Hahella ganghwensis sp. nov., isolated from tidal flat sediment

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A marine bacterial strain, designated FR1050^T, was isolated from a sediment sample of getbol (Korean tidal flat). Phylogenetic investigations based on 16S rRNA gene sequence analysis showed that the isolate formed a robust monophyletic clade with *Hahella chejuensis* within the γ -*Proteobacteria*. Sequence similarity between strain FR1050^T and the type strain of *Hahella chejuensis* was 94.7%. Cells were Gram-negative, aerobic, rod-shaped, motile and halophilic; optimum growth occurred at sea salt concentrations of 4–6%. The major fatty acids were C_{18:1} ω 9c (39.0%) and C_{16:0} (18.1%). The DNA G+C content was 44 mol%. The polyphasic data obtained showed that strain FR1050^T is affiliated to the genus *Hahella* but represents a novel species for which the name *Hahella ganghwensis* sp. nov. is proposed. The type strain is FR1050^T (=KCTC 12277^T=JCM 12486^T).

The genus *Hahella* was proposed by Lee *et al.* (2001) to accommodate a red-pigmented marine bacterial strain that formed a distinct phyletic line within the γ -*Proteobacteria* with low (<90%) 16S rRNA gene sequence similarity to other recognized bacterial species. The only species of the genus, *Hahella chejuensis*, was isolated from a marine sediment sample from Korea (Marado, Cheju) and produced abundant extracellular polysaccharides. During the course of a study on marine microbial diversity, a *Hahella*-like strain, designated FR1050^T, was isolated from a Korean sediment sample and was the subject of a taxonomic investigation. Based on its polyphasic properties, strain FR1050^T is considered to represent a novel species, for which the name *Hahella ganghwensis* sp. nov. is proposed.

A marine sediment sample was collected from the getbol (Korean tidal flat) of Ganghwa Island, Korea (37° 35' 31.9" N 126° 27' 24.5" E). The sample was diluted with sterilized artificial sea water (ASW; Lyman & Fleming, 1940), spread onto a plate that contained marine agar 2216 (MA; Difco) and incubated at 25 °C for 3 weeks. The isolate was routinely

cultured on MA and maintained as a glycerol suspension (20%, w/v) at –80 °C. *H. chejuensis* KCTC 2396^T cultured on MA at 30 °C was used as a reference strain.

16S rRNA gene sequence analysis of strain FR1050^T was performed using universal primers (Lane, 1991) as described by Chun & Goodfellow (1995), and an almost complete sequence was obtained (1454 bp). Phylogenetic analyses were performed using the Fitch–Margoliash (Fitch & Margoliash, 1967), maximum-likelihood (Felsenstein, 1993), maximum-parsimony (Fitch, 1971) and neighbour-joining (Saitou & Nei, 1987) methods. Evolutionary distance matrices were generated according to Jukes & Cantor (1969). The resulting neighbour-joining tree topology was evaluated by bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. Alignment and phylogenetic analyses were carried out using the jPHYDIT program (available at <http://chunlab.snu.ac.kr/jphydit/>) and PAUP 4.0 (Swofford, 1998) as described by Chun *et al.* (2000). Preliminary sequence comparison against the 16S rRNA gene sequences held in GenBank indicated that the getbol isolate belonged to the γ -*Proteobacteria*. The resultant sequence was then aligned manually based on 16S rRNA gene sequence secondary structure (Gutell, 1994) with representative sequences of the γ -*Proteobacteria* obtained from GenBank. Only unambiguously aligned nucleotide positions (1381 bp)

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain FR1050^T is AY676463.

Fig. 1. Neighbour-joining tree based on nearly complete 16S rRNA gene sequence analysis showing relationships between strain FR1050^T and members of the γ -Proteobacteria. Numbers at nodes indicate percentages of bootstrap support (>50%) based on 1000 resamplings. Solid circles indicate that the corresponding nodes (groupings) were also recovered in Fitch–Margoliash, maximum-likelihood and maximum-parsimony trees. *Helicobacter pylori* ATCC 43504^T (U01330) was used as an outgroup (not shown). Bar, 0.1 nucleotide substitution per position.

were used to construct phylogenetic trees. On the basis of 16S rRNA gene sequence similarity values, the closest relatives were *H. chejuensis* KCTC 2396^T (94.7%), *Zooshikella ganghwensis* JC2044^T (90.1%) and *Microbulbifer hydrolyticus* DSM 11525^T (90.7%). No other recognized bacterial species showed more than 90% 16S rRNA gene sequence similarity. This close relationship between strain FR1050^T and *H. chejuensis* KCTC 2396^T was also evident in the phylogenetic trees (Fig. 1). Strain FR1050^T and *H. chejuensis* formed a monophyletic clade with 100% bootstrap support and this grouping was recovered in all the phylogenetic trees employed in this study. *Z. ganghwensis* JC2044^T was recovered as a sister group to the *Hahella* clade containing strain FR1050^T in the neighbour-joining, Fitch–Margoliash and maximum-likelihood trees. However, the branching patterns of the other genera varied depending on the tree-building methods employed and were supported by relatively low bootstrap values. It is evident from phylogenetic analysis that strain FR1050^T is affiliated to the genus *Hahella* with a novel species status.

For phenotypic tests, strain FR1050^T and *H. chejuensis* KCTC 2396^T were grown on MA at 30 °C. Cellular morphologies were observed by differential interference microscopy (Nikon) and scanning electron microscopy (JEOL) using cells grown at 25 °C for 3 days. Motility was examined using wet mounts. The pH range (3–12) for growth was determined using MA. The requirement for NaCl (0–10%) and sea salts (0–11%, Sigma) for growth was tested using synthetic ZoBell medium (ZoBell, 1941; 15 g Bacto agar, 5 g Bacto peptone, 1 g yeast extract, 0.1 g ferric citrate in 1000 ml distilled water). Sea salts were not added to the synthetic medium when the growth range for NaCl concentration was tested. The getbol isolate was halophilic, requiring media containing 1–10% (w/v) artificial sea salts for growth (optimum 4–6%), and was unable to grow on ZoBell medium containing 0–10% (w/v) NaCl alone. Growth at various temperatures was examined on MA at 4–50 °C. Growth under anaerobic conditions was checked in an anaerobic chamber (1% CO₂, 10% H₂, 80% N₂; Sheldon Manufacturing) using anaerobically prepared MA,

trypticase soy agar (Difco; supplemented with 1% sea salts) and nutrient agar (Difco; supplemented with 1% sea salts). Biochemical tests were performed using the API 20NE, API 20E and API ZYM kits (bioMérieux). Strips were inoculated with a heavy bacterial suspension in ASW or AUX medium (bioMérieux) supplemented with 2% sea salts. Catalase and oxidase activities were determined using 3% (v/v) hydrogen peroxide and Kovacs reagent (Kovacs, 1956), respectively. Results from these biochemical and physiological tests are given in the species description and in Table 1. In contrast to the result given by Lee *et al.* (2001), the type strain of *H. chejuensis*, together with strain FR1050^T, showed no growth under anaerobic conditions when the media were prepared anaerobically. The reported anaerobic growth of *H. chejuensis* may have resulted from residual oxygen in the media tested by Lee *et al.* (2001). Based on the result obtained here, an emended description for the genus *Hahella* is given below.

Cellular fatty acids of strain FR1050^T and *H. chejuensis* KCTC 2396^T were analysed as methyl esters by GLC according to the instructions of the Microbial Identification System (MIDI). Fatty acid methyl esters were prepared from biomass grown on MA at 30 °C for 2 days. The DNA G+C content was determined by thermal denaturation as described by Mandel & Marmur (1968). The DNA G+C ratio of strain FR1050^T was 44 mol% and the cellular fatty acid profile is given in Table 2. Although the culture conditions for growth of strain FR1050^T and *H. chejuensis* KCTC 2396^T were identical, their fatty acid compositions were very different, especially for C_{18:1}ω7c, C_{18:1}ω9c, C_{17:0} 10-methyl and a mixture of iso-C_{16:0} 2OH and/or C_{16:1}ω7c.

Our phylogenetic analysis indicates that strain FR1050^T is affiliated to the genus *Hahella*. However, the low 16S rRNA gene sequence similarity between strain FR1050^T and *H. chejuensis* KCTC 2396^T (94.7%) indicates that the getbol isolate represents a different species. In addition, many phenotypic characteristics differentiate strain FR1050^T from *H. chejuensis* (Tables 1 and 2). Based on the polyphasic evidence presented here, the getbol isolate merits novel

Table 1. Phenotypic characteristics that differentiate strain FR1050^T from *H. chejuensis*

+, Positive; –, negative; w, weakly positive. Both strains were positive for catalase, oxidase, hydrolysis of aesculin and gelatin, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and utilization of D-glucose and mannose; negative for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, production of acetoin, trypsin, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, N-acetyl- β -glucosaminidase, α -mannosidase, α -fucosidase, indole, urease, production of H₂S, tryptophan deaminase and utilization of rhamnose, melibiose and arabinose. Data are from this study and Lee *et al.* (2001).

Characteristic	Strain FR1050 ^T	<i>H. chejuensis</i>
Pigmentation	Cream	Red
Temperature range for growth (°C)	15–40	10–45
Sea salts requirement	+	–
Nitrate reduction	–	+
Acid production from glucose	–	+
Utilization of:		
Citrate	w	–
D-Mannitol	–	+
D-Sorbitol	–	+
Inositol	–	+
Malate	+	–
Maltose	–	+
Sucrose	–	+
Enzyme activity (API ZYM):		
Lipase (C14)	+	w
Valine arylamidase	w	+
Cystine arylamidase	–	w
α -Glucosidase	+	–
β -Glucosidase	–	w
DNA G + C content (mol%)	44	55

species in the genus *Hahella*. The name *Hahella ganghwensis* sp. nov. is therefore proposed for strain FR1050^T.

Description of *Hahella ganghwensis* sp. nov.

Hahella ganghwensis (gang.hwen'sis. N.L. fem. adj. *ganghwensis* pertaining to Ganghwa Island, Republic of Korea, the geographical origin of the type strain of the species).

Gram-negative, oxidase- and catalase-positive, aerobic and halophilic. Colonies on MA are circular, smooth, convex with entire margin, slightly cream-coloured and approximately 1 mm in diameter after 5 days at 30 °C. Produces slightly brown pigment after 5 days on MA at 40 °C. Cells are motile rods, 0.4–0.5 × 1.0–1.5 µm in size. Spores are not formed. Growth occurs in 1–10 % (w/v) sea salts (optimum 4–6 %). Does not grow without sea salts. Growth occurs at pH 5–10 (optimum 7–8) and at 15–40 °C (optimum 35 °C). Utilizes N-acetylglucosamine, but not amygdalin, gluconate, caprate or adipate. Other physiological and biochemical

Table 2. Cellular fatty acid composition (%) of strain FR1050^T

Only fatty acids representing at least 1 % of the total fatty acids are presented. –, Not detected.

Fatty acid	Strain FR1050 ^T	<i>H. chejuensis</i>
Saturated fatty acids		
C _{12:0}	2.4	1.3
C _{14:0}	2.4	2.8
C _{16:0}	18.1	12.5
C _{16:0} N alcohol	7.2	6.5
C _{18:0}	1.1	2.8
Unsaturated fatty acids		
C _{16:1} ω9c	8.8	3.2
C _{17:1} ω8c	1.4	–
C _{18:1} ω7c	<1	9.4
C _{18:1} ω9c	39.0	19.8
C _{18:3} ω6c	9.0	10.7
Hydroxy fatty acid		
C _{12:0} 3-OH	3.8	2.5
Methyl fatty acid		
C _{17:0} 10-methyl	–	14.4
Summed feature*		
3 (iso-C _{16:0} 2-OH/C _{16:1} ω7c)	3.3	11.9

*Summed features are groups of two or three fatty acids that could not be separated by GLC with the MIDI system.

characteristics are given in Table 1. Major fatty acids are C_{18:1}ω9c (39.0 %) and C_{16:0} (18.1 %); the complete fatty acid profile is given in Table 2. The DNA G + C content is 44 mol%.

The type strain, FR1050^T (=KCTC 12277^T =JCM 12486^T), was isolated from sediment of getbol, the Korean tidal flat.

Emended description of the genus *Hahella*

The description of the genus *Hahella* remains that given by Lee *et al.* (2001), with the following modifications. Aerobes. Reduction of nitrate to nitrite is variable. Require sea salts or NaCl for growth.

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