The genus *Psychrobacter* was first described by Juni & Heym (1986), and at the time of writing comprises 24 recognized species: *Psychrobacter immoblis* (Juni & Heym, 1986), *P. frigidicola*, *P. urativorans* and *P. phenylpyruvicus* (Bowman et al., 1996), *P. glacincola* (Bowman et al., 1997), *P. pacificensis* (Maruyama et al., 2000), *P. proteolyticus* (Denner et al., 2001), *P. submarinus* and *P. marincola* (Romanenko et al., 2002), *P. faecalis* (Kämpfer et al., 2002), *P. pulmonis* (Vela et al., 2003), *P. jeotgali* (Yoon et al., 2003), *P. luti* and *P. fozii* (Bozal et al., 2003), *P. okhotakensis* (Yumoto et al., 2003), *P. maritimus* and *P. arenosus* (Romanenko et al., 2004), *P. vallis* and *P. aquaticus* (Shivaji et al., 2005), *P. nivimaris* (Heuchert et al., 2004), *P. alimentarius* (Yoon et al., 2005a), *P. cibarius* (Jung et al., 2005) and *P. aquimaris* and *P. namhaensis* (Yoon et al., 2005b). In the study, we report on the detailed taxonomic characterization of a *Psychrobacter*-like bacterial strain, SW-238T, which was isolated from sea water of the South Sea in Korea.

Strain SW-238T was isolated by the standard dilution plating technique on marine agar 2216 (MA; Difco) at 30 °C. The type strains of 10 *Psychrobacter* species were used as reference strains for DNA–DNA hybridization: *P. nivimaris* DSM 16093T, *P. proteolyticus* DSM 13887T, *P. vallis* DSM 15337T, *P. aquaticus* DSM 15339T, *P. marincola* DSM 14160T and *P. submarinus* DSM 14161T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany, *P. alimentarius* JG-100T, *P. aquimaris* SW-210T and *P. namhaensis* SW-242T were obtained from the studies of Yoon et al. (2005a, b) and *P. pacificensis* IFO 16270T was obtained from the Institute for Fermentation, Osaka (IFO), Osaka, Japan. Cell morphology was examined by light microscopy (Nikon E600) and transmission electron microscopy. The presence of flagella was determined by transmission electron microscopy using cells from exponentially growing cultures. Gram reaction was determined by using the bioMérieux Gram Stain kit according to the manufacturer’s instructions. The pH range for growth was determined in marine broth 2216 (MB; Difco) adjusted to various pH values (initial pH 4.5–10.5 at intervals of 0.5 pH units). The pH was adjusted prior to sterilization by the addition of HCl and Na₂CO₃. Growth in the absence of NaCl was investigated in trypticase soy broth lacking NaCl. Growth at various NaCl concentrations was investigated in MB or trypticase soy broth (Difco). Growth at various temperatures (4–45 °C) was measured on MA. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber on MA and on MA supplemented with nitrate, both of which had been prepared anaerobically using nitrogen. Catalase and oxidase activities and hydrolysis of casein, starch, and Tweens 20, 40, 60 and 80 were determined as described by Cowan & Steel (1965). Hydrolysis of
aesculin and nitrate reduction were determined as described by Lanyi (1987). Hydrolysis of gelatin and urea was determined as described by Lanyi (1987) with a modification that artificial sea water was used instead of distilled water. The artificial sea water contained (per litre of distilled water) 23.6 g NaCl, 0.64 g KCl, 4.53 g MgCl₂.6H₂O, 5.94 g MgSO₄.7H₂O and 1.3 g CaCl₂.2H₂O (Bruns et al., 2001). Hydrolysis of hypoxanthine, tyrosine and xanthine was investigated on MA with the substrate concentrations described by Cowan & Steel (1965). H₂S production was tested as described by Bruns et al. (2001). Acid production from carbohydrates was determined as described by Leifson (1963). Enzyme activity was determined by using the API ZYM system (bioMérieux). Utilization of substrates as sole carbon and energy sources was tested according to the method of Yurkov et al. (1994). Requirements for yeast extract and vitamins for growth were investigated in liquid medium (Yurkov et al., 1994), omitting yeast extract and vitamin B₁₂ but supplementing with 0.1 % (w/v) acetate as the sole carbon and energy sources. Yeast extract and vitamins were added to the medium at the following concentrations (per litre): yeast extract (0.005 g), p-aminobenzoic acid (1 mg), biotin (10 μg), thiamine hydrochloride (1 mg) and vitamin B₁₂ (1 mg). Susceptibility to antibiotics was tested on MA plates using antibiotic discs containing the following compounds: polymyxin B, 100 U; streptomycin, 50 μg; penicillin G, 20 U; chloramphenicol, 100 μg; ampicillin, 10 μg; cephalothin, 30 μg; gentamicin, 30 μg; novobiocin, 5 μg; erythromycin, 15 μg; tetracycline, 30 μg. Other physiological tests were performed with the API 20E system (bioMérieux).

Cell biomass for DNA extraction and for respiratory lipoquinone analysis was obtained from cultivation in MB at 30 °C. Chromosomal DNA was isolated and purified according to the method described by Yoon et al. (1996), with the modification that RNase T1 was used together with RNase A to minimize contamination with RNA. The 16S rRNA gene was amplified by PCR using two universal primers as described by Yoon et al. (1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described by Yoon et al. (2003). Respiratory lipoquinones were analysed by using the method of Komagata & Suzuki (1987), using reversed-phase HPLC. For fatty acid methyl ester analysis, cell mass was harvested from MA plates after incubation for 3 days at 30 °C. The fatty acid methyl esters were extracted and prepared according to the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The DNA G+C content was determined by the method of Tamaoka & Komagata (1984) with a modification that DNA was hydrolysed and the resultant nucleotides were analysed by reversed-phase HPLC. DNA–DNA hybridization was performed fluorometrically by the method of Ezaki et al. (1989) using photobiotin-labelled DNA probes and microdilution wells. Hybridization was performed with five replications for each sample. The highest and lowest values obtained in each sample were excluded and the means of the remaining three values were quoted as DNA–DNA relatedness values.

The almost complete 16S rRNA gene sequence of strain SW-238ᵀ, comprising 1495 nt (approximately 96 % of the Escherichia coli 16S rRNA gene sequence), was analysed. Comparative 16S rRNA gene sequence analyses revealed
Table 1. Differential phenotypic characteristics of *Psychrobacter celer* sp. nov. and some *Psychrobacter* species

Species: 1, *P. celer* sp. nov. (*n* = 1 strain); 2, *P. pacificensis* (*n* = 6; data from Maruyama et al., 2000; this study); 3, *P. nivimaris* (*n* = 1; Heuchert et al., 2004; this study); 4, *P. proteolyticus* (*n* = 1; Denner et al., 2001; Bozal et al., 2003; this study); 5, *P. aquimarina* (*n* = 1; Yoon et al., 2005b); 6, *P. namhaensis* (*n* = 1; Yoon et al., 2005); 7, *P. vallis* (*n* = 1; Shivaji et al., 2005); 8, *P. aquaticus* (*n* = 1; Shivaji et al., 2005); 9, *P. alimentarius* (*n* = 2; Yoon et al., 2005a); 10, *P. submarinus* (*n* = 1; Romanenko et al., 2002); 11, *P. marincola* (*n* = 1; Romanenko et al., 2002). +, Positive; −, negative; ND, not determined; W, weakly positive; V, variable; data in parentheses are for the type strain. All species are positive for catalase, oxidase and sensitivity to gentamicin (10\(\mu\)g per disc) and polymyxin B (300 U per disc). All species are negative for Gram-stain, spore formation, motility, hydrolysis of aesculin and starch, acid production from maltose and D-mannitol, utilization of D-glucose and arginine dihydrolase, lysine decarboxylase and ornithine decarboxylase activities (in the case of hydrolysis of starch, acid production from maltose and sensitivity to gentamicin and polymyxin B by *P. pacificensis*, this holds true for the type strain).

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<td>Cocci or short rods</td>
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that strain SW-238<sup>T</sup> was phylogenetically affiliated to the genus *Psychrobacter*. In the neighbour-joining tree based on 16S rRNA gene sequences, strain SW-238<sup>T</sup> fell within the radiation of the cluster comprising *Psychrobacter* species (Fig. 1). Levels of 16S rRNA gene sequence similarity between strain SW-238<sup>T</sup> and the type strains of recognized *Psychrobacter* species ranged from 94·8 to 97·9 % (Fig. 1). Sequence similarities to other species included in the phylogenetic analysis were below 92·6 % (Fig. 1).

Morphological, cultural, physiological and biochemical characteristics of strain SW-238<sup>T</sup> are given under the species description below or are shown in Table 1, together with those of some phylogenetically related *Psychrobacter* species. Strain SW-238<sup>T</sup> did not require yeast extract or vitamins for growth in minimal salt medium, but it grew better when yeast extract was added to the medium. The predominant respiratory lipoquinone found in strain SW-238<sup>T</sup> was ubiquinone-8 (Q-8) at a peak area ratio of approximately 85 %. The major components (>1 %) of the fatty acids detected in strain SW-238<sup>T</sup> were C<sub>18 : 1</sub>ω9c (62·3 %), C<sub>17 : 1</sub>ω8c (11·0 %), C<sub>18 : 0</sub>ω9c and/or anteiso-C<sub>18 : 0</sub> (3·1 %), C<sub>16 : 1</sub>ω7c and/or iso-C<sub>15 : 0</sub> ω2-OH (2·9 %), C<sub>19 : 0</sub>ω9c and/or C<sub>19 : 1</sub>ω11c (2·8 %), C<sub>12 : 0</sub> ω3-OH (2·8 %), C<sub>12 : 0</sub> (2·5 %), C<sub>10 : 0</sub> (1·5 %), C<sub>13 : 0</sub> ω3-OH and/or iso-C<sub>15 : 1</sub> (1·5 %), iso-C<sub>16 : 1</sub> and/or C<sub>14 : 0</sub> ω3-OH (1·5 %), C<sub>18 : 0</sub> (1·5 %), C<sub>16 : 0</sub> ω3-OH (1·2 %) and C<sub>16 : 1</sub>ω9c (1·2 %). This fatty acid profile was similar to those of other *Psychrobacter* species shown previously, although there were differences in the proportions of some fatty acids that might have been caused by different cultivation conditions (e.g. Maruyama *et al*., 2000; Romanenko *et al*., 2002; Bozal *et al*., 2003; Yoon *et al*., 2005a, b). The DNA G + C content of strain SW-238<sup>T</sup> was 47·6 mol%. These chemotaxonomic properties support the result of monothetic phylogenetic classification that strain SW-238<sup>T</sup> may represent a member of the genus *Psychrobacter*. Strain SW-238<sup>T</sup> was distinguishable from some phylogenetically related *Psychrobacter* species based on differences in phenotypic characteristics (Table 1). Levels of DNA–DNA relatedness between strain SW-238<sup>T</sup> and the type strains of 10 *Psychrobacter* species that showed 16S rRNA gene sequence similarity values of greater than 97 % to strain SW-238<sup>T</sup> were in the range 6–21 %. The genetic distinctiveness, together with differential phenotypic properties and 16S rRNA gene sequence similarity data, was sufficient to categorize strain SW-238<sup>T</sup> as representing a novel *Psychrobacter* species (Wayne *et al*., 1987; Stackebrandt & Goebel, 1994). Therefore, on the basis of the data presented, strain SW-238<sup>T</sup> should be placed in the genus *Psychrobacter* as a member of a novel species, for which the name *Psychrobacter celer* sp. nov. is proposed.

**Description of *Psychrobacter celer* sp. nov.**

*Psychrobacter celer* (ce’ler. L. masc. adj. *celer* rapid, pertaining to fast growth).

Cells are cocccobacilli (0·9–1·1×1·2–2·0 μm). Colonies are circular, smooth, glistening, raised, cream-coloured and

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<td>C&lt;sub&gt;16 : 1&lt;/sub&gt;ω7c</td>
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The type strain, SW-238T (KCTC 12313T = JCM 12601T), was isolated from sea water of the South Sea in Korea.

Acknowledgements

This work was supported by the 21C Frontier programme of Microbial Genomics and Applications (grant MG02-0401-001-1-0-0) from the Ministry of Science and Technology (MOST) of the Republic of Korea.

References


