Hahella antarctica sp. nov., isolated from Antarctic seawater

Kiyoung Lee,[1] Hong Kum Lee[2] and Jang-Cheon Cho[1]

Correspondence
Jang-Cheon Cho
chojc@inha.ac.kr

[1]Division of Biology and Ocean Sciences, Inha University, Yonghyun-Dong, Incheon 402-751, Republic of Korea

[2]Polar BioCenter, Korea Polar Research Institute, KOPRI, Songdo Techno Park, Incheon 406-840, Republic of Korea

A Gram-negative, psychrotolerant, chemoheterotrophic, aerobic, cream-coloured bacterium, designated IMCC3113^T, was isolated from coastal seawater from the Antarctic. On the basis of 16S rRNA gene sequence similarity analyses, the strain was most closely related to the type strains of *Hahella chejuensis* (93.0 %) and *Hahella ganghwensis* (92.1 %) in the *Gammaproteobacteria*. Phylogenetic investigations using 16S rRNA gene sequences showed that this Antarctic marine isolate formed a robust monophyletic clade with the two *Hahella* species but constituted a distinct phyletic line in the clade. The DNA G+C content of strain IMCC3113^T was 56.4 mol% and the major respiratory quinone was Q-9. Several phenotypic and physiological characteristics, including the temperature range and NaCl optimum for growth, several enzyme activities and the cellular fatty acid composition, served to differentiate the strain from the two *Hahella* species. Therefore strain IMCC3113^T represents a novel species of the genus *Hahella*, for which the name *Hahella antarctica* sp. nov. is proposed. The type strain is IMCC3113^T (=KCCM 42675^T =NBRC 102683^T).

The genus *Hahella* (Lee *et al.*, 2001) in the family *Hahellaceae* of the *Gammaproteobacteria* currently contains two species, *Hahella chejuensis* (Lee *et al.*, 2001) and *Hahella ganghwensis* (Baik *et al.*, 2005), which were isolated from marine sediments. *H. chejuensis* has been reported as a red-pigmented bacterial species, showing antibacterial and algicidal activities associated with the presence of antibiotic prodiginines (Jeong *et al.*, 2005; Kim *et al.*, 2007). *H. ganghwensis* is a cream-coloured, chemoheterotrophic bacterium that requires sea salts for growth (Baik *et al.*, 2005). This study focuses on the taxonomic study of a non-pigmented bacterial strain, designated IMCC3113^T, isolated from Antarctic coastal seawater. On the basis of its taxonomic properties, strain IMCC3113^T is considered to represent a novel species in the genus *Hahella*.

Strain IMCC3113^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) at 20 °C for 1 month. After the optimum growth temperature of the strain had been determined, cultures were maintained routinely on MA or marine broth 2216 (MB; Difco) at 25 °C and preserved as a glycerol suspension (10 %, v/v) at −75 °C.

The methods used for DNA extraction, PCR and 16S rRNA gene sequencing have been described elsewhere (e.g. Cho & Giovannoni, 2003). The resultant almost-complete 16S rRNA gene sequence (1485 bp) of strain IMCC3113^T was aligned with its nearest neighbours by using the ARB software package (Ludwig *et al.*, 2004). The 16S rRNA gene sequence similarity between strain IMCC3113^T and other related species was calculated using the alignment based on the secondary structure of the 16S rRNA with the ARB software. On the basis of 16S rRNA gene sequence similarities, the strain was most closely related to *H. chejuensis* KCTC 2396^T (93.0 %), *H. ganghwensis* KCTC 12277^T (92.1 %) and *Oleiphilus messinensis* DSM 13489^T (90.6 %). No other bacterial species with validly published names exceeded 90 % 16S rRNA gene sequence similarity. To clarify the phylogenetic position of the strain, 1294 unambiguously aligned nucleotide positions, determined from 16S rRNA gene sequences of 28 members of the *Oceanospirillales*, were used for phylogenetic analyses in PAUP* 4.0 beta 10 (Swofford, 2002). Phylogenetic trees were generated using neighbour joining (Saitou & Nei, 1987) with Jukes–Cantor distance corrections (Jukes & Cantor, 1969), maximum parsimony (Fitch, 1971) and maximum likelihood (Felsenstein, 1981). The robustness of the neighbour-joining and maximum-likelihood trees was confirmed by bootstrap analyses based on 1000 and 100

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IMCC3113^T is EF495227.

Transmission electron micrographs of cells of strain IMCC3113^T are available as a supplementary figure with the online version of this paper.

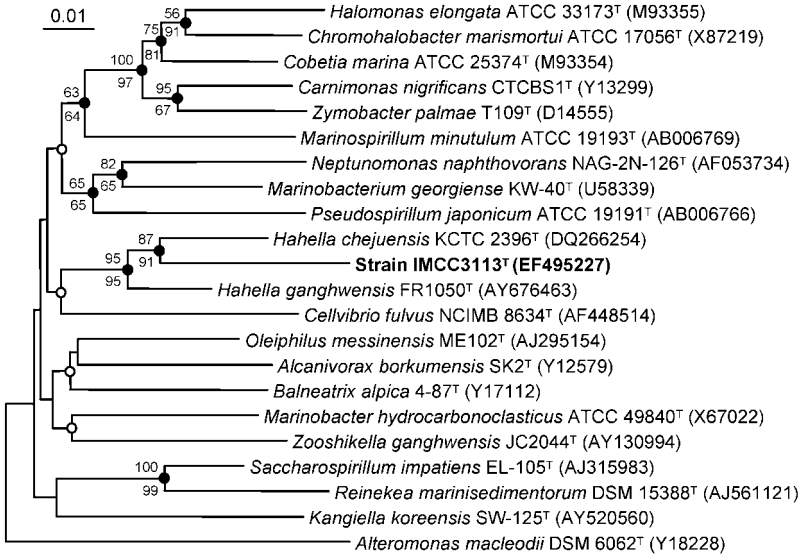

Fig. 1. Neighbour-joining phylogenetic tree, based on 16S rRNA gene sequences, showing the relationships between strain IMCC3113^T and representatives of the *Gammaproteobacteria*. Bootstrap percentages (>50 %) from both the neighbour-joining approach (above nodes) and the maximum-parsimony approach (below nodes) are shown. Filled and open circles indicate nodes recovered reproducibly by all treeing methods or by two treeing methods, respectively. Bar, 0.01 substitutions per nucleotide position.

resamplings of the sequences, respectively. In all of the phylogenetic trees generated in this study (Fig. 1), strain IMCC3113^T, *H. chejuensis* KCTC 2396^T and *H. ganghwensis* KCTC 12277^T formed a monophyletic clade with strong bootstrap support. The branching-order patterns between the three strains in the clade were recovered consistently in all of the phylogenetic trees. This monophyletic clade was clustered with *Cellvibrio fulvus* NCIMB 8634^T in the neighbour-joining and maximum-likelihood trees. However, this phylogenetic relationship was not supported by bootstrap analyses. This phylogenetic inference, coupled with 16S rRNA gene sequence similarities of

Table 1. Characteristics that differentiate strain IMCC3113^T from the type strains of the two *Hahella* species

Data for *H. chejuensis* KCTC 2396^T and *H. ganghwensis* KCTC 12277^T were taken from Lee *et al.* (2001) and Baik *et al.* (2005), respectively. All three strains are positive for catalase, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase and acid phosphatase. All three strains are negative for indole production, arginine dihydrolase, urease, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase. +, Positive; −, negative; w, weakly positive.

Characteristic	IMCC3113^T	*H. chejuensis* KCTC 2396^T	*H. ganghwensis* KCTC 12277^T
Isolation source	Antarctic seawater	Sea sediment	Tidal flat sediment
Cell size (length × width, μm)	0.9–5.9 × 0.4–0.8	1.6–9.0 × 0.5–0.7	1.0–1.5 × 0.4–0.5
Pigmentation	Cream	Red	Cream
Motility	−	+	+
Temperature range for growth (°C)	3–25	10–45	15–40
Optimum NaCl concentration (%) for growth	2	2	4–6
Anaerobic growth	w	−*	−
Nitrate reduction	+	+	−
Acid production from glucose	−	+	−
Hydrolysis of:			
Aesculin	−	+	+
Gelatin	−	+	+
Enzyme activity			
Oxidase	−	+	+
Lipase (C14)	−	w	+
Valine arylamidase	−	+	w
Cystine arylamidase	−	w	−
Naphthol-AS-BI-phosphohydrolase	−	+	+
α-Glucosidase	+	−	+
DNA G+C content (mol%)	56.4	55	44

*Data from Baik *et al.* (2005).

<97 % (Wayne *et al.*, 1987) between strain IMCC3113^T and the two *Hahella* species, suggested that the strain should be assigned to the genus *Hahella* as a representative of a novel species.

Phenotypic and physiological characterization was carried out according to a previous study (Choo *et al.*, 2007) and standard methods (Smibert & Krieg, 1994), using MA as the basal medium at 25 °C, unless otherwise specified. The cell morphology was examined using liquid cultures grown aerobically in MB for 2 days. The colony morphology was observed using colonies grown on MA for 5 days. Flagellar motility was investigated using wet mounts prepared from fresh cultures grown in MB at 25 °C for 2 days. The growth temperature range and optimum were tested from 3 to 42 °C. The pH range and optimum for growth were examined on MA adjusted to pH values from 4.0 to 12.0. The NaCl concentrations and 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). Biochemical tests and carbon-source oxidation tests were carried out using API 20NE and API ZYM strips (bioMérieux) and in GN2 microplates (Biolog), according to the manufacturers' instructions, using inoculation with bacterial suspensions in artificial seawater medium. Ten different antimicrobial agents (listed in the species description) were tested using the diffusion plate method (Jorgensen *et al.*, 1999). The DNA G+C content was determined using HPLC (Mesbah *et al.*, 1989). Cellular fatty acid methyl esters were prepared from cultures grown on MA at 25 °C for 4 days and then analysed, according to the MIDI Microbial Identification System, by the Korean Culture Center of Microorganisms (Seoul, Republic of Korea). Respiratory quinones were analysed, using reversed-phase HPLC, by the Korean Culture Center of Microorganisms (Komagata & Suzuki, 1987).

The phenotypic and biochemical characteristics determined for strain IMCC3113^T are given in Table 1 and the species description. The DNA G+C content of the strain was 56.4 mol% and the major cellular fatty acid constituents are given in Table 2. Overall, cells of strain IMCC3113^T were Gram-negative, psychrotolerant, aerobic, chemoheterotrophic, non-motile, granule-containing and irregularly rod-shaped. Transmission electron micrographs of the cells are shown in Supplementary Fig. S1 (available in IJSEM Online). The phylogenetic analyses in this study showed that strain IMCC3113^T belonged to the genus *Hahella*. However, strain IMCC3113^T and the two *Hahella* species could be differentiated from each other on the basis of the levels of 16S rRNA gene sequence similarity (92.1–93.0 %) and several phenotypic properties, including cell size, the temperature range for growth, oxidase activity, nitrate reduction, several enzyme activities and the proportions of major fatty acids, as shown in Tables 1 and 2. Therefore strain IMCC3113^T represents a novel species of the genus *Hahella*, for which the name *Hahella antarctica* sp. nov. is proposed.

Description of *Hahella antarctica* sp. nov.

Hahella antarctica (an.tarc′ti.ca. N.L. fem. adj. *antarctica* of the Antarctic, where the type strain was isolated).

Gram-negative, non-motile, aerobic, psychrotolerant and chemoheterotrophic. Cells are straight or irregular rods, 0.9–5.9 μm long and 0.4–0.8 μm wide. Colonies grown on MA at 25 °C for 5 days are 0.5–1.0 mm in diameter, circular, pulvinate with entire margins, dry, hard and cream-coloured. Growth occurs at 3–25 °C (optimum, 25 °C), pH 5–10 (optimum, pH 7) and with 0.5–5.0 % NaCl (optimum, 2.0 %). Other phenotypic and physiological characteristics are given in Table 1. Oxidizes the following carbon substrates (Biolog GN2 microplates): melibiose, acetic acid, *cis*-aconitic acid, citric acid, D-galacturonic acid, D-gluconic acid, D-glucuronic acid, β-hydroxybutyric acid, propionic acid, bromosuccinic acid, glucuronamide, L-aspartic acid, L-histidine, hydroxy-L-proline, L-leucine, L-serine and inosine. Does not oxidize the following carbon substrates: α-cyclodextrin, dextrin, Tween 40, Tween 80, α-D-glucose, maltose, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, L-arabinose, D-arabitol, cellobiose, i-erythritol, D-fructose, L-fucose, D-galactose, gentiobiose, *myo*-inositol, α-D-lactose, lactulose, D-mannitol, D-mannose, methyl β-D-glucoside, D-psicose, raffinose, L-rhamnose, D-sorbitol, sucrose, trehalose, turanose, xylitol, pyruvic acid methyl ester, succinic acid monomethyl ester, α-ketobutyric acid, α-ketovaleric acid, formic acid, D-galactonic acid lactone, D-glucosaminic acid, α-hydroxybutyric acid, γ-hydroxybutyric acid,

Table 2. Cellular fatty acid compositions (%) of strain IMCC3113^T and the type strains of the two *Hahella* species

Data for reference strains were taken from Baik *et al.* (2005). Only fatty acids amounting to at least 1 % of the total cellular fatty acid content of one or more of the strains are shown. All species were grown on MA. –, Not detected.

Fatty acid	IMCC3113^T	*H. chejuensis* KCTC 2396^T	*H. ganghwensis* KCTC 12277^T
$C_{10:0}$	2.8	–	–
$C_{12:0}$	3.2	1.3	2.4
$C_{12:0}$ 3-OH	7.1	2.5	3.8
$C_{14:0}$	1.3	2.8	2.4
$C_{16:0}$	22.5	12.5	18.1
$C_{16:0}$ *N* alcohol	1.5	6.5	7.2
$C_{16:1}\omega 9c$	0.7	3.2	8.8
$C_{17:0}$ 10-methyl	3.8	14.4	–
$C_{17:1}\omega 8c$	0.2	–	1.4
$C_{18:0}$	0.9	2.8	1.1
$C_{18:1}\omega 7c$	10.4	9.4	<1
$C_{18:1}\omega 9c$	8.2	19.8	39.0
$C_{18:3}\omega 6c$	3.6	10.7	9.0
Summed feature 3*	31.6	11.9	3.3

*Comprises $C_{16:1}\omega 7c$ and/or iso-$C_{15:0}$ 2-OH.

p-hydroxyphenylacetic acid, α-ketoglutaric acid, DL-lactic acid, malonic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, succinamic acid, L-ornithine, L-phenylalanine, L-pyroglutamic acid, D-serine, DL-carnitine, γ-aminobutyric acid, urocanic acid, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butanediol, glycerol, DL-α-glycerol phosphate, α-D-glucose 1-phosphate, D-glucose 6-phosphate, L-alaninamide, D- and L-alanine, L-alanyl glycine, L-asparagine, L-glutamic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-proline and L-threonine. Susceptible to gentamicin (10 μg), kanamycin (30 μg), penicillin G (10 μg), rifampicin (50 μg), streptomycin (10 μg) and tetracycline (30 μg), but resistant to ampicillin (10 μg), chloramphenicol (25 μg), erythromycin (15 μg) and vancomycin (30 μg). Cellular fatty acid profile is given in Table 2. Major respiratory quinone is Q-9. DNA G+C content is 56.4 mol%.

The type strain, IMCC3113^T (=KCCM 42675^T=NBRC 102683^T), was isolated from a surface seawater sample from Maxwell Bay, King George Island, western Antarctica.

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