**Psychrobacter aestuarii** sp. nov., isolated from a tidal flat sediment

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A Gram-negative-staining, non-motile, non-spore-forming and strictly aerobic bacterial strain, SC35T, was isolated from tidal flat sediment collected from the South Sea, Korea, and subjected to a taxonomic study using a polyphasic approach. The organism grew optimally at 20–30 °C and with 1–2 % (w/v) NaCl. Strain SC35T contained ubiquinone-8 as the predominant respiratory lipoquinone and C18 : 1 w9c as the major fatty acid. The DNA G + C content was 48.5 mol %. A phylogenetic tree based on 16S rRNA gene sequences showed that strain SC35T formed a lineage within the genus *Psychrobacter* (94.3–96.5 % sequence similarity), forming a distinct branch in a clade also containing *Psychrobacter pacificensis* NIBH P2K6T and *Psychrobacter celer* SW-238T. On the basis of phenotypic and phylogenetic data, strain SC35T (=KCTC 22503T =JCM 16343T) was placed in the genus *Psychrobacter* as the type strain of a novel species, for which the name *Psychrobacter aestuarii* sp. nov. is proposed.

The genus *Psychrobacter* was first described by Juni & Heym (1986) as a group of psychrotolerant, aerobic, Gram-negative, non-motile, oxidase-positive cocccobacilli belonging to the class *Gammaproteobacteria*. At the time of writing, the genus comprises 30 recognized species (http://www.bacterio.cict.fr/s/psychrobacter.html). Members of the genus *Psychrobacter* have been isolated from a variety of low-temperature marine environments, including Antarctic sea ice, ornithogenic soil and sediments, the stomach contents of the Antarctic krill *Euphausia*, seawater (north-western Pacific Ocean, 300 m depth), the deep sea and the internal tissues of marine ascidian and crustacean species (Bowman et al., 1996, 1997; Maruyama et al., 2000; Romanenko et al., 2002, 2004; Yumoto et al., 2003; Shivaji et al., 2004, 2005; Bozal et al., 2003; Heuchert et al., 2004; Denner et al., 2001; Romanenko et al., 2009), low-temperature Arctic permafrost (Bakermans et al., 2006), moderate-temperature marine environments (Yoon et al., 2005b, 2005c) and H2O2-containing wastewater (Yumoto et al., 2009). Other sources of members of the genus *Psychrobacter* include pigeon faeces, fish, processed meat and poultry products, fermented seafood and infected lamb (González et al., 2000; Juni & Heym, 1986; Kämpfer et al., 2002; Vela et al., 2003; Yoon et al., 2003, 2005a; Jung et al., 2005). In this study, we report on the detailed taxonomic characterization of a *Psychrobacter*-like bacterial strain, SC35T.

Strain SC35T was isolated from the tidal flat sediment of Suncheon Bay (34° 52’ N 127° 30’ E), Republic of Korea, using the standard dilution plating technique. Isolation was achieved using marine agar (MA; Difco) at 20 °C for 7 days. The isolate was routinely cultured on MA and maintained at −80 °C as a suspension in marine broth (MB; Difco) containing glycerol (20 %, w/v).

Bacterial DNA preparation and PCR amplification and sequencing of 16S rRNA gene were carried out as described by Chun & Goodfellow (1995). The resultant sequence of strain SC35T was aligned manually against 16S rRNA gene sequences from type strains of the genus *Psychrobacter* obtained from the GenBank database. Phylogenetic trees were inferred from the regions available for all sequences (positions 38–1450; *Escherichia coli* numbering system) using neighbour-joining (Saitou & Nei, 1987) and the Fitch–Margoliash (Fitch & Margoliash, 1967) methods. Evolutionary distance matrices were generated according to Jukes & Cantor (1969). The resultant neighbour-joining topology was evaluated by bootstrap analyses (Felsenstein,
based on 1000 resamplings. Alignment and phylogenetic analyses were carried out using the PHYLIP program (http://plaza.snu.ac.kr/~jchun/phydit/) and PAUP 4.0 (Swofford, 1998) as described by Chun et al. (2000).

The preliminary 16S rRNA gene sequence comparison indicated that strain SC35\(^{T}\) was closely related to the genus *Psychrobacter*. The newly determined sequence was then aligned manually with those of representatives of the genus *Psychrobacter*. Strain SC35\(^{T}\) showed 16S rRNA gene sequence similarity values of 96.5\% with *Psychrobacter pacificensis* NIBH P2K6\(^{T}\), 96.3\% with *P. celer* SW-238\(^{T}\) and *P. nivimaris* 88/2-7\(^{T}\) and 94.3–95.6\% with other type strains of the genus *Psychrobacter*. The neighbour-joining tree (Fig. 1) shows that strain SC35\(^{T}\) formed a distinct branch with the clade comprising *P. pacificensis* NIBH P2K6\(^{T}\) and *P. celer* SW-238\(^{T}\), which was also found in the Fitch–Margoliash tree (data not shown). On the basis of the 16S rRNA gene sequence similarity data and the results of the phylogenetic analysis, it is clear that strain SC35\(^{T}\) belongs to the genus *Psychrobacter* (Wayne et al., 1987). As 16S rRNA gene sequence similarities between strain SC35\(^{T}\) and recognized species of the genus *Psychrobacter* were below the level indicative of separation at the species level (97\%; Stackebrandt & Goebel, 1994), strain SC35\(^{T}\) represents a novel genomic species of the genus *Psychrobacter*.

Growth on various standard bacteriological media was tested for nutrient agar (NA), tryptic soy agar (TSA) and R2A agar (all from Difco) according to the manufacturer’s instructions. Cells grown at 30 °C for 2 days were observed using differential interference microscopy (BX50; Olympus) and scanning electron microscopy (S-4800; Hitachi). Cells

![Fig. 1. Neighbour-joining phylogenetic tree based on nearly complete 16S rRNA gene sequences showing relationships between strain SC35\(^{T}\) and representatives of related taxa. Bootstrap values (>50\%) based on 1000 resamplings are shown at branch nodes. Bar, 0.01 substitutions per nucleotide position.](image-url)
of strain SC35^T grown on MA at 30 °C for 2 days were used for the physiological and biochemical tests. Motility was examined by observing cells in wet mounts using a phase-contrast microscope (DS-Fi1; Nikon). Growth with 0–10 % (w/v) NaCl, in increments of 1.0 %, was investigated in tryptic soy broth (TSB; Difco) prepared according to the formula of the medium except that NaCl was excluded. The pH range for growth was determined in ZoBell liquid medium (ZoBell, 1941; 15 g Bacto agar, 5 g Bacto peptone, 1 g yeast extract, 0.1 g ferric citrate, 1 l distilled water) adjusted to pH 3–11 in increments of 1 pH unit by the addition of HCl or NaOH and sterilized by filtration. Growth at 4–50 °C and in an anaerobic chamber (CO_2/H_2/N_2, 10 : 10 : 80; Sheldon Manufacturing) were determined on MA, using incubation times of up to 1 week. Catalase and oxidase activities were determined using 3 % (v/v) hydrogen peroxide and Kovacs' reagent (Kovacs, 1956), respectively. Acid production from L-arabinose, D-galactose, D-glucose, lactose and L-rhamnose were tested as described by Yamaguchi & Yokoe (2000). Nitrate reduction was tested on MB medium. H_2S production was determined on Kligler iron agar (Difco). Hydrolysis of carboxymethylcellulose (0.5 %, w/v), casein (2 % skim milk, w/v), egg yolk (10 %, w/v), starch (0.2 %, w/v) and Tweens 20, 40 and 80 (1 %, w/v) was tested as described by Smibert & Krieg (1994) using MA as the basal medium. Deamination of L-phenylalanine was examined with the method of Richard & Kiredjian (1995). Hydrolysis of L-tyrosine (0.5 %, w/v) and xylan (1 %, w/v) was tested using MA as the basal medium (Barrow & Feltham, 1993). DNase activity was determined on DNase test agar (Difco). Other biochemical tests and enzymic activities were performed using the API 20E, API 20NE and API ZYM systems (bioMérieux) and the GN2 MicroPlate system (Biolog) according to the manufacturers’ instructions. Antibiotic resistance was determined by the disc diffusion method using commercial antibiotic-impregnated discs (BBL Becton Dickinson) on TSA at 30 °C. The results were interpreted according to published guidelines (CLSI, 2003) after 5 days of incubation.

Cells of strain SC35^T were strictly aerobic, Gram-negative-staining, motile coccobacilli (see Supplementary Fig. S1, available in IJSEM Online). Colonies grown on MA at 30 °C for 2 days were circular, convex and smooth with entire margins, approximately 1.0–2.0 mm in diameter (pH 7). The strain grew well on MA, TSA and NA and grew slightly on R2A. On MA, strain SC35^T was able to grow at 4–37 °C. The detailed results of the physiological and biochemical analyses are given in Table 1 and the species description. Table 1 also shows that there were several phenotypic characters that differentiated strain SC35^T from the type strains of phylogenetically related species.

Cellular fatty acids of strain SC35^T grown on MA at 30 °C for 3 days were prepared in duplicate and analysed as methyl esters by GLC according to the instructions of the Microbial Identification System (MIDI, 1999). For G+C content calculations, genomic DNA was prepared in duplicate and analysed by the thermal denaturation method of Marmur & Doty (1962). Isoprenoid quinone analysis was performed by using reversed-phase TLC according to Collins (1985).

The cellular fatty acid profiles of strain SC35^T and its two closest phylogenetic neighbours are presented in Supplementary Table S1. The predominant fatty acid of strain SC35^T was C_{18:1}ω9c (48.8 %), which was similar to that of other Psychrobacter species except for Psychrobacter cryohalolentis (C_{18:1}ω7c) and Psychrobacter arcticus (C_{18:0}) (Bakermans et al., 2006). The DNA G+C content of strain SC35^T was 48.5 ± 0.4 mol%, which was a similar value to that found for other species of the genus Psychrobacter. The quinone analysis revealed a spot that corresponded to ubiquinone-8.

The phylogenetic, chemotaxonomic and phenotypic data clearly indicate that strain SC35^T represents a novel genomic species within the genus Psychrobacter, for which the name Psychrobacter aestuarii sp. nov. is proposed.

Description of Psychrobacter aestuarii sp. nov.

Psychrobacter aestuarii (aes.tu’a.ri.i. L. gen. n. aestuarii of the tidal flat, from where the type strain was isolated).

Cells are Gram-negative-staining, strictly aerobic, non-motile cocccobacilli, 0.5–0.8 x 0.7–1.6 μm. Grows best on media such as MA, TSA and NA and weakly on R2A agar. Colonies on MA are cream-coloured, circular, convex, smooth and opaque with entire margins and approximately 1.0–2.0 mm in diameter after 2 days at 30 °C (pH 7). Growth occurs with 0–4 % (w/v) NaCl (optimum 1–2 %), at pH 7–9 (optimum pH 7–8) and at 4–37 °C (optimum 20–30 °C). Oxidase- and catalase-positive. Does not produce acetoin, H_2S, indole, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase or urease. Does not reduce nitrate to nitrite. Hydrolyses aesculin, Tweens 20, 40 and 80 and tyrosine, but not carboxymethylcellulose, casein, DNA, egg yolk, aesculin, gelatin, starch or xylan. Utilizes acetate, citrate and malate. Does not produce acid from L-arabinose, D-galactose, D-glucose, lactose or L-rhamnose. With API ZYM, only positive for leucine arylamidase. With Biolog GN2, utilizes acetic acid, cis-aconitic acid, L-alanine, γ-aminobutyric acid, L-asparagine, bromosuccinic acid, α-cyclodextrin, L-glutamic acid, glycogen, β-hydroxybutyric acid, p-hydroxyphenylacetic acid, α-ketoglutaric acid, DL-lactic acid, L-leucine, pyruvic acid methyl ester, succinic acid monomethyl ester, L-phenylalanine, L-proline, L-pyroglutamic acid and succinic acid and weakly utilizes L-alanyl glycine, alaninamide, D-alanine, 2-aminoethanol, citric acid, L-ornithine, phenylethylamine, quinic acid and Tweens 40 and 80; does not use the other substrates. Sensitive to (μg per disc, unless otherwise indicated): amikacin (30), ampicillin (10), gentamicin (10), kanamycin (30), penicillin (10 U) and streptomycin (10), but resistant
Table 1. Differential phenotypic characteristics of strain SC35T and related species of the genus Psychrobacter

Taxa: 1, strain SC35T; 2, P. pacificensis (n=3; data from Maruyama et al., 2000; this study); 3, P. celer KCTC 12313T (n=1; Yoon et al., 2005c; this study); 4, P. jeotgalii (n=2; Yoon et al., 2003); 5, P. salsus (n=5; Shivaji et al., 2004); 6, P. submarinus DSM 14161T (n=1; Romanenko et al., 2002); 7, P. marincola DSM 14160T (n=1; Romanenko et al., 2002). +, Positive; w, weakly positive; v, variable; −, negative; ND, not determined. Data in parentheses are for the type strain.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<td>Anaerobic growth</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<td>Urease</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Tryptophan deaminase</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Growth with NaCl (%)</td>
<td>0</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<td>10</td>
<td>−</td>
<td>−</td>
<td>+</td>
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<td>+</td>
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<td>+</td>
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<td>15</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>ND</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>(−)</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Tween 80</td>
<td>+</td>
<td>(−)</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>+</td>
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<td>Acid production from: 1-Arabinose</td>
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<td>(−)</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>δ-Galactose</td>
<td>−</td>
<td>(+)</td>
<td>+</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>−</td>
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<tr>
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<td>(+)</td>
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<td>−</td>
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<td>Lactose</td>
<td>−</td>
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<td>−</td>
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<td>+</td>
<td>−</td>
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<td>−</td>
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<td>Utilization of: Acetate</td>
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<td>V (−)</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Citrate</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<td>−</td>
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<tr>
<td>Malate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>V (w)</td>
<td>ND</td>
<td>−†</td>
<td>−†</td>
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<tr>
<td>Enzyme activities (API ZYM) Acid phosphatase</td>
<td>−</td>
<td>(+)</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>−†</td>
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<tr>
<td>Naphthol-AS-BI-phosphohydrolase</td>
<td>−</td>
<td>(−)</td>
<td>+</td>
<td>V (w)</td>
<td>ND</td>
<td>+†</td>
<td>+†</td>
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<tr>
<td>Sensitivity to (µg per disc): Ampicillin (10)</td>
<td>+</td>
<td>(−)</td>
<td>W</td>
<td>ND</td>
<td>(+)</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Streptomycin (10)</td>
<td>+</td>
<td>(+)</td>
<td>+</td>
<td>ND</td>
<td>(+)</td>
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<td>+</td>
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<tr>
<td>Tetracycline (30)</td>
<td>−</td>
<td>(−)</td>
<td>−</td>
<td>ND</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Major fatty acids (&gt;10% of total fatty acids)</td>
<td>C16:0,9c</td>
<td>C16:1,9c</td>
<td>C18:1,9c</td>
<td>C18:1,9c</td>
<td>C18:1,9c</td>
<td>C18:1,9c</td>
<td>C18:1,9c</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>48.5</td>
<td>43–44</td>
<td>47.6</td>
<td>43–44</td>
<td>44–45</td>
<td>46.7</td>
<td>50.7</td>
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</table>

*Data from Yoon et al. (2005c).
†Data from Bakermans et al. (2006).

To chloramphenicol (30), erythromycin (15), nalidixic acid (30), polymyxin B (300 U), tetracycline (30) and vancomycin (30). The major fatty acid is C18:1ω9c.

The type strain, SC35T (=KCTC 22503T=JCM 16343T), was isolated from tidal flat sediment of the South Sea, Republic of Korea. The DNA G+C content of the type strain is 48.5 ± 0.4 mol%.

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References


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