Sejongia marina sp. nov., isolated from Antarctic seawater

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A Gram-negative, psychrotolerant, chemoheterotrophic, aerobic, non-gliding, non-motile, yellow-pigmented bacterium, designated IMCC3228^T, was isolated from coastal seawater of the Antarctic. On the basis of 16S rRNA gene sequence comparisons, the strain was most closely related to the genera *Sejongia* (95.3–96.1 %) and *Chryseobacterium* (94.2–95.9 %) in the family *Flavobacteriaceae*. Phylogenetic trees generated using several treeing algorithms based on 16S rRNA gene sequences showed that this Antarctic marine isolate formed a distinct phyletic line within the genus *Sejongia*. The DNA G+C content of the strain was 35.0 mol% and the major respiratory quinone was MK-6. Several phenotypic and chemotaxonomic characteristics, including temperature and NaCl optima for growth, oxidase activity and the proportions of major cellular fatty acids, served to differentiate the strain from the recognized species of the genus *Sejongia*. Therefore strain IMCC3228^T represents a novel species of the genus *Sejongia*, for which the name *Sejongia marina* sp. nov. is proposed. The type strain is IMCC3228^T (=KCCM 42689^T=NBRC 103143^T).

The genus *Sejongia* (Yi *et al.*, 2005) in the family *Flavobacteriaceae* (Bernardet *et al.*, 2002; Reichenbach, 1989) was proposed for two yellow-pigmented strains isolated from terrestrial samples from the Antarctic. The genus currently contains two species, *Sejongia antarctica* and *Sejongia jeonii*, which are aerobic, psychrotolerant, non-motile and non-gliding bacteria. This study focuses on the taxonomic study of a yellow-pigmented bacterial strain, designated IMCC3228^T, isolated from coastal seawater of the Antarctic. On the basis of taxonomic traits, strain IMCC3228^T represents a novel species within the genus *Sejongia*.

Strain IMCC3228^T was isolated from a seawater sample collected from the coast of King George Island, Weaver Peninsula, Antarctica (62° 14′ S 58° 47′ E). Isolation of the strain was performed using the standard dilution-plating method on marine agar 2216 (MA; Difco) kept at 8 °C for 2 months. After the optimum growth temperature of the strain had been determined, cultures were maintained routinely on MA at 15 °C and preserved as glycerol suspensions (10 %, v/v) at −75 °C.

The methods used for DNA extraction, PCR amplification and sequencing of the 16S rRNA gene were as described elsewhere (Cho & Giovannoni, 2003). The resulting almost-complete 16S rRNA gene sequence (1471 bp) of strain IMCC3228^T was aligned with the nearest relatives by using the ARB software package (Ludwig *et al.*, 2004). The levels of 16S rRNA gene sequence similarity between strain IMCC3228^T and related type species were calculated using the alignment based on the secondary structure of the 16S rRNA gene in the ARB software package. Preliminary sequence comparisons with 16S rRNA gene sequences deposited in the GenBank database (Altschul *et al.*, 1997) and on the EzTaxon server (http://www.eztaxon.org) indicated that strain IMCC3228^T was closely related to the genera *Sejongia* and *Chryseobacterium* in the family *Flavobacteriaceae*. The strain was most closely related to *S. antarctica* AT1013^T (96.1 %), *Chryseobacterium formosense* CCUG 49271^T (95.9 %), *Chryseobacterium indologenes* ATCC 29897^T (95.6 %), *Chryseobacterium soldanellicola* KCTC 12382^T (95.6 %) and *S. jeonii* AT1047^T (95.3 %). To clarify the phylogenetic position of the strain, 1091 unambiguously aligned nucleotide positions (determined from the 16S rRNA gene sequences of 18 members of the class *Bacteroidetes*) were used for phylogenetic analyses in PAUP* 4.0 beta 10 (Swofford, 2002). Phylogenetic trees were generated using the neighbour-joining (Saitou & Nei, 1987) algorithm with the Jukes–Cantor distance model (Jukes & Cantor, 1969) and also the maximum-parsimony

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IMCC3228^T is EF554366.

Transmission electron micrographs of cells of strain IMCC3228^T are available as a supplementary figure with the online version of this paper.

(Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) algorithms. The robustness of the neighbour-joining and maximum-likelihood trees was confirmed by using bootstrap analyses based on 1000 and 100 resamplings of the sequences, respectively. In all of the phylogenetic trees generated by the three treeing methods (Fig. 1), strain IMCC3228^T, *S. antarctica* AT1013^T and *S. jeonii* AT1047^T formed a monophyletic clade. This monophyletic relationship between strain IMCC3228^T and the two *Sejongia* species was also shown consistently in several ARB trees generated using different phylogenetic masks. Although the bootstrap support for this monophyletic clade was relatively low (67 % in the neighbour-joining tree), the clade was clearly separated from other genera in the family *Flavobacteriaceae*. This phylogenetic inference together with the level of 16S rRNA gene sequence similarity (Wayne *et al.*, 1987) between strain IMCC3228^T and the two recognized *Sejongia* species (<97 %) suggested that strain IMCC3228^T represented a novel species in the genus *Sejongia*.

Phenotypic and physiological characterization were carried out as described previously (Choo *et al.*, 2007) and according to standard methods (Smibert & Krieg, 1994), using MA as the basal medium at 15 °C, unless otherwise indicated. Cell morphology was examined using cultures grown aerobically on MA for 5 days. The colony morphology was checked using colonies grown on MA for 7 days and for 3 weeks. Flagellar-based motility was investigated using wet mounts made from fresh cultures grown on marine broth (MB) at 15 °C for 3 days. Gliding motility was determined by using phase-contrast microscopy with cells incubated for 17 h on microscope slides coated with MA (0.7 % agar), according to the method described by Bowman (2000). The presence of flexirubin-type pigments was investigated using the bathochromatic shift test with a 20 % (w/v) KOH solution (Bernardet *et al.*, 2002; McCammon & Bowman, 2000). Cellular pigments were extracted with acetone/methanol (1 : 1, v/v) and their absorption spectra were determined using a scanning UV/visible spectrophotometer (Optizen 2120UV; Mechasis). The growth temperature range and optimum were tested from 3 to 42 °C on MA. The pH range and optimum for growth were tested on MA adjusted to pH values ranging from 4.0 to 12.0. The NaCl concentrations tolerated and the NaCl optimum for growth were determined in NaCl-free artificial seawater medium (Choo *et al.*, 2007) supplemented with 5.0 g peptone, 1.0 g yeast extract and various concentrations of NaCl (0–15 %, w/v). The degradation of macromolecules was tested by incubating cultures at 15 °C for 4 weeks on MA containing macromolecules. The following macromolecules were tested: starch (0.2 %, w/v), casein (10 % skim milk, w/v), elastin (0.5 %, w/v), chitin (0.5 %, w/v), agar (1.5 %, w/v) and carboxymethylcellulose (0.2 %, w/v). Hydrolysis was determined by the formation of clear zones around the colonies either by non-staining or after flooding with the appropriate staining solution (Teather & Wood, 1982). Biochemical tests and carbon-source assimilation tests were carried out using API 20NE and API ZYM strips (bioMérieux) and in GN2 microplates (Biolog), according to the manufacturer's instructions and using bacterial suspensions prepared with artificial seawater medium for the inoculations. Ten different kinds of antimicrobial agents (listed in the species description) were tested using the diffusion plate method (Jorgensen *et al.*, 1999). The DNA G+C content was analysed by using HPLC (Mesbah *et al.*, 1989). Cellular fatty acid methyl esters were prepared from cultures grown on MA at 15 °C for 5 days and were analysed by the Korean Culture Center of Microorganisms (KCCM; Seoul, Republic of Korea) using the MIDI

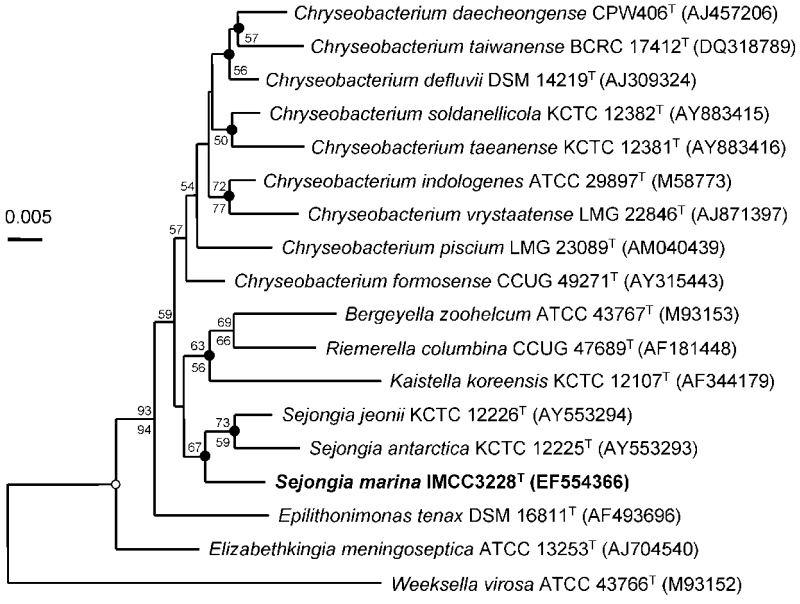

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain IMCC3228^T and representative species of the family *Flavobacteriaceae*. Bootstrap percentages (>50 %) from both the neighbour-joining tree (above nodes) and the maximum-likelihood tree (below nodes) are shown. Filled and open circles at the nodes indicate nodes recovered by all three treeing methods or by two treeing methods, respectively. Bar, 0.005 substitutions per nucleotide position.

Microbial Identification System. Respiratory quinones were analysed by the KCCM, using reversed-phased HPLC (Komagata & Suzuki, 1987).

The results of the phenotypic and biochemical analyses of strain IMCC3228^T are given in Table 1 and the species description. The major cellular fatty acid constituents (>1 %) are listed in Table 2 and the minor constituents (<1 %) are given in the species description. In summary, cells of strain IMCC3228^T were Gram-negative, psychrotolerant, aerobic, chemoheterotrophic, oxidase-negative, catalase-positive, non-motile, non-gliding, yellow-pigmented and rod-shaped (see Supplementary Fig. S1 in IJSEM Online). As shown in Tables 1 and 2, strain IMCC3228^T showed characteristics similar to those of the two recognized *Sejongia* species, including weak anaerobic growth, the absence of gliding and flagellar motility, the DNA G+C content and the presence of branched fatty acids as major fatty acid constituents. However, strain IMCC3228^T and the two *Sejongia* species could be differentiated not only on the basis of 16S rRNA gene sequence similarity (95.3–96.1 %) but also by several phenotypic properties, including growth at 30 °C, the optimum NaCl concentration for growth, oxidase activity, indole production, several enzyme activities and the proportions of several major fatty acids. Therefore strain IMCC3228^T represents a novel species of the genus *Sejongia*, for which the name *Sejongia marina* sp. nov. is proposed.

Description of *Sejongia marina* sp. nov.

Sejongia marina (ma.ri′na. L. fem. adj. *marina* of the sea, marine).

Gram-negative, non-gliding, non-motile, aerobic, psychrotolerant and chemoheterotrophic. Cells are straight or slightly curved rods with rounded ends and are 0.6–1.2 μm long and 0.5–0.8 μm wide. Colonies grown on MA for 7 days are 0.2–1.0 mm in diameter, circular, convex, shiny with entire margins, butyrous and yellow in colour. Old colonies (grown for 3 weeks) become dark yellowish and viscous. Growth occurs at 3–25 °C (optimum, 15 °C), pH 6–10 (optimum, pH 7) and with 0–3.5 % NaCl (optimum, 0.5 %). Oxidizes the following carbon substrates (Biolog GN2 microplates): α-cyclodextrin, dextrin, Tweens 40 and 80, α-D-glucose, maltose, succinic acid monomethyl ester, α-ketobutyric acid, α-ketovaleric acid, L-alaninamide, D-alanine, L-alanine, L-alanyl glycine, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-leucine, L-proline, L-serine and L-threonine. Does not oxidize the following carbon substrates: *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, D-cellobiose, i-erythritol, D-fructose, L-fucose, D-galactose, gen-

Table 1. Characteristics that differentiate strain IMCC3228^T from the two other recognized species of the genus *Sejongia*

Taxa: 1, IMCC3228^T; 2, *S. antarctica* AT1013^T; 3, *S. jeonii* AT1047^T. Data are from this study and Yi *et al.* (2005). All strains are weakly positive for anaerobic growth, positive for hydrolysis of gelatin and aesculin and positive for degradation of starch, catalase, alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and α-glucosidase. All strains are negative for the following: flagellar and gliding motility; flexirubin-type pigments; arginine dihydrolase; urease; degradation of carboxymethylcellulose, chitin and agar; β-galactosidase, α-galactosidase, β-glucuronidase, β-glucosidase, α-mannosidase, *N*-acetyl-β-glucosaminidase and α-fucosidase. +, Positive; −, negative.

Characteristic	1	2	3
Isolation source	Antarctic seawater	Antarctic soil	Antarctic moss
Cell size (length × width, μm)	0.6–1.2 × 0.5–0.8	1.0–3.1 × 0.4–0.5	1.0–3.1 × 0.4–0.5
Growth at 30 °C	−	−	+
Optimum NaCl for growth (%)	0.5	0	0
Carotenoid-pigment peaks (nm)	450 (major), 425, 477	452 (major), 480	452 (major), 480
Nitrate reduction	+	−	−
Indole production	−	+	+
Acid production from glucose	−	+	+
Enzyme activities			
α-Chymotrypsin	+	−	−
Cystine arylamidase	+	−	−
Esterase (C4)	+	−	−
Lipase (C14)	+	−	−
Oxidase	−	+	+
Trypsin	+	−	−
Degradation of:			
Casein	−	+	+
Elastin	+	−	+
DNA G+C content (mol%)	35	34	36

Table 2. Cellular fatty acid content (%) of strain IMCC3228^T and the species of the genus *Sejongia*

Strains: 1, strain IMCC3228^T; 2, *S. antarctica* AT1013^T; 3, *S. jeonii* AT1047^T. Data are from this study and Yi *et al.* (2005). Only those fatty acids amounting to at least 1 % of the total cellular fatty acid content of at least one of the species are shown. –, Not detected.

Fatty acid	1	2	3
iso-$C_{12:0}$	–	0.5	1.0
iso-$C_{13:0}$	0.6	2.5	2.9
anteiso-$C_{13:0}$	0.5	3.2	3.6
iso-$C_{14:0}$	0.8	1.5	5.0
$C_{15:0}$	–	2.6	1.5
$C_{15:0}$ 2-OH	2.9	1.9	1.9
iso-$C_{15:0}$	17.0	13.6	12.2
iso-$C_{15:0}$ 3-OH	3.2	1.0	1.3
anteiso-$C_{15:0}$	33.8	15.2	24.2
anteiso-$C_{15:1}$	–	6.6	0.0
iso-$C_{16:0}$	0.6	2.8	5.7
iso-$C_{16:0}$ 3-OH	2.9	5.1	9.0
iso-$C_{16:1}$	2.3	3.6	9.1
$C_{17:0}$ 2-OH	5.9	3.3	2.3
iso-$C_{17:0}$ 3-OH	5.0	5.6	4.4
$C_{17:0}$ cyclo	1.2	–	–
$C_{17:1}\omega 9c$	1.4	–	–
iso-$C_{17:1}\omega 9c$	8.8	21.3	8.6
anteiso-$C_{17:1}\omega 9c$	2.3	2.5	1.9
$C_{18:1}\omega 5c$	1.5	1.5	0.8
iso-$C_{19:1}$	1.5	–	–

tiobiose, *myo*-inositol, α-D-lactose, lactulose, D-mannitol, D-mannose, D-melibiose, methyl β-D-glucoside, D-psicose, D-raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose, turanose, xylitol, pyruvic acid methyl ester, acetic acid, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxybutyric acid, β-hydroxybutyric acid, γ-hydroxybutyric acid, *p*-phenylacetic acid, α-ketoglutaric acid, DL-lactic acid, malonic acid, propionic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, L-histidine, hydroxy-L-proline, L-ornithine, L-phenylalanine, L-pyroglutamic acid, D-serine, DL-carnitine, γ-aminobutyric acid, urocanic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL-α-glycerol phosphate, α-D-glucose 1-phosphate or D-glucose 6-phosphate. Susceptible to chloramphenicol, erythromycin, penicillin G, rifampicin, streptomycin, tetracycline and vancomycin but resistant to ampicillin, gentamicin and kanamycin. Other physiological and biochemical characteristics are given in Table 1. The major cellular fatty acid constituents (>1 %) are given in Table 2. Traces (<1 %) of the following fatty acids are also present: anteiso-$C_{17:1}$, iso-$C_{14:0}$, iso-$C_{16:0}$, iso-$C_{13:0}$, anteiso-$C_{13:0}$, iso-$C_{14:0}$ 3-OH, iso-$C_{19:0}$, $C_{13:0}$ 3-OH and/or iso-$C_{15:1}$, iso-$C_{14:1}$ and $C_{16:0}$ 3-OH (0.09 %). The major respiratory quinone is MK-6. The DNA G+C content is 35.0 mol%.

The type strain, IMCC3228^T (=KCCM 42689^T=NBRC 103143^T), was isolated from a sample of surface seawater from Maxwell Bay, King George Island, West Antarctica.

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