic tree showing the relationships of strain KOPRI 21160^T, other species of the genus *Polaribacter* and representatives of the family *Flavobacteriaceae*. The tree was based on an alignment of 1372 bp 16S rRNA gene sequences and reconstructed by using the neighbour-joining method. Filled circles indicate that the corresponding branches are also recovered using both in the maximum-likelihood and maximum-parsimony methods. Open circles indicate that the corresponding branches are also recovered using the maximum-likelihood method. Bootstrap values (expressed as percentages of 1000 replications) greater than 70 % are shown at nodes. GenBank accession numbers of the 16S rRNA gene sequences are given in parentheses. Bar, 0.01 substitutions per nucleotide position.

7 days. API ZYM strips were incubated for 4 h at 25 °C for KOPRI 21160^T and *P. butkevichii* KCTC 12100^T and at 4 °C for *P. irgensii* KCTC 23136^T.

Susceptibility of the three strains to antibiotics was determined by the disc diffusion method on MA plates after 3 days of incubation at 25 °C for strain KOPRI 21160^T and *P. butkevichii* KCTC 12100^T and after 7 days of incubation at 4 °C for *P. irgensii* KCTC 23136^T using laboratory-prepared discs (6 mm in diameter). Strains were regarded as susceptible when the diameter of the inhibition zone was greater than 8 mm, weakly susceptible when the diameter was less 8 mm and resistant when there was no inhibition zone.

The phenotypic characteristics of strain KOPRI 21160^T are given in the species description and in Fig. S1 (available in IJSEM Online). The differentiating characteristics of strain KOPRI 21160^T and the two reference strains are presented in Table 1.

For isoprenoid quinone, fatty acid methyl ester (FAME) and polar lipid analyses, strain KOPRI 21160^T and *P. butkevichii* KCTC 12100^T were incubated on MA at 25 °C for 3 days, while *P. irgensii* KCTC 23136^T was incubated on MA at 4 °C for 7 days. Isoprenoid quinones of the three strains were extracted from freeze-dried cells according to Komagata & Suzuki (1988). The quinones were purified via preparative TLC (silica gel F254; Merck) and identified by HPLC (L-5000; Hitachi). The only respiratory quinone of the three strains was menaquinone-6 (MK-6). MK-6 is the major or only respiratory quinone in all members of the family *Flavobacteriaceae* (Bernardet, 2011).

For FAME analysis, cells of the same physiological age were obtained from the three strains according to the standard protocol of the Microbial Identification System (MIDI) (Sasser, 2001). FAMEs were also extracted according to Sasser (2001), separated by gas chromatography (HP 6890N; Agilent) and identified with the Sherlock software package (MIDI Sherlock system 4.1, TSBA library version 4.0; MIDI). The major cellular fatty acids of strain KOPRI 21160^T were iso-$C_{15:0}$ (18.1 %), iso-$C_{15:0}$ 3-OH (17.9 %) and $C_{15:1}\omega6c$ (10.4 %). The fatty acid composition of the reference strains was very similar to that of strain KOPRI 21160^T with only minor variations in the respective proportions of the fatty acids (Table 2).

Polar lipids were extracted from the three strains and analysed by two-dimensional TLC (silica gel F254) according to Tindall (1990) and Altenburger *et al.* (1996). The total lipids were detected by spraying one plate with 5 % ethanolic molybdophosphoric acid while other plates were sprayed with ninhydrin, molybdenum blue and α-naphthol to specifically detect amino lipids, phospholipids and glycolipids, respectively. Phosphatidylethanolamine, four unidentified polar lipids and two unidentified aminolipids were detected in strain KOPRI 21160^T. The two reference strains each contained only one unidentified aminolipid; in addition, *P. butkevichii* KCTC

Table 1. Differentiating properties of strain KOPRI 21160^T and the type strains of closely related species of the genus *Polaribacter*

Strains: 1, KOPRI 21160^T; 2, *P. butkevichii* KCTC 12100^T; 3, *P. irgensii* KCTC 23136^T. All data are from this study, except the DNA G+C content of the reference strains (data from Gosink *et al.*, 1998; Nedashkovskaya *et al.*, 2005). All strains are catalase- and oxidase-positive, grow with 5 % NaCl but do not grow with 0 % NaCl, have MK-6 as the only respiratory quinone, do not produce flexirubin-type pigments and are susceptible to (μg per disc) rifampicin (1.5) and lincomycin (2.5), but resistant to ampicillin (2.5), doxycycline (0.25), penicillin G (2.5), tetracycline (0.5), gentamicin (2.5) and streptomycin (2.5). In the API 20E strip, all strains are positive for hydrolysis of gelatin but negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease and tryptophan deaminase activities, citrate utilization, H_2S and indole production, and acid production from D-glucose, inositol, D-sorbitol, L-rhamnose, sucrose, melibiose, amygdalin and L-arabinose. +, Positive; W, weakly positive; −, negative.

Characteristic	1	2	3
Growth temperature (°C)	4–37	4–37	4–10
Growth with 0.5 % NaCl	+	+	−
Hydrolysis of:			
Casein	−	+	−
DNA	+	+	−
Starch	+	+	−
CMC	−	+	−
Tween 20	+	+	−
Tween 40	+	+	−
Tween 60	+	W	−
Tween 80	+	W	−
β-Galactosidase activity	+	−	−
Acetoin production	−	+	−
Acid production from D-mannitol	+	−	−
Antibiotic susceptibility (μg per disc):			
Chloramphenicol (0.5)	W	W	−
Gramicidin (2.5)	W	W	−
Erythromycin (0.5)	+	−	+
DNA G+C content (mol%)	30	32.4	31

Table 2. Cellular fatty acid composition (%) of strain KOPRI 21160^T and the type strains of closely related species of the genus *Polaribacter*

Strains: 1, KOPRI 21160^T; 2, *P. butkevichii* KCTC 12100^T; 3, *P. irgensii* KCTC 23136^T. All data from this study. Cells in the same physiological state were used for the analysis of FAMEs. Fatty acids comprising less than 1 % of the total in all strains are not shown. TR, Trace (less than 1 %); –, not detected.

Fatty acid	1	2	3
Saturated			
$C_{13:0}$	TR	1.5	–
$C_{14:0}$	TR	1.1	–
$C_{15:0}$	6.5	7.7	2.0
iso-$C_{13:0}$	5.5	7.8	2.9
iso-$C_{14:0}$	3.8	6.5	2.4
iso-$C_{15:0}$	18.1	17.2	16.6
iso-$C_{16:0}$	1.0	TR	TR
anteiso-$C_{15:0}$	8.4	4.0	2.9
$C_{15:0}$ 2-OH	1.1	TR	TR
$C_{15:0}$ 3-OH	6.2	7.6	3.7
$C_{16:0}$ 3-OH	1.3	1.3	1.5
iso-$C_{15:0}$ 3-OH	17.9	16.1	22.6
iso-$C_{16:0}$ 3-OH	4.3	3.0	3.2
iso-$C_{17:0}$ 3-OH	2.1	1.2	1.7
10-Methyl $C_{18:0}$ TSBA	0.9	1.1	TR
Unsaturated			
$C_{15:1}\omega6c$	10.4	10.7	9.1
$C_{17:1}\omega6c$	1.2	1.3	1.2
$C_{18:1}\omega5c$	TR	–	1.0
iso-$C_{15:1}$ G	3.9	3.3	11.6
iso-$C_{16:1}$ H	1.7	1.6	1.4
Summed features*			
1	–	–	1.3
2	–	–	1.5
3	3.0	3.5	7.6

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 1 consisted of iso-$C_{15:1}$ H and/or $C_{13:0}$ 3-OH. Summed feature 2 consisted of iso-$C_{16:1}$ I and/or $C_{14:0}$ 3-OH. Summed feature 3 consisted of $C_{16:1}\omega7c$ and/or iso-$C_{15:0}$ 2-OH.

12100^T contained one unidentified phospholipid while *P. irgensii* KCTC 23136^T contained one unidentified phosphoaminolipid (Fig. 2).

Although strain KOPRI 21160^T shared more than 97.0 % 16S rRNA gene sequence similarity with *P. butkevichii* KCTC 12100^T and *P. irgensii* KCTC 23136^T, the DNA–DNA hybridization values were below 70 %, demonstrating that strain KOPRI 21160^T represents a novel species in the genus *Polaribacter* (Stackebrandt & Goebel, 1994) that can be distinguished from its closest neighbours by the phenotypic properties listed in Table 1.

Therefore, on the basis of polyphasic analysis, strain KOPRI 21160^T is considered to represent a novel species of the genus *Polaribacter*, for which the name *Polaribacter sejongensis* sp. nov. is proposed. In addition, emended descriptions of the genus *Polaribacter*, *Polaribacter butkevichii* and *Polaribacter irgensii* are also proposed on the basis of new data obtained in this study.

Emended description of the genus *Polaribacter* Gosink *et al.* 1998 emend. Fukui *et al.* 2013

The description is as given by Gosink *et al.* (1998) and Fukui *et al.* (2013) with the following amendments. Some strains occur in temperate marine environments and are mesophilic. MK-6 is the only or major respiratory quinone in all analysed strains.

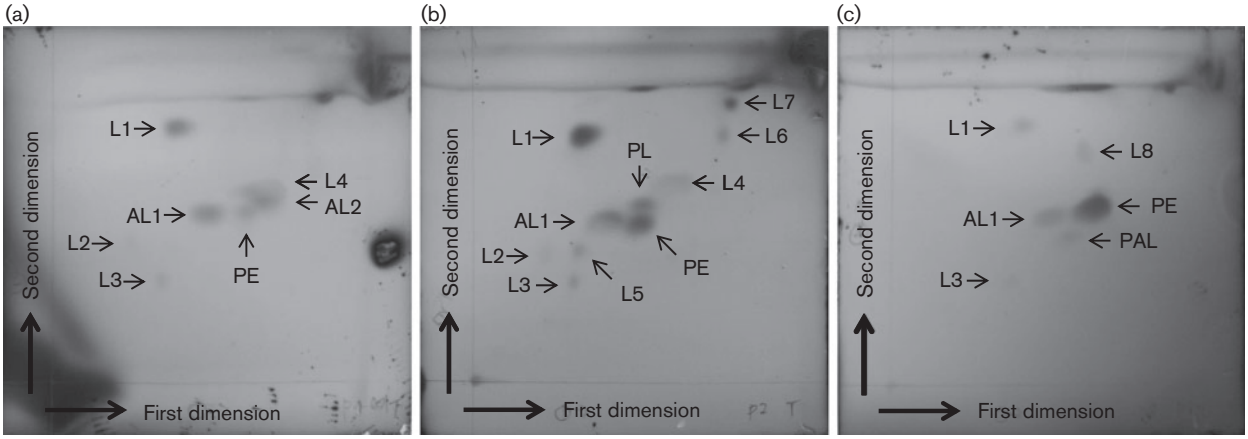

Fig. 2. Thin-layer chromatography of the total polar lipids of strain KOPRI 21160^T (a), *P. butkevichii* KCTC 12100^T (b) and *P. irgensii* KCTC 23136^T (c). PE, phosphatidylethanolamine; AL1–2, unidentified aminolipids; PL, unidentified phospholipid; L1–8, unidentified polar lipids; PAL, unidentified phosphoaminolipid.

Emended description of *Polaribacter butkevichii* Nedashkovskaya *et al.* 2005

The description is as given by Nedashkovskaya *et al.* (2005) with the following amendments. CMC and Tweens 20 and 60 are hydrolysed. The major polar lipids are phosphatidylethanolamine, one unidentified aminolipid, one unidentified phospholipid and one unidentified lipid. Minor amounts of six other unidentified lipids are also present.

Emended description of *Polaribacter irgensii* Gosink *et al.* 1998

The description is as given by Gosink *et al.* (1998) with the following amendments. Catalase- and oxidase-positive. Growth occurs with 5 % NaCl. Susceptible to (μg per disc) erythromycin (0.5), rifampicin (1.5) and lincomycin (2.5), but resistant to ampicillin (2.5), chloramphenicol (0.5), gramicidin (2.5), doxycycline (0.25), penicillin G (2.5), tetracycline (0.5), gentamicin, (2.5) and streptomycin (2.5). Casein, DNA, starch, CMC and Tweens 20, 40, 60 and 80 are not hydrolysed. In the API 20E strip, positive for hydrolysis of gelatin but negative for β-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease and tryptophan deaminase activities, citrate utilization, H_2S, acetoin and indole production, and acid production from D-glucose, mannitol, inositol, D-sorbitol, L-rhamnose, sucrose, melibiose, amygdalin and L-arabinose. The major polar lipid is phosphatidylethanolamine. Significant or minor amounts of one unidentified aminolipid, one unidentified phosphoaminolipid and three unidentified polar lipids are also present. MK-6 is the only respiratory quinone.

Description of *Polaribacter sejongensis* sp. nov.

Polaribacter sejongensis (se.jong.en′sis. N.L. masc. adj. *sejongensis* of or belonging to Sejong, the name of the Antarctic station where the type strain was isolated).

Cells are Gram-staining-negative, strictly aerobic, catalase- and oxidase-positive rods, 0.8–5 μm in length and 0.4–0.8 μm in diameter, non-motile. Coccoid bodies and gas vesicles are observed in ageing cultures but coiled cells are not observed. Colonies are light yellow, circular with entire edges and convex, with a diameter of 1 mm after 3 days of incubation on MA. Flexirubin-type pigments are not produced. Growth occurs at 4–37 °C (optimum, 25 °C), at pH 7.0–8.5 (optimum, pH 7.5) and in the presence of 0.5–5 % (w/v) NaCl (optimum, 3 %). DNA, starch and Tweens 20, 40, 60 and 80 are hydrolysed, but casein and CMC are not. In the API 20 E strip, positive for β-galactosidase activity, hydrolysis of gelatin and acid production from mannitol, but negative for all other tests. In the API 20 NE strip, positive for β-glucosidase and β-galactosidase activities, but negative for all other tests. In the API ZYM strip, alkaline phosphatase, leucine arylamidase, valine arylamidase, acid phospatase and naphthol-AS-BI-phosphohydrolase activities are present; weak esterase (C4) and esterase lipase (C8) activities are present; the other enzyme activities are absent. Susceptible to (μg per disc) rifampicin (1.5), lincomycin (2.5) and erythromycin (0.5); weakly susceptible to chloramphenicol (0.5) and gramicidin (2.5); and resistant to ampicillin (2.5), doxycycline (0.25), penicillin G (2.5), tetracycline (0.5), gentamicin, (2.5) and streptomycin (2.5). The only respiratory quinone is MK-6. The major cellular fatty acids (>10 %) are iso-$C_{15:0}$, iso-$C_{15:0}$ 3-OH and $C_{15:1}\omega6c$. The major polar lipids are phosphatidylethanolamine, two unidentified aminolipids and one unidentified lipid. Minor amounts of three other unidentified lipids are also present.

The type strain is KOPRI 21160^T (=KCTC 23670^T=JCM 18092^T), isolated from a soil sample collected near the King Sejong Station on King George Island, Antarctica. The DNA G+C content of the type strain is 30.0 mol%.

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