Planococcus halocryophilus sp. nov., an extreme sub-zero species from high Arctic permafrost

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A novel aerobic, Gram-positive, motile, coccoid bacterial strain, designated Or1^T, was isolated from permafrost active-layer soil collected from the Canadian high Arctic. Strain Or1^T was capable of growth over a broad temperature range, including sub-zero growth (below −10 to 37 °C), and at high salinity (0–19 % NaCl), growing optimally at 25 °C, at pH 7.0–8.0 and in the presence of 2 % NaCl. Its taxonomic and phylogenetic position was determined by using a polyphasic approach, which indicated that strain Or1^T was a member of the genus *Planococcus*. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain Or1^T belonged to the genus *Planococcus*, differing by 0.4–3.6 % from the type strains of all recognized *Planococcus* species, and was related most closely to *Planococcus antarcticus* CMS 26or^T (98.8 % similarity) and *Planococcus donghaensis* JH1^T (99.6 %). However, DNA–DNA hybridization experiments showed that strain Or1^T had low genomic relatedness to *Planococcus antarcticus* CMS 26or^T (18 %) and *Planococcus donghaensis* JH1^T (46 %). The major menaquinones of strain Or1^T were MK-7 (55 %), MK-8 (36 %) and MK-6 (9 %) and the major fatty acids were anteiso-$C_{15:0}$, $C_{16:1}\omega7c$ alcohol and anteiso-$C_{17:0}$. The DNA G+C content of strain Or1^T was 40.5 mol%. Differential phenotypic, phylogenetic and genomic data suggest that strain Or1^T represents a novel species of the genus *Planococcus*, for which the name *Planococcus halocryophilus* sp. nov. is proposed. The type strain is Or1^T (=DSM 24743^T=JCM 17719^T).

Following the description of the genus *Planococcus* by Migula (1894), several amendments to this description were made by Nakagawa *et al.* (1996) and more recently by Yoon *et al.* (2010). At the time of writing, the genus *Planococcus* comprised nine recognized species: *Planococcus citreus* (Kocur *et al.*, 1970), *Planococcus kocurii* (Hao & Komagata, 1985), *Planococcus antarcticus* (Reddy *et al.*, 2002), *Planococcus rifietoensis* (Romano *et al.*, 2003), *Planococcus maritimus* (Yoon *et al.*, 2003), *Planococcus maitriensis* (Alam *et al.*, 2003), *Planococcus columbae* (Suresh *et al.*, 2007), *Planococcus donghaensis* (Choi *et al.*, 2007) and *Planococcus salinarum* (Yoon *et al.*, 2010). Five additional species originally allocated to the genus *Planococcus*, namely *Planococcus okeanokoites* (Nakagawa *et al.*, 1996), *Planococcus mcmeekinii* (Junge *et al.*, 1998), *Planococcus alkanoclasticus* (Engelhardt *et al.*, 2001), *Planococcus psychrophilus* (Reddy *et al.*, 2002) and *Planococcus stackebrandtii* (Mayilraj *et al.*, 2005), were subsequently transferred to the genus *Planomicrobium* (Yoon *et al.*, 2001; Dai *et al.*, 2005; Jung *et al.*, 2009). All members of the genus *Planococcus* are Gram-positive, aerobic cocci that are able to grow at moderately low temperatures and high salt concentrations, and have been predominantly isolated from cold and/or saline environments (Arctic, Antarctic, marine). Chemotaxonomic, phylogenetic and physiological characterization and classification of a novel *Planococcus*-like strain, Or1^T, presented in this paper indicate that it represents a novel species of the genus *Planococcus* with the broadest cold and salt tolerance reported to date, most notably growing at sub-zero temperatures.

Bacterial strain Or1^T was isolated from active-layer soil, at a depth of <1 m, sampled from a soil core (Eur3) taken at Eureka, Ellesmere Island, in the Canadian high Arctic (Steven *et al.*, 2007). Strain Or1^T was isolated from 5 g aseptically collected permafrost (Juck *et al.*, 2005; Steven *et al.*, 2008) along with several other permafrost bacteria following enrichment in R2A media (Difco) supplemented with sodium acetate (0.4 g l^{-1}) incubated at 5 °C. Following 11 months of incubation, 100 µl culture was transferred to R2A agar solid media at 5 °C (Difco), resulting in the isolation of individual colonies, including strain Or1^T. Routine cultivation of strain Or1^T was carried out in trypticase soy agar (TSA; Difco) or broth (TSB; Difco) at room temperature (23–25 °C), and the strain was maintained in 25 % glycerol stocks at −80 °C. Interest in the extreme cold growth of strain Or1^T prompted sequencing of the complete genome (454 pyrosequence+Illumina consensus sequence) referred to in parts of this paper (N. C. S. Mykytczuk and others, unpublished results).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Or1^T is JF742665.

A summary of the morphological and physiological traits of strain Or1^T compared with those of the type strains of recognized *Planococcus* species is presented in Table 1. Cell morphology and Gram reaction of strain Or1^T were determined microscopically by using a Nikon Eclipse E600 microscope on cells grown in TSB. Gram staining was performed with a BD Gram stain kit and spore formation with the Schaeffer and Fulton Spore Stain kit (Fluka Analytical, Sigma-Aldrich). Cells of strain Or1^T were consistently Gram-positive and no spores were observed following staining of colonies grown for 48 and 72 h, and up to 1 week. Micrographs were also obtained by transmission and scanning electron microscopy with samples prepared as described by Vali *et al.* (2004). Cells of strain Or1^T were coccoid, typically 0.8–1.2 μm in diameter, arranged singly or as diplococci (Fig. 1). Motility was assessed by microscopic examination of wet mounts over the period of incubation at both room temperature and at 5 °C. Cells of strain Or1^T were motile under the observed conditions, as with most recognized *Planococcus* species (Table 1). Initial screening of the genome sequence indicated the presence of a flagellar synthesis operon, but this has not yet been confirmed as functional (N. C. S. Mykytczuk and others, unpublished results). Colony morphology was determined on TSA plates following 2–3 days growth at 25 °C, which resulted in the formation of orange, round, convex colonies with a raised centre. Colony morphology changed with increasing salt concentration and at sub-zero temperatures (only TSA plates with 5 % sucrose were tested at −5 °C) leading to smaller or more irregular, umbonate colonies, respectively.

Further physiological and biochemical characterizations of strain Or1^T were completed under specified growth conditions and are summarized relative to recognized *Planococcus* species in Table 1. Growth temperature limits for strain Or1^T were determined on TSA media incubated at 0–30 °C (increments of 5 °C), and also at 32, 35, 37, 38, 39 and 40 °C. Growth occurred at all temperatures up to and including 37 °C. Growth at sub-zero temperatures was determined in TSB liquid cultures supplemented with NaCl and glycerol for −5 °C (5 % NaCl, 2 % glycerol) and −10 °C (12 % NaCl, 5 % glycerol). Growth was confirmed by an increase in turbidity monitored spectrophotometrically at OD 480 nm (wavelength adjusted for glycerol), viable plate counting on TSA plates, as well as an accumulation of sedimented cells over weeks and months of incubation at −5 and −10 °C, respectively. Growth below −10 °C appeared possible, but additional media amendments were required and were not explored as part of this study. The unique sub-zero growth capacity appears to be linked to the ability of strain Or1^T to tolerate a high-salinity brine that remains liquid at low temperatures and corresponds with genome-encoded traits conferring cold and salinity tolerance (N. C. S. Mykytczuk and others, unpublished results). The pH range for growth of strain Or1^T was determined on R2A agar or TSA plates adjusted with either HCl or $NaCO_3$ to give a final pH ranging from 4.5 to 12.0 at 0.5 pH unit increments, and incubated at 25 °C. Growth was observed within the range pH 6.0–11.0, but not at higher or lower pH. Salt tolerance was determined on TSA media supplemented with 0–22 % (w/v) NaCl in increments of 1 or 2 % and incubated at room temperature for up to 2 weeks. Growth was observed at NaCl concentrations of 0–19 %, with optimal growth at 1.5 % NaCl. This range is similar to that of just one recognized *Planococcus* species, *Planococcus maritimus*, with most other species showing only moderate halotolerance (Table 1).

Strain Or1^T was catalase-positive, as determined by the addition of 3 % H_2O_2 to an aliquot of active culture, with the production of bubbles indicating a positive reaction. Strain Or1^T was also oxidase-positive, as determined by using a BD BBL DrySlide according to the manufacturer's instructions (Becton Dickinson). Biolog Gen III Micro plates (Biolog) were used to test the ability of strain Or1^T to metabolize major classes of biochemicals and other physiological properties, including the use of various sugars, amino acids, carboxylic acids, reducing power and chemical sensitivities. Growth on different carbon sources was assessed and strain Or1^T was capable of utilizing glucose, rhamnose, fructose, glycerol, mannose, galactose, maltose, glucosamine, glutamine, ribose, trehalose, cellobiose, mannitol, sucrose, lactose, lactic acid, pectin, gelatin, dextrin, alanine, serine, arginine, acetic acid, glutamic acid and gluconic acid, but not raffinose, melibiose, inositol methyl pyruvate, aminobutyric acid, ketobutyric acid or bromosuccinic acid when these were provided as the only carbon source.

Results of antibiotic sensitivity included in the Biolog microplates revealed that strain Or1^T was sensitive to troleandomycin, rifamycin SV, mynocycline, lincomycin, vancomycin and nalidixic acid. Additional antibiotic sensitivity tests were performed by the addition of antibiotics to a final concentration of 100 mg ml^{-1} in TSA medium. Strain Or1^T was resistant to tetracycline, polymyxin B, penicillin G and erythromycin, but sensitive to amoxicillin and streptomycin. These antibiotic sensitivities matched the profiles reported for some *Planococcus* species, such as *Planococcus maitriensis* (Alam *et al.*, 2003), but did not fully match the susceptibility profile of related species, including *Planococcus antarcticus* (Reddy *et al.*, 2002).

For characteristics not tested by the Biolog system, additional screening of acid production from carbohydrates and physiological traits were determined by using API 20E test strips according to the manufacturer's instructions (bioMérieux). Strain Or1^T produced acid from glucose, mannitol, sucrose, rhamnose and L-arabinose, but not from inositol, sorbitol, melibiose or amygdalin. Aesculin, starch and Tween 80 were not hydrolysed, while gelatin was hydrolysed. Strain Or1^T was positive for β-galactosidase, citrate utilization, arginine decarboxylase, lysine decarboxylase, hydrogen sulfide and indole production, but negative for the Vogues–Proskauer test (acetylmethylcarbinol production),

Table 1. Comparison of the phenotypic and chemotaxonomic characteristics of strain Or1^T and the type strains of all recognized *Planococcus* species

Strains: 1, Or1^T (data from this study); 2, *P. antarcticus* DSM 14505^T (Reddy *et al.*, 2002); 3, *P. kocurii* DSM 20747^T (Hao & Komagata, 1985; Reddy *et al.*, 2002); 4, *P. maritimus* JCM 11543^T (Yoon *et al.*, 2003); 5, *P. maitriensis* DSM 15305^T (Alam *et al.*, 2003); 6, *P. rifietoensis* DSM 15069^T (Romano *et al.*, 2003); 7, *P. citreus* DSM 20549^T (Hao & Komagata, 1985; Reddy *et al.*, 2002); 8, *P. columbae* PgEx11^T (Suresh *et al.*, 2007); 9, *P. donghaensis* JH1^T (Choi *et al.*, 2007); 10, *P. salinarum* ISL-16^T (Yoon *et al.*, 2010). All strains are positive for the Gram reaction and catalase activity and have coccoid cell morphology. All taxa are negative for spore formation and urease. +/−, Weakly positive; V, variable reaction; NR, not reported.

Characteristic	1	2	3	4	5	6	7	8	9	10
Growth temperature range (°C)	<−10 to 37	0–30	4–37	4–41	0–40	0–30	5–42	8–42	4–37	4–38
Motility	+	+	+	+	+	NR	+	+	+	−
pH range	6.0–11	6.0–12	NR	5.0–8.0	6.0–12	6.0–10.5	NR	6.0–11	7.0–8.0	6.0–7.5
NaCl tolerance (%)	19	12	3.3	17	12.5	15	10	14	12	13
Oxidase	+	−	−	−	−	+	−	−	+	+
Nitrate reduction	−	−	−	−	+	−	−	+	−	−
Hydrolysis of:										
Gelatin	+	+	V	+	+	+	+	−	−	−
Starch	−	−	−	−	−	−	−	−	+	−
Casein	−	NR	NR	+	+	−	NR	−	+	+
Tween 40	+	NR	NR	−	−	+	+	+	NR	+
Acid production from:										
Glucose	+	+	V	+	−	−	+	−	−	−
Mannitol	+	−	−	+	−	−	−	−	−	−
Sucrose	+	+	−	−	+/−	−	−	+	+	−
Peptidoglycan	L-Lys–D-Glu	L-Lys–D-Glu	L-Lys–D-Glu	L-Lys–D-Glu	L-Lys–D-Glu	L-Lys–D-Glu	L-Lys–D-Glu	L-Lys–D-Glu	L-Lys–D-Glu	NR
Menaquinone(s)	MK-7, MK-8, MK-6	MK-7, MK-8	MK-7, MK-8	MK-8, MK-7, MK-6	MK-8, MK-7	MK-8	MK-8, MK-7	MK-7, MK-8	MK-7, MK-8	MK-8, MK-7
DNA G+C content (mol%)	40.5	42	40–43	48	39	48	48–51	50.5	47	48.3

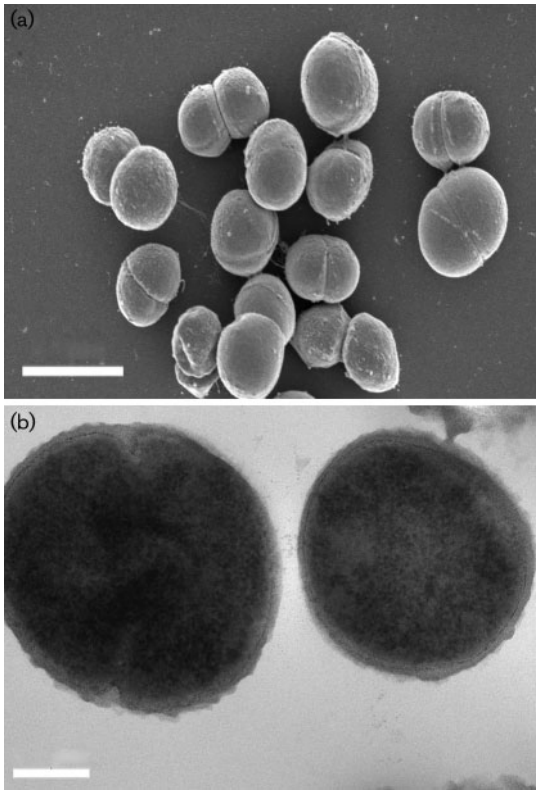

Fig. 1. Scanning (a) and transmission (b) electron micrographs of cells of strain Or1^T grown at 25 °C. Single cocci (0.8–1.2 μm in diameter) and diplococci are observed and cell division septa at different stages are apparent. Bars: a, 1.5 μm; b, 0.2 μm.

urease, ornithine carboxylase, tryptophan deaminase and nitrate reduction. For several of the biochemical characteristics described above, validation of necessary enzymes and pathways was performed by using the draft genome for strain Or1^T and allowed for accurate differentiation between weak or variable results (N. C. S. Mykytczuk and others, unpublished results).

Initially, the 16S rRNA gene sequence was determined from DNA isolated from a single colony of strain Or1^T. The almost-complete 16S rRNA gene sequence was PCR-amplified and sequenced as outlined by Steven *et al.* (2008). In brief, DNA was isolated by using the Gram-positive protocol of the DNeasy Blood and Tissue kit (Qiagen) and the 16S rRNA gene was PCR-amplified by using primers 27F and 1492R (Lane, 1991). Subsequently, the complete 16S rRNA gene sequence (1550 bp) was extracted from the draft genome sequence (N. C. S. Mykytczuk and others, unpublished results) and used for phylogenetic analyses. The two sequences were identical in their alignment, the submitted sequence being ~130 bp longer.

The phylogenetic position of strain Or1^T compared with members of the genus *Planococcus* and the closely related *Planomicrobium* clade was determined from 16S rRNA gene sequence alignments (CLUSTAL W) and phylogenetic and molecular evolutionary analyses conducted in MEGA version 5 (Tamura *et al.*, 2011). Trees were constructed by using the neighbour-joining method (Saitou & Nei, 1987) and Jukes–Cantor modelling (Jukes & Cantor, 1969) with 1000 bootstrap iterations (Fig. 2). A similar topology was also obtained for the tree constructed with the maximum-likelihood algorithm. The 16S rRNA gene sequence of strain Or1^T contained the same signature nucleotides (T and A at positions 183 and 190, respectively; *Escherichia coli* 16S rRNA gene sequence numbering) as those defined for the genus *Planococcus*, and clustered separately from the related *Planomicrobium* clade (Dai *et al.*, 2005). The phylogenetic similarity of strain Or1^T to recognized *Planococcus* species was determined from pairwise sequence comparisons by using the EzTaxon database (www.eztaxon.org; Chun *et al.*, 2007); relative to all *Planococcus* species, strain Or1^T showed highest 16S rRNA gene sequence divergence from *Planococcus columbae* PgEx11^T(96.4 %) and highest similarity to strains clustering in the same branch, namely *Planococcus donghaensis* JH1^T (99.6 %) and *Planococcus antarcticus* CMS 26or^T (98.8 %). Although these levels of 16S rRNA gene sequence similarity are very high, significant differences were found between strain Or1^T and these two most closely related species.

In particular, DNA–DNA hybridization experiments revealed the unique position of strain Or1^T relative to *Planococcus antarcticus* CMS 26or^T (=DSM 22276^T) and *Planococcus donghaensis* JH1^T (=DSM 14505^T). Cells were disrupted by using a French pressure cell (Thermo Spectronic) and the crude DNA lysate was purified by chromatography on hydroxyapatite as described by Cashion *et al.* (1977). DNA–DNA hybridization was carried out as described by De Ley *et al.* (1970) and Huß *et al.* (1983) by using a UV/VIS spectrophotometer equipped with a Peltier-thermostatted cell holder (Cary 100 Bio). Levels of DNA–DNA relatedness between strain Or1^T and the type strains of *Planococcus donghaensis* and *Planococcus antarcticus* were 46.0 and 18.2 %, respectively. As the threshold for defining members of the same species according to DNA–DNA relatedness is $\geqslant$70 % (Wayne *et al.*, 1987; Stackebrandt & Goebel, 1994), these results support the suggestion that strain Or1^T does not belong to either of these phylogenetically related species. Furthermore, the low DNA–DNA hybridization values between strain Or1^T and the type strains of *Planococcus donghaensis* and *Planococcus antarcticus*, despite their close relationship at the 16S rRNA gene sequence level, is in accordance with the observations of Nakagawa *et al.* (1996), who demonstrated that although *Planococcus kocurii*, *Planococcus citreus* and *Planomicrobium okeanokoites* exhibit high 16S rRNA gene sequence similarity (99 %), they can be identified as distinct species on the basis of phenotypic differences and low DNA–DNA reassociation values (15–27 %). It should be noted that in our phylogenetic analyses (Fig. 2), the above species are not as similar when compared based on more

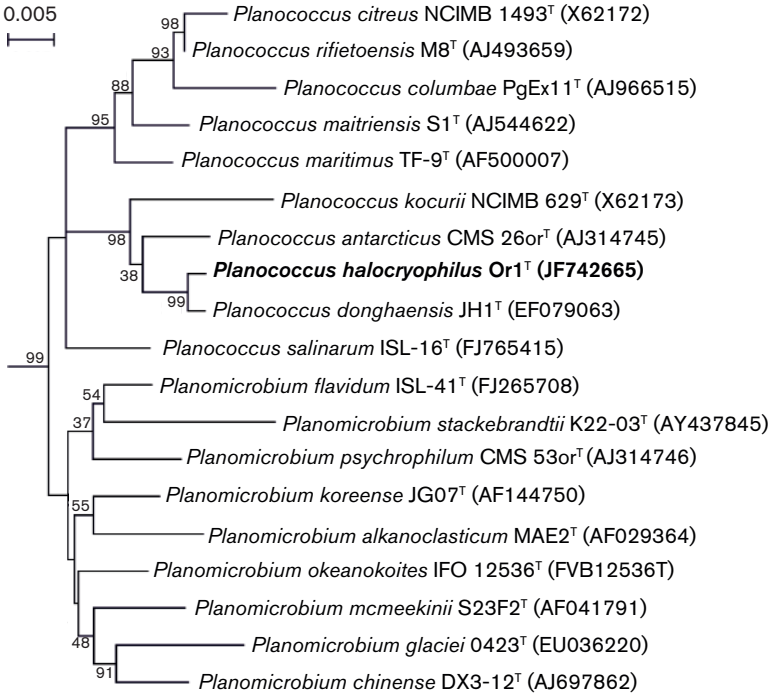

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences (1428 bp) showing the position of strain Or1^T among related taxa. Bootstrap values (expressed as percentages of 1000 replications) are shown at branch nodes. Similar topologies were recovered in the tree generated with the maximum-likelihood algorithm. The tree was rooted with *Exiguobacterium aurantiacum* NCDO 2321^T (X70316) and *Exiguobacterium sibiricum* 255-15^T (CP001022) (not shown). Bar, 0.005 substitutions per nucleotide position.

complete sequence information, yielding lower values as determined via the EzTaxon database (96.8–97.6 % 16S rRNA gene sequence similarity). Nonetheless, these data support the disparity observed between high phylogenetic similarity and genome sequence dissimilarity.

Chemotaxonomic characteristics of strain Or1^T included analyses of respiratory quinones, carried out by Dr Brian Tindall, and peptidoglycan structure, which was determined by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany. The predominant menaquinones of strain Or1^T were MK-7 (55 %), MK-8 (36 %) and MK-6 (9 %). Both MK-7 and MK-8 are included in the description of the genus *Planococcus* (Nakagawa *et al.*, 1996); however, only one recognized species, *Planococcus maritimus*, possesses small amounts of MK-6 (Table 1). The menaquinone profiles of strain Or1^T and *Planococcus maritimus* JCM 11543^T are more similar to that of *Planomicrobium koreense* than to those of other *Planococcus* species, supporting existing evidence that there is heterogeneity and/or cross-over in the properties of the genera *Planococcus* and *Planomicrobium* (Yoon *et al.*, 2003; Dai *et al.*, 2005). Peptidoglycan structure determination was carried out as described by Schleifer & Kandler (1972) with TLC followed by quantitative analysis by GC and GC-MS (320-MS Quadrupole GC-MS; Varian) according to MacKenzie (1987). The peptidoglycan of strain Or1^T was designated type A4α according to Schleifer & Kandler (1972), and type A11.33 according to the DSMZ catalogue of strains, and the cross-link peptide was L-Lys–D-Glu. Cellular fatty acid profiles were obtained from cultures of strain Or1^T harvested from TSB following 3 days of cultivation, determined previously to be the mid- to late exponential growth phase at 25 °C. Fatty acids were extracted and fatty acid methyl esters were prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990) at Keystone Laboratories, Edmonton, Canada. The fatty acids were separated by using an Agilent GC (model 6890N) and were identified by using Sherlock version 6.0 via the RTSBA6 database. The fatty acid profile of strain Or1^T comprised (each constituting $\geqslant$0.5 % of the total): saturated fatty acids $C_{16:0}$ (7.0 %) and $C_{18:0}$ (2.9 %); branched fatty acids anteiso-$C_{15:0}$ (46.2 %), iso-$C_{15:0}$ (1.7 %), anteiso-$C_{17:0}$ (18.3 %), iso-$C_{14:0}$ (1.7 %), iso-$C_{16:0}$ (2.9 %), iso-$C_{17:0}$ (2.3 %), iso-$C_{17:1}\omega10c$ (0.9 %) and iso-$C_{18:0}$ (1.2 %); unsaturated fatty acids $C_{16:1}\omega7c$ alcohol (2.1 %), $C_{16:1}\omega11c$ (4.8 %) and $C_{18:1}\omega9c$ (0.8 %); and summed feature 4 (iso-$C_{17:1}$ and/or anteiso-$C_{17:1}$; 5.2 %). This profile is similar to those of recognized *Planococcus* species, although there were differences in the proportions of some fatty acids. Table 2 presents the first compilation of all fatty acid abundance data presented in the literature for the genus *Planococcus*, with some species showing a broad range, probably due to differences in cultivation conditions and extraction procedures. The DNA G+C content was determined according the method described by Gonzalez & Saiz-Jimenez (2002), giving a value of 40.3 mol% (mean of three assays). This was in agreement with the G+C content obtained from the strain Or1^T genome sequence, calculated as [G+C]/[A+T+C+G]$\times$100 (40.5 mol%). This falls within the

Table 2. Cellular fatty acid profile of strain Or1^T compared with those of the type strains of all recognized *Planococcus* species

Strains: 1, Or1^T (data from this study); 2, *P. antarcticus* DSM 14505^T (Reddy *et al.*, 2002); 3, *P. kocurii* DSM 20747^T (Hao and Komagata, 1985; Nakagawa *et al.*, 1996; Engelhardt *et al.*, 2001; Yoon *et al.*, 2003); 4, *P. maritimus* JCM 11543^T (Yoon *et al.*, 2003; Suresh *et al.*, 2007); 5, *P. maitriensis* DSM 15305^T (Alam *et al.*, 2003; Suresh *et al.*, 2007); 6, *P. rifietoensis* DSM 15069^T (Romano *et al.*, 2003; Suresh *et al.*, 2007); 7, *P. citreus* DSM 20549^T (Hao & Komagata, 1985; Nakagawa *et al.*, 1996; Engelhardt *et al.*, 2001; Yoon *et al.*, 2003; Alam *et al.*, 2003; Suresh *et al.*, 2007); 8, *P. columbae* PgEx11^T (Suresh *et al.*, 2007); 9, *P. donghaensis* JH1^T (Choi *et al.*, 2007); 10, *P. salinarum* ISL-16^T (Yoon *et al.*, 2010). Values are percentages of the total fatty acids and only fatty acids comprising >0.5 % are shown. Ranges are shown for species for which different data are presented in the literature. −, Not detected.

Fatty acid	1	2	3*	4	5	6	7	8	9	10
Straight-chain										
$C_{14:0}$	0.5	−	−	−	0–0.9	0–6.7	0–1.2	−	−	−
$C_{15:0}$	−	14	6–13	0–3.1	0–2.5	0–7.2	0–6.7	−	1.7	−
$C_{16:0}$	7.0	4.2	1–2.7	0.8–4.5	6.8–7.2	3.0–17	0–19	1.5	6.3	−
$C_{17:0}$	−	1.0	−	0–2.5	4.5–5.3	−	0–5.5	1.8	1.3	−
$C_{18:0}$	2.9	−	0–2.2	−	0–4.0	−	0–4.9	−	−	−
Branched										
anteiso-$C_{14:0}$	−	−	−	−	−	0–7.7	−	−	−	−
iso-$C_{14:0}$	1.2	1.0	6–16	2.1–13	2.4–4.1	0–5.1	1–9.4	11	3.4	4.2
iso-$C_{15:0}$	1.7	1.3	6–14	9.5–23	2.8–5.3	0–18	0–6.7	25	4.7	2.7
anteiso-$C_{15:0}$	46	43	40–48	31–38	27–31	43–51	35–62	35	44	45
iso-$C_{16:0}$	2.9	4.0	4–11	4.1–18	9.2	5–9.3	2.9–8.6	12	9.4	7.5
iso-$C_{17:0}$	2.3	−	0–3.0	3.1–7.6	−	0–8.8	−	5	6.4	2.5
anteiso-$C_{17:0}$	18	9.5	2–14	4.4–7	6.6–7.2	5.4–7.6	0–14	4.3	16	11
iso-$C_{17:1}\omega 10c$	0.9	−	0–2.5	0.8–4.5	−	−	0–3.2	−	0.7	2.0
iso-$C_{18:0}$	1.2	−	−	0–2.1	−	−	−	−	0.4	0.5
Unsaturated										
$C_{16:1}\omega 7c$ alcohol	2.1	−	1–11	3.3–8.9	1.6–2.6	0–4.3	0–9.8	4.7	1.6	17
$C_{16:1}\omega 9c$	−	−	−	−	3.8–5.2	−	−	−	−	−
$C_{16:1}\omega 11c$	4.9	−	0–2.5	0.6–0.7	−	−	0–9.8	−	1.3	1.4
$C_{18:1}\omega 9c$	−	−	−	−	0–4.2	−	0–5.6	−	−	−
Summed feature 4†	5.2	4.2	−	1.3–3.1	−	−	−	−	1.9	6.0

*$C_{15:1}$ (double bond position not shown) also present (Nakagawa *et al.*, 1996).
†Summed feature 4 comprises iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B, which could not be separated by GC with the MIDI system.

range of DNA G+C values reported for the genus *Planococcus* (39–51.2 mol%, Table 1) (Nakagawa *et al.*, 1996; Yoon *et al.*, 2010).

The physiological, chemotaxonomic and systematic molecular characteristics of strain Or1^T are similar to those of recognized species of the genus *Planococcus* (Nakagawa *et al.*, 1996; Yoon *et al.*, 2010; Table 1) and therefore it should be placed in the genus *Planococcus*. Strain Or1^T could be differentiated from recognized *Planococcus* species based on growth characteristics, namely extreme sub-zero growth and halotolerance, hydrolysis and acid production from various substrates, and its phylogenetic position, which is supported by DNA–DNA hybridization analysis. Therefore, strain Or1^T is considered to represent a novel species of the genus *Planococcus*, for which the name *Planococcus halocryophilus* sp. nov. is proposed.

As strain Or1^T is able to grow at sub-zero temperatures, it is probably an active member of the active-layer microbial community. Its high halotolerance probably provides the adaptive advantage for surviving within pockets of liquid brine (Deming, 2002), one of the only sources of liquid water at sub-zero temperatures in active-layer and permafrost soils. Although several recognized *Planococcus* species are psychrotolerant, strain Or1^T appears to have the broadest growth temperature and salinity range, both necessary traits allowing growth in extreme cryoenvironments.

Description of *Planococcus halocryophilus* sp. nov.

Planococcus halocryophilus [ha.lo.cryo.phi′lus. Gr. n. *hals halos* salt; Gr. n. *kruos* icy cold; N.L. adj. *philus -a -um* (from Gr. adj. *philos -ê -on*) friend, loving; N.L. masc. adj. *halocryophilus* salt and cold loving, referring to the sub-zero growth ability and salt-tolerant features of this species and the environment within which it can survive].

Cells are Gram-positive, aerobic, non-spore-forming, motile cocci, 0.8–1.2 μm in diameter, and occur singly or

in pairs. Colonies on TSA are bright orange, opaque, smooth, glistening, of low convexity in the centre, circular and 2.0–3.0 mm in diameter after 3 days of cultivation at 25 °C. Psychrotolerant; grows at −10 to 37 °C (optimally at 25 °C). Halotolerant; grows in the presence of 0–19 % NaCl, but high salinity is not essential for growth. The growth pH range is 6.0–11.0, with optimal growth at pH 7.0–8.0. Utilizes glucose, rhamnose, fructose, glycerol, mannose, galactose, maltose, glucosamine, glutamine, ribose, trehalose, cellobiose, mannitol, sucrose, lactose, lactic acid, pectin, gelatin, dextrin, alanine, serine, arginine, acetic acid, glutamic acid and gluconic acid. Acid is produced from glucose, mannitol, sucrose, rhamnose and L-arabinose. Positive for Tween 80 and gelatin hydrolysis, β-galactosidase, citrate utilization, arginine decarboxylase, lysine decarboxylase, hydrogen sulfide and indole production. Resistant to tetracycline, polymyxin B, penicillin G and erythromycin, but sensitive to troleandomycin, rifamycin SV, mynocycline, lincomycin, vancomycin, nalidixic acid, amoxicillin and streptomycin. The cell-wall peptidoglycan is of type L-Lys–D-Glu, and the predominant menaquinones are MK-7, MK-8 and MK-6. The predominant fatty acids (>10 % of the total) are anteiso-$C_{15:0}$ and anteiso-$C_{17:0}$.

The type strain, Or1^T (=DSM 24743^T=JCM 17719^T), was isolated from permafrost active-layer soil at Eureka, Ellesmere Island, in the Canadian high Arctic. The DNA G+C content of the type strain is 40.5 mol%.

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