Marinicola seohaensis gen. nov., sp. nov., isolated from sea water of the Yellow Sea, Korea

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A Gram-negative, non-flagellated, non-spore-forming and rod-shaped bacterial strain, SW-152^T, was isolated from sea water of the Yellow Sea in Korea, and subjected to a polyphasic taxonomic study. Strain SW-152^T grew optimally at 30 °C and in the presence of 2–3 % (w/v) NaCl. It contained MK-7 as the predominant menaquinone and iso-C_{15:0} and iso-C_{15:1} as the major fatty acids. Polar lipids detected in strain SW-152^T were phosphatidylethanolamine, diphosphatidylglycerol and unidentified lipids. The DNA G + C content was 40.3 mol%. Phylogenetic trees based on 16S rRNA gene sequences exhibited that strain SW-152^T forms a distinct evolutionary lineage within the *Cytophaga*–*Flavobacterium*–*Bacteroides* (CFB) group. Strain SW-152^T exhibited low 16S rRNA similarity levels of less than 89.4 % to members belonging to the CFB group. Phenotypic properties of strain SW-152^T differentiate it from phylogenetically related taxa. On the basis of phenotypic and phylogenetic data, strain SW-152^T (= KCTC 12312^T = JCM 12600^T) was classified in a novel genus and species, *Marinicola seohaensis* gen. nov., sp. nov.

Members of the *Cytophaga*–*Flavobacterium*–*Bacteroides* (CFB) group are widely distributed in nature, particularly marine environments (Glöckner *et al.*, 1999; Kirchman, 2002). The CFB group has been known to play an important role in carbon cycling in the marine environments (Kirchman, 2002). However, the taxonomy of the CFB group has been confused, and phylogenetic differentiation of some members belonging to the CFB group is still not clear-cut (Suzuki *et al.*, 2001; Bernardet *et al.*, 2002). Taxonomic re-evaluations and reclassifications of many taxa belonging to the CFB group have been performed recently (Nakagawa & Yamasato, 1996; Bernardet *et al.*, 1996; Johansen *et al.*, 1999; McCammon & Bowman, 2000; Barbeyron *et al.*, 2001; Suzuki *et al.*, 2001; Sakamoto *et al.*, 2002). During the last few years, developments in bacterial systematics have increased continuously the number of novel taxa assigned to the CFB group (Bowman *et al.*, 2003). In this study, we describe a slightly halophilic bacterial strain, SW-152^T, which was isolated from sea water in the Yellow Sea, Korea. The result of a 16S rRNA gene sequence comparison indicated that the strain should be considered to be a member of the CFB group. The aim of the present work was to determine the exact taxonomic

position of strain SW-152^T by a polyphasic taxonomic characterization.

Sea water collected from the Yellow Sea, Korea, was used as the source for isolation of bacterial strains. Strain SW-152^T was isolated by the standard dilution plating technique at 25 °C on marine agar 2216 (MA; Difco). To investigate its morphological characteristics, strain SW-152^T was routinely cultivated at 30 °C on MA. Cell morphology was examined by light microscopy (Nikon E600) and transmission electron microscopy (TEM). The presence of flagella was examined by TEM using cells from exponentially growing cultures. Gliding motility was determined as described by Bowman (2000). Gram reaction was determined using the bioMérieux Gram Stain kit according to the manufacturer's instructions. Growth at various temperatures (4–45 °C) was measured on MA. The pH range for growth was determined in marine broth 2216 (MB; Difco) that was adjusted to various pH values (pH 4.5–10.0 at intervals of 0.5 pH units). Growth under anaerobic conditions was determined after incubation in a Forma anaerobic chamber on MA and MA supplemented with nitrate, both of which had been prepared anaerobically using nitrogen. Catalase and oxidase activities and hydrolysis of casein, starch and Tweens 20, 40, 60 and 80 were determined as described by Cowan & Steel (1965). Hydrolysis of hypoxanthine, tyrosine and xanthine was tested on MA using the substrate concentrations described by Cowan & Steel (1965). Hydrolysis of aesculin, gelatin and urea and

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Abbreviation: CFB, *Cytophaga*–*Flavobacterium*–*Bacteroides*.

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nitrate reduction were studied as described previously (Lanyi, 1987) with the modification that artificial sea water was used for preparation of media. The artificial sea water contained (per litre of distilled water) 23.6 g NaCl, 0.64 g KCl, 4.53 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 5.94 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 1.3 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (Bruns *et al.*, 2001). H_2S production was tested as described previously (Bruns *et al.*, 2001). Presence of flexirubin pigment was investigated as described by Reichenbach (1992). Acid production from carbohydrates was determined as described by Leifson (1963). Growth on several substrates was tested in a basal medium containing 0.2 g NaNO_3 , 0.2 g NH_4Cl and 0.05 g yeast extract in 1 litre artificial sea water (Bruns *et al.*, 2001) as described by Suzuki *et al.* (2001). Susceptibility to antibiotics was determined on MA plates by using antibiotic discs with the following concentrations: penicillin G (20 U), carbenicillin (100 μg), gentamicin (10 μg), kanamycin (30 μg), lincomycin (15 μg), neomycin (30 μg), polymyxin B (300 U), streptomycin (10 μg), tetracycline (30 μg), ampicillin (10 μg), oleandomycin (15 μg) and chloramphenicol (100 μg). Other physiological tests were performed with the API 20E system (bioMérieux).

Cell biomass for isoprenoid quinone and polar lipid analyses and for DNA extraction was obtained after cultivation for 3 days in MB at 30 °C. Isoprenoid quinones were analysed as described by Komagata & Suzuki (1987) using reversed-phase HPLC. Chromosomal DNA isolation and purification were performed according to the method described by Yoon *et al.* (1996), with the exception that ribonuclease T1 was treated in combination with ribonuclease A to minimize the contamination of RNA. For fatty acid methyl ester (FAME) analysis, a loop of cell mass was harvested from agar plates after incubation for 3 days on MA at 30 °C. The FAMEs 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 reversed-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 was performed as described by Yoon *et al.* (2003). Alignment of sequences was carried out with CLUSTAL W software (Thompson *et al.*, 1994). Gaps at the 5' and 3' ends of the alignment were omitted from further analyses. Phylogenetic trees were inferred by using three tree-making algorithms, the neighbour-joining (Saitou & Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Kluge & Farris, 1969) methods implemented within the PHYLIP package (Felsenstein, 1993). Evolutionary distance matrices for the neighbour-joining method were calculated by the algorithm of Jukes & Cantor (1969) using the program DNADIST. The stability of relationships was assessed by a bootstrap analysis based on 1000 resamplings of the

neighbour-joining dataset by using the programs SEQBOOT, DNADIST, NEIGHBOR and CONSENSE of the PHYLIP package.

Morphological, cultural, physiological and biochemical characteristics of strain SW-152^T are shown in Table 1 or given in the genus and species descriptions (see below). Strain SW-152^T contained menaquinone-7 (MK-7) as the predominant isoprenoid quinone at peak area ratio of approximately 91 %. Strain SW-152^T had a fatty acid profile that contained large amounts of iso-branched- and hydroxy fatty acids. The major fatty acid components (>1 %) detected in strain SW-152^T were as follows: iso-C_{15:0} (33.5 %), iso-C_{15:1} (20.5 %), iso-C_{17:0} 3-OH (11.2 %), iso-C_{16:0} 3-OH (7.2 %), iso-C_{15:0} 3-OH (5.6 %), iso-C_{13:0} (5.2 %), iso-C_{15:0} 2-OH and/or C_{16:1} ω7c (4.8 %), anteiso-C_{15:0} (2.4 %), C_{16:0} 3-OH (1.8 %), iso-C_{16:0} (1.2 %) and C_{15:0} (1.1 %). Polar lipids detected in strain SW-152^T were phosphatidylethanolamine, diphosphatidylglycerol, an unidentified glycolipid and an unidentified phospholipid. An amino-group-containing lipid that was ninhydrin-positive was also detected. The DNA G+C content of strain SW-152^T was 40.3 mol%.

An almost-complete 16S rRNA gene sequence of strain SW-152^T determined in this study comprised 1472 nt. Sequence searches with public databases revealed that the 16S rRNA gene sequence of strain SW-152^T shows highest sequence similarity to those of the CFB group. In the neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, strain SW-152^T formed an independent phylogenetic lineage within the evolutionary radiation enclosed by members of the CFB group (Fig. 1). Strain SW-152^T exhibited 16S rRNA gene sequence similarity levels of less than 89.4 % to all species used in the phylogenetic analysis (Fig. 1).

The phylogenetic analyses based on 16S rRNA gene sequences indicated that strain SW-152^T does not fall within the radiation encompassed by a recognized genus but forms a distinct evolutionary lineage within the CFB group. Although strain SW-152^T joined *Reichenbachia agariperforans* by a bootstrap resampling value of 69.3 % and these two formed a cluster with *Persicobacter diffluens* and [*Flexibacter*] *aggregans*, 16S rRNA gene sequence similarity values between strain SW-152^T and the three species were very low (<89.4 %). Strain SW-152^T is differentiated from several phylogenetically related taxa by some phenotypic characteristics as shown in Table 1. Accordingly, it is appropriate that strain SW-152^T be allocated in a new genus within the CFB group. On the basis of the phenotypic and phylogenetic data, we propose the creation of a novel genus and species, *Marinicola seohaensis* gen. nov., sp. nov., for strain SW-152^T.

Description of *Marinicola* gen. nov.

Marinicola (Ma.ri.ni'co.la. L. adj. *marinus* of the sea; L. masc. suffix *-cola* inhabitant; N.L. masc. n. *Marinicola* inhabitant of the sea).

Table 1. Differential phenotypic characteristics of strain SW-152^T and some related taxa

Taxa: 1, strain SW-152^T; 2, genus *Reichenbachia*; 3, genus *Persicobacter*; 4, [*Flexibacter*] *aggregans*. Data from this study, Reichenbach (1989), Nakagawa *et al.* (1997), Bowman *et al.* (2003) and Nedashkovskaya *et al.* (2003). +, Positive; –, negative; ND, not determined. Data in parentheses are for type strains. All taxa are Gram-negative, strictly aerobic, rod-shaped, motile by means of gliding, oxidase-positive, negative for casein hydrolysis and require Na⁺ or sea water for growth.

Characteristic	1	2	3	4
Pigment colour	Strong orange	Orange	Pink to orange	Yellow
Catalase	+	+	–	+
Flexirubin	+	+	–	–
Optimal growth temperature (°C)	30	25–28	25–30	ND
Maximum growth temperature (°C)	40	35	<45	35–45
Growth at 4 °C	+	+	–	–
Max. NaCl tolerance (%)	8	6	6	ND
Carbohydrate utilization	–	–	+	+
Hydrolysis of:				
Agar	–	+	+	+
Gelatin	–	+	+	–
Starch	–	+	+	–
Predominant menaquinone	MK-7	MK-7	MK-7	ND
Major fatty acids	iso-C _{15:0} , iso-C _{15:1}	iso-C _{15:0} , C _{16:1}	ND	ND
DNA G+C content (mol%)	40.3	44.5	40–42 (42)	37–42 (37)

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of strain SW-152^T and some other related taxa. The numbers on the branches indicate the bootstrap value of 1000 resamplings (greater than 50%). Bar, 0.01 substitution per nucleotide position. Dots represent that the corresponding nodes are also recovered in the maximum-likelihood tree. *Chlorobium limicola* DSM 245^T was used as an outgroup.

Cells are Gram-negative, non-flagellated, non-spore-forming and rod-shaped. Strictly aerobic. Motile by means of gliding. Flexirubin pigment is produced. Catalase- and oxidase-positive. Predominant menaquinone is MK-7. Major fatty acids are iso-C_{15:0} (33.5%) and iso-C_{15:1} (20.5%). Major phospholipids are phosphatidylethanolamine and diphosphatidylglycerol. DNA G + C content is 40.3 mol%.

The type species is *Marinicola seohaensis*.

Description of *Marinicola seohaensis* sp. nov.

Marinicola seohaensis (seo.ha.en'sis. N.L. masc. adj. *seohaensis* of Seohae, the Korean name for the Yellow Sea in Korea, from where the organism was isolated).

Exhibits the following properties in addition to those given in the genus description. Cells are Gram-negative, strictly aerobic rods, 0.2–0.3 × 2.0–4.0 µm. Colonies are circular, convex, glistening, smooth, strong orange in colour and 1–2 mm in diameter after incubation for 3 days on MA at 30 °C. Growth occurs at 4 and 40 °C, with an optimum temperature of 30 °C; growth does not occur above 41 °C. Optimal growth occurs in the presence of 2–3% (w/v) NaCl; growth does not occur in the absence of NaCl and in the presence of greater than 9% (w/v) NaCl. Optimal pH for growth is between 7.0 and 8.0; growth is observed at pH 5.5, but not at pH 5.0. Urease-negative. Tweens 20, 40, 60 and 80 are weakly hydrolysed. Aesculin, hypoxanthine, xanthine and tyrosine are not hydrolysed. Nitrate is not reduced. H₂S and indole are not produced. Growth occurs on Casamino acids, peptone and tryptone as the sole carbon and nitrogen sources, but does not occur on D-glucose, sucrose, D-ribose, DL-aspartate, L-glutamic acid, L-leucine or L-proline. No acid is produced from L-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, lactose, maltose, D-mannose, D-melezitose, melibiose, D-raffinose, L-rhamnose, D-ribose, sucrose, D-trehalose, D-xylose, adonitol, myo-inositol, D-mannitol or D-sorbitol. Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are absent. When assayed with the API ZYM system, alkaline phosphatase, esterase lipase (C 8), leucine arylamidase, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase and N-acetyl-β-glucosaminidase are present and esterase (C 4) and valine arylamidase are weakly present, but lipase (C 14), cystine arylamidase, trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, α-mannosidase and α-fucosidase are absent. Susceptible to carbenicillin, lincomycin, oleandomycin and chloramphenicol, but not to penicillin G, gentamicin, kanamycin, neomycin, polymyxin B, streptomycin, tetracycline or ampicillin. Polar lipids are phosphatidylethanolamine, diphosphatidylglycerol, an unidentified glycolipid, an unidentified phospholipid and a ninhydrin-positive lipid. DNA G + C content is 40.3 mol%. Other phenotypic properties are given in Table 1.

The type strain, SW-152^T (= KCTC 12312^T = JCM 12600^T), was isolated from sea water of the Yellow Sea, Korea.

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