Hongiella marincola sp. nov., isolated from sea water of the East Sea in Korea

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Two Gram-negative, non-motile, non-spore-forming, rod-shaped strains, SW-2^T and SW-26, were isolated from sea water of the East Sea in Korea. These organisms grew optimally at 37 °C and in the presence of 2–3 % (w/v) NaCl. They did not grow without NaCl or in the presence of > 9 % (w/v) NaCl. Strains SW-2^T and SW-26 were characterized chemotaxonomically as having MK-7 as the predominant isoprenoid quinone and iso-C_{15:0} as the major fatty acid. The DNA G + C content of strains SW-2^T and SW-26 was 43 mol%. A neighbour-joining tree based on 16S rRNA gene sequences showed that strains SW-2^T and SW-26 fell within the *Cytophaga*–*Flavobacterium*–*Bacteroides* group and formed a coherent cluster with *Hongiella* species. Strains SW-2^T and SW-26 showed a 16S rRNA gene sequence similarity value of 99.9 % and a mean DNA–DNA relatedness level of 87 % to each other. Levels of 16S rRNA gene sequence similarity between strains SW-2^T and SW-26 and the type strains of two *Hongiella* species ranged from 94.2 to 96.6 %. On the basis of phenotypic and chemotaxonomic properties and phylogenetic distinctiveness, strains SW-2^T and SW-26 should be placed in the genus *Hongiella* as members of a novel species, for which the name *Hongiella marincola* sp. nov. is proposed. The type strain is SW-2^T (=KCTC 12180^T=DSM 16067^T).

The genus *Hongiella* was proposed by Yi & Chun (2004) with three species, *Hongiella mannitolivorans*, *Hongiella halophila* and *Hongiella ornithinivorans*. However, *H. halophila* has been transferred to the genus *Algoriphagus* as *Algoriphagus halophilus* (Nedashkovskaya *et al.*, 2004). In this study, we describe two Gram-negative, slightly halophilic, rod-shaped strains, SW-2^T and SW-26, that were isolated from sea water from Hwajinpo Beach of the East Sea in Korea. As a result of 16S rRNA gene sequence comparison, these organisms were considered to be related phylogenetically to the *Cytophaga*–*Flavobacterium*–*Bacteroides* group and, in particular, to the genus *Hongiella*. Accordingly, the aim of the present work was to determine the exact taxonomic positions of strains SW-2^T and SW-26 by using a combination of phenotypic properties, detailed phylogenetic analysis based on 16S rRNA gene sequences and genomic relatedness.

Strains SW-2^T and SW-26 were isolated by the dilution-plating technique on marine agar 2216 (MA; Difco). Cell

mass of strains SW-2^T and SW-26 for isoprenoid quinone analysis and DNA extraction was obtained from cultures in marine broth 2216 (MB; Difco) at 37 °C. For fatty acid methyl ester analysis, cell mass of strains SW-2^T and SW-26 was obtained from agar plates after cultivation for 3 days at 37 °C on MA. Light microscopy (E600 microscope; Nikon) and transmission electron microscopy were used to examine cell morphology. The presence of flagella was determined by transmission electron microscopy using cells from exponentially growing cultures. Gram reaction was determined by using a bioMérieux Gram Stain kit according to the manufacturer's instructions. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber with anaerobically prepared MA. Growth in the absence of NaCl was investigated in trypticase soy broth without NaCl. Growth at various NaCl concentrations was investigated in MB. Growth at various temperatures (4–50 °C) was measured on MA. Catalase and oxidase activities and hydrolysis of casein, starch and Tween 80 were determined as described by Cowan & Steel (1965). Hydrolysis of gelatin, hypoxanthine, tyrosine and xanthine was investigated on MA plates with the substrate concentrations described previously (Cowan & Steel, 1965). Hydrolysis of aesculin and nitrate reduction were determined as described by Lanyi (1987), with the modification that artificial sea

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains SW-2^T and SW-26 and the newly determined 16S rRNA gene sequence of *Cyclobacterium marinum* DMS 745^T are AY533663, AY533664 and AY533665, respectively.

water was used instead of distilled water. Artificial sea water contained the following [(1 distilled water)⁻¹]: 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). H₂S production was tested as described by Bruns *et al.* (2001). Hydrolysis of birchwood xylan (Sigma) was determined on solid medium and in liquid marine salts basal medium (Baumann & Baumann, 1981) that contained 0.5 % (w/v) xylan as the sole carbon source. The API ZYM system (bioMérieux) was used to determine the activity of some enzymes. Acid production from carbohydrates was determined as described by Leifson (1963). Utilization of substrates as sole carbon and energy sources was tested according to the method of Baumann & Baumann (1981), using supplementation with 2 % (v/v) Hutner's mineral base (Cohen-Bazire *et al.*, 1957) and 1 % (v/v) vitamin solution (Staley, 1968). For *in vivo* pigment absorption-spectrum analysis, cultures were washed twice by centrifugation using a MOPS buffer (MOPS-NaOH, 0.01 M; KCl, 0.1 M; MgCl₂, 0.001 M; pH 7.5) and disrupted by sonication with a Branson Sonifier 450. After removal of cell debris, the absorption spectrum of the supernatant was examined on a Beckman Coulter DU800 spectrophotometer.

Chromosomal DNA was isolated and purified according to the method described previously (Yoon *et al.*, 1996), except that ribonuclease T₁ was used together with ribonuclease A. Isoprenoid quinones were extracted and analysed as described by Komagata & Suzuki (1987) using reverse-phase HPLC. For quantitative analysis of cellular fatty acid compositions, a loop of cell mass was harvested and 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 the modification that DNA was hydrolysed and the resultant nucleotides were analysed by reverse-phase HPLC.

The 16S rRNA gene was amplified by PCR using two universal primers as described previously (Yoon *et al.*, 1998). Sequencing of the amplified 16S rRNA gene and phylogenetic analysis were performed as described previously (Yoon *et al.*, 2003). 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 remaining three values were used to calculate similarity values. The DNA relatedness values quoted are the means of these three values.

The 16S rRNA gene sequences of strains SW-2^T and SW-26 determined in this study each comprised 1476 nt, representing approximately 96 % of the *Escherichia coli* 16S rRNA gene sequence. The 16S rRNA gene sequences of strains SW-2^T and SW-26 were similar (99.9 % similarity, with only two nucleotide differences). The neighbour-joining tree based on 16S rRNA gene sequences showed that strains SW-2^T and SW-26 formed a coherent cluster with *Hongiella* species (Fig. 1). Strains SW-2^T and SW-26 exhibited 16S rRNA gene sequence similarity levels of 94.2 and 96.6 %, respectively, to the type strains of *H. mannitolivorans* and *H. ornithinivorans*. Sequence similarities to all other species included in the phylogenetic analysis were below 94.9 % (Fig. 1).

Morphological, physiological and biochemical characteristics are shown in Table 1 or are given in the species description (see below). Strains SW-2^T and SW-26 did not grow in the presence of 8 and 9 % (w/v) NaCl, respectively. Strain SW-2^T produced acid from D-fructose, but strain SW-26 did not. Strains SW-2^T and SW-26 had MK-7 as the predominant isoprenoid quinone. Members of the genus *Hongiella* were also observed to have MK-7 as the predominant isoprenoid quinone (Yi & Chun, 2004). Strains SW-2^T and SW-26 had cellular fatty acid profiles that

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic positions of strains SW-2^T and SW-26 and representatives of some other related taxa. Bootstrap values (1000 replications) of 50 % or greater are shown as percentages at each node. Bar, 0.01 substitutions per nucleotide position.

Table 1. Differential phenotypic characteristics of *H. marincola*, *H. mannitolivorans* and *H. ornithinivorans*

Species: 1, *H. marincola*; 2, *H. mannitolivorans*; 3, *H. ornithinivorans*. +, Positive; –, negative; w, weakly positive; v, variable reaction. All species are Gram-negative rods with round ends and are non-motile. All of the species test positive for the following: oxidase, catalase, amylase, hydrolysis of aesculin, alkaline phosphatase, leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, valine arylamidase, trypsin, α -chymotrypsin, *N*-acetyl- β -glucosaminidase and utilization of D-cellobiose, D-glucose, D-mannose, D-raffinose, D-trehalose, D-xylose, lactose and sucrose. All of the species test negative for the following: spore formation, hydrolysis of agar and urea, H₂S production, lipase (C14), α -mannosidase, α -fucosidase, β -glucuronidase and utilization of acetate, benzoate, citrate, D-sorbitol, D-galactose and L-arabinose.

Characteristic	1	2*	3*
Pigment absorption max. (nm)	486	480	480
Nitrate reduction to nitrite	v (–)†	+	–
Gelatinase	–	+	w
Tweenase	+	–	+
Hydrolysis of casein	+	–	–
Utilization of:			
D-Fructose	+	–	+
D-Mannitol	–	+	–
Succinate	v (+)†	–	–
Enzyme activity (with API ZYM system):			
Esterase (C4)	+	–	–
Esterase lipase (C8)	+	–	–
Cystine arylamidase	+	–	–
α -Galactosidase	–	+	+
β -Galactosidase	+	+	w
α -Glucosidase	+	–	+
β -Glucosidase	w	–	+
Growth with 0% NaCl	–	+	+
Growth at 45 °C	+	–	+
DNA G+C content (mol%)	43	42	38

*Data from Yi & Chun (2004).

†The reaction of the type strain, SW-2^T, is indicated in parentheses.

contained large amounts of branched, unsaturated and hydroxy fatty acids (Table 2). These fatty acid profiles were similar to those of the type strains of two *Hongiella* species described previously (Table 2). Strains SW-2^T and SW-26 contained iso-C_{15:0} as the major fatty acid (Table 2). The DNA G+C contents of strains SW-2^T and SW-26 were the same, i.e. 43 mol%. Strains SW-2^T and SW-26 exhibited a mean DNA–DNA relatedness value of 87% when their DNA was used individually as a labelled DNA probe. This value indicates that strains SW-2^T and SW-26 are members of the same genomic species (Wayne *et al.*, 1987).

16S rRNA gene sequence analysis indicated that strains SW-2^T and SW-26 have the closest phylogenetic affiliations to the genus *Hongiella* (Fig. 1). Chemotaxonomic analysis

Table 2. Cellular fatty acid composition (%) of *H. marincola* strains SW-2^T and SW-26, *H. mannitolivorans* strain JC2050^T and *H. ornithinivorans* strain JC2052^T

Strains: 1, *H. marincola* SW-2^T; 2, *H. marincola* SW-26; 3, *H. mannitolivorans* JC2050^T (data from Yi & Chun, 2004); 4, *H. ornithinivorans* JC2052^T (data from Yi & Chun, 2004). Fatty acids representing $\geq 1\%$ of the total fatty acid content of at least one of the strains are shown.

Fatty acid	1	2	3	4
Straight-chain fatty acid:				
C _{15:0}	4.4	2.4	1.7	0.7
Branched fatty acids:				
anteiso-C _{11:0}	0	0	1.7	0
iso-C _{14:0}	1.4	1.1	1.2	0.5
iso-C _{15:0}	34.3	35.6	28.7	29.2
iso-C _{15:1} G	0	0	1.0	1.1
anteiso-C _{15:0}	4.9	4.3	4.6	4.9
iso-C _{16:0}	6.7	5.1	3.7	3.6
iso-C _{16:0} G	0	0	0	1.9
iso-C _{16:1} H	1.8	1.4	2.8	1.6
iso-C _{17:1} ω 9c	2.4	3.6	5.6	7.8
Unsaturated fatty acids:				
C _{15:1} ω 6c	2.0	1.3	2.5	1.1
C _{16:1} ω 5c	0	2.6	1.3	1.7
C _{17:1} ω 6c	3.6	2.7	3.1	2.6
Hydroxy fatty acids:				
iso-C _{15:0} 3-OH	3.4	3.3	3.6	3.4
iso-C _{16:0} 3-OH	3.6	1.9	4.0	2.3
C _{16:0} 3-OH	0.6	0	0.6	0.5
C _{17:0} 2-OH	0.8	0.6	0.7	1.6
iso-C _{17:0} 3-OH	7.8	8.8	7.9	11.1
Summed features*:				
3	13.7	15.5	13.7	14.0
4	3.1	3.3	4.8	4.9

*Summed features represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contained one or more of C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH. Summed feature 4 contained one or more of an unknown fatty acid of iso-C_{17:1} I and/or anteiso-C_{17:1} B.

confirms the result of monothetic classification based on 16S rRNA gene sequence analysis. The predominant isoprenoid quinone and cellular fatty acid profile of strains SW-2^T and SW-26 were most similar to those of *Hongiella* species, although the genus *Algoriphagus*, its phylogenetic neighbour, has a fatty acid profile similar to that of the genus *Hongiella* (Yi & Chun, 2004; Nedashkovskaya *et al.*, 2004). Accordingly, in view of these combined chemotaxonomic and phylogenetic analyses, strains SW-2^T and SW-26 are considered as members of the genus *Hongiella*. Strains SW-2^T and SW-26 are differentiated from the two *Hongiella* species by some physiological and biochemical characteristics, as shown in Table 1. The levels of 16S rRNA gene sequence similarity warrant the separation of strains SW-2^T

and SW-26 from the two *Hongiella* species described previously (Stackebrandt & Goebel, 1994). Therefore, on the basis of phenotypic and chemotaxonomic data and phylogenetic distinctiveness, strains SW-2^T and SW-26 should be classified within the genus *Hongiella* as a novel species, for which the name *Hongiella marincola* sp. nov. is proposed.

Description of *Hongiella marincola* sp. nov.

Hongiella marincola (ma.rin'co.la. L. gen. n. *maris* of the sea; L. n. *incola* inhabitant; N.L. n. *marincola* inhabitant of the sea).

Cells are rods, 0.4–0.6 × 2.0–3.0 µm on MA. Non-motile. Colonies are circular, smooth, low convex, glistening, moderately reddish-orange in colour and 1.0–2.0 mm in diameter after 3 days incubation at 37 °C on MA. Optimal growth temperature is 37 °C. Growth occurs at 10 and 45 °C, but not at 4 °C or above 46 °C. Optimal pH for growth is between 6.5 and 7.5. Growth is observed at pH 5.5, but not at pH 5.0. Optimal growth occurs in the presence of 2–3 % (w/v) NaCl. No growth occurs in the presence of >9 % (w/v) NaCl. Growth does not occur under anaerobic conditions on MA. Tyrosine is hydrolysed weakly. Hypoxanthine, xanthine and birchwood xylan are not hydrolysed. Acid is produced from D-xylose, melibiose, L-arabinose, D-melezitose, D-glucose, D-galactose, D-mannose, D-cellobiose, lactose, sucrose, maltose, D-trehalose and D-raffinose, but not from D-sorbitol, *myo*-inositol, D-ribose, D-mannitol, adonitol or L-rhamnose. Acid production from D-fructose is variable (positive for type strain). Formate is not utilized. The predominant isoprenoid quinone is MK-7. The major fatty acids are iso-C_{15:0}, iso-C_{17:0} 3-OH, iso-C_{16:0} and C_{16:1ω7c} and/or iso-C_{15:0} 2-OH. The DNA G + C content is 43 mol% (HPLC). Other phenotypic characteristics are given in Table 1.

The type strain, SW-2^T (=KCTC 12180^T=DSM 16067^T), was isolated from sea water of the East Sea of Korea. The reference strain is SW-26 (=KCTC 12181=DSM 16068).

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