Kangiella geojedonensis sp. nov., isolated from seawater

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A Gram-stain-negative, non-motile, non-spore-forming bacterial strain, YCS-5^T, was isolated from seawater off the southern coast of Korea. Strain YCS-5^T grew optimally at 30 °C and in the presence of 2 % (w/v) NaCl. Phylogenetic analyses based on 16S rRNA gene sequences revealed that strain YCS-5^T fell within the clade comprising *Kangiella* species. Strain YCS-5^T exhibited 16S rRNA gene sequence similarity values of 96.6, 95.7 and 97.9 % to the type strains of *Kangiella koreensis*, *Kangiella aquimarina* and *Kangiella japonica*, respectively, and less than 89.8 % to strains of other species used in the phylogenetic analysis. Strain YCS-5^T contained Q-8 as the predominant ubiquinone and iso-C_{17:0}, iso-C_{15:0}, iso-C_{11:0} 3-OH and iso-C_{17:1}ω9c as the major fatty acids. The polar lipid profile of strain YCS-5^T was similar to that of *K. koreensis* SW-125^T, with phosphatidylglycerol and an unidentified aminolipid as major polar lipids. The DNA G + C content was 47 mol%. The mean DNA–DNA relatedness value between strain YCS-5^T and *K. japonica* JCM 16211^T was 12 %. Differential phenotypic properties and the phylogenetic and genetic distinctiveness of strain YCS-5^T demonstrated that this strain is distinguishable from other *Kangiella* species. On the basis of the data presented, strain YCS-5^T is considered to represent a novel species of the genus *Kangiella*, for which the name *Kangiella geojedonensis* sp. nov. is proposed; the type strain is YCS-5^T (=KCTC 23420^T=CCUG 60526^T).

The genus *Kangiella* was first proposed by Yoon *et al.* (2004) with the description of two novel species, *Kangiella koreensis* and *Kangiella aquimarina*. Subsequently, two further *Kangiella* species with validly published names, *Kangiella japonica* (Romanenko *et al.*, 2010) and *Kangiella spongicola* (Ahn *et al.*, 2011), have been described. Phylogenetic analysis based on 16S rRNA gene sequences showed that the genus *Kangiella* belongs to the *Gammaproteobacteria* (Yoon *et al.*, 2004). Members of the genus *Kangiella* have been isolated from marine environments (Yoon *et al.*, 2004; Romanenko *et al.*, 2010). In this study, we describe a bacterial strain, designated YCS-5^T, which was isolated from seawater off the southern coast of Korea. Comparative 16S rRNA gene sequence analysis indicated that strain YCS-5^T can be affiliated to the genus *Kangiella*. The aim of the present work was to determine the exact taxonomic position of strain YCS-5^T by using a polyphasic approach that included the determination of chemotaxonomic and phenotypic properties and detailed phylogenetic investigations based on 16S rRNA gene sequences.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain YCS-5^T is HQ340606.

A supplementary figure is available with the online version of this paper.

Strain YCS-5^T was isolated by the standard dilution plating technique on marine agar 2216 (MA; Difco) at 25 °C. *K. koreensis* (type species of the genus *Kangiella*) SW-125^T, which was used as a reference strain for polar lipid analysis, was obtained from our previous study (Yoon *et al.*, 2004). *K. japonica* JCM 16211^T, which was used as a reference strain for DNA–DNA hybridization, was obtained from the Japan Collection of Microorganisms, Saitama, Japan. The morphological, physiological and biochemical characteristics of strain YCS-5^T were investigated using cells that were routinely cultivated on MA at 30 °C. Cell morphology was examined by using light microscopy (DP70; Olympus) and transmission electron microscopy (CM-20; Philips). Flagellation was determined by transmission electron microscopy of cells from an exponentially growing culture. For this purpose, cells were negatively stained with 1 % (w/v) phosphotungstic acid and grids were examined after being air-dried. The Gram reaction was determined by using the bioMérieux Gram stain kit according to the manufacturer's instructions. Growth in the absence of NaCl and in the presence of 0.5, 1.0, 2.0 and 3.0 % (w/v) NaCl was investigated by using trypticase soy broth prepared according to the formula of the Difco medium except that NaCl

was excluded and that 0.45 % (w/v) $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ or 0.06 % (w/v) KCl was added. Growth at various NaCl concentrations (2.0–18.0 %, w/v, in increments of 1.0 %) was investigated in marine broth 2216 (MB; Difco). Growth at various temperatures (4, 10, 20, 25, 28, 30, 35, 37, 40 and 45 °C) was measured on MA. The pH range for growth was determined in MB adjusted to various pH values (pH 4.5–9.5, in increments of 0.5 pH unit) by the addition of HCl or Na_2CO_3 ; pH values were verified after autoclaving. Growth under anaerobic conditions was determined after incubation in a Forma anaerobic chamber on MA and on MA supplemented with potassium nitrate (0.1 %, w/v), both of which had been prepared anaerobically under a nitrogen atmosphere. Catalase and oxidase activities were determined as described by Cowan & Steel (1965). Hydrolysis of casein, starch, hypoxanthine, tyrosine and xanthine was tested on MA using the substrate concentrations described by Cowan & Steel (1965). Hydrolysis of aesculin, gelatin, Tweens 20, 40, 60 and 80, and urea and nitrate reduction were investigated as described previously (Lányi, 1987) with the modification that artificial seawater was used for preparation of media. The artificial seawater contained (l^{-1} 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). Acid production from carbohydrates was determined as described by Leifson (1963). Susceptibility to antibiotics was tested on MA plates using antibiotic discs of the following: ampicillin (10 µg), chloramphenicol (100 µg), penicillin G (20 U), polymyxin B (100 U), streptomycin (50 µg) and tetracycline (30 µg). Other physiological and biochemical tests were performed with the API 20E and API ZYM systems (bioMérieux).

Cell biomass of strain YCS-5^T for DNA extraction and for the analyses of isoprenoid quinones and polar lipids was obtained from cultures grown for 5 days in MB at 30 °C. Cell biomass of *K. koreensis* SW-125^T for polar lipid analysis and cell biomass of *K. japonica* JCM 16211^T for DNA extraction were obtained from cultures grown for 5 days in MB at 30 °C. Chromosomal DNA was isolated and purified as described previously (Yoon *et al.*, 1996), with the exception that RNase T1 was used in combination with RNase A to minimize contamination with RNA. 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 by Yoon *et al.* (2004). Isoprenoid quinones were extracted according to the method of Komagata & Suzuki (1987) and analysed using reversed-phase HPLC and a YMC ODS-A (250 × 4.6 mm) column. For fatty acid methyl ester analysis, cell mass of strain YCS-5^T was harvested from MA plates after incubation for 7 days at 30 °C. Fatty acids were saponified, methylated and extracted using the standard protocol of the MIDI (Sherlock Microbial Identification System, version 4.0). Fatty acids were analysed by GC (Hewlett Packard 6890) and identified by using the TSBA40 database of the Microbial Identification System (Sasser, 1990). Polar lipids were extracted according to the procedures described by Minnikin *et al.* (1984) and identified by two-dimensional TLC followed

by spraying with molybdophosphoric acid, molybdenum blue, ninhydrin and α -naphthol reagents (Minnikin *et al.*, 1984; Komagata & Suzuki, 1987) and Dragendorff's reagent (Sigma). 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. DNA–DNA hybridization was performed fluorometrically at 45 °C 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 for each sample were excluded and the means of the remaining three values are quoted as DNA–DNA relatedness values.

Morphological, cultural, physiological and biochemical characteristics of strain YCS-5^T are given in the species description (see below) or in Table 1. The almost complete 16S rRNA gene sequence of strain YCS-5^T determined in this study comprised 1461 nt (approx. 96 % of the *Escherichia coli* 16S rRNA sequence). In the neighbour-joining phylogenetic

Table 1. Differential phenotypic characteristics of *Kangiella geojedonensis* YCS-5^T and the type strains of three *Kangiella* species

Strains: 1, *K. geojedonensis* YCS-5^T; 2, *K. koreensis* SW-125^T; 3, *K. aquimarina* SW-154^T; 4, *K. japonica* KMM 3899^T. Data for reference strains are from Yoon *et al.* (2004) and Romanenko *et al.* (2010). All strains are Gram-stain-negative and rod-shaped. All strains are positive for catalase, oxidase, hydrolysis of casein, gelatin, tyrosine and Tweens 20, 40 and 80, activity of esterase lipase (C8), leucine arylamidase and naphthol-AS-BI-phosphohydrolase (weak for *K. japonica* KMM 3899^T), and susceptibility to chloramphenicol and polymyxin B. All strains are negative for motility, nitrate reduction (under aerobic conditions), H_2S and indole production, hydrolysis of aesculin and starch, activity of lipase (C14), cystine arylamidase, α -chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, *N*-acetyl- β -glucosaminidase, α -mannosidase and α -fucosidase, and susceptibility to tetracycline.

Characteristic	1	2	3	4
Growth at/in:				
4 °C	–	+	–	+
45 °C	–	–	+	–
15 % (w/v) NaCl	+	–	–	–
Enzyme activity (API ZYM)				
Alkaline phosphatase	+	+	+	–
Esterase (C4)	+	+	+	–
Valine arylamidase	–	+	+	+
Trypsin	–	+	+	–
Acid phosphatase	+	–	+	–
Susceptibility to antibiotics				
Ampicillin	–	+	+	+
Penicillin G	–	+	+	+
Streptomycin	–	+	+	+
DNA G + C content (mol%)	47	44	44	45.8

tree based on 16S rRNA gene sequences, strain YCS-5^T fell within the clade comprising *Kangiella* species, clustering coherently with *K. japonica* KMM 3899^T and *K. spongicola* A79^T (Fig. 1). This cluster also joined the cluster comprising the type strains of *K. koreensis* and *K. aquimarina* with a bootstrap resampling value of 100 % (Fig. 1). Strain YCS-5^T exhibited 16S rRNA gene sequence similarity values of 96.6, 95.7 and 97.9 % to the type strains of *K. koreensis*, *K. aquimarina* and *K. japonica*, respectively, and less than 89.8 % to strains of other species used in the phylogenetic analysis.

The predominant isoprenoid quinone detected in strain YCS-5^T was ubiquinone-8 (Q-8), which is the same as that found in members of the genus *Kangiella* (Yoon *et al.*, 2004; Romanenko *et al.*, 2010). The cellular fatty acid profile of strain YCS-5^T is shown in Table 2, together with those of type strains of two *Kangiella* species that were analysed under identical conditions and using the same methods (Yoon *et al.*, 2004). The major fatty acids (>10 % of total fatty acids) detected in strain YCS-5^T were iso-C_{17:0}, iso-C_{15:0}, iso-C_{11:0} 3-OH and iso-C_{17:1}ω9c (Table 2). The fatty acid profile of strain YCS-5^T was similar to those of *K. koreensis* SW-125^T and *K. aquimarina* SW-154^T, even though there were differences in the proportions of some fatty acids, particularly iso-C_{17:0} and iso-C_{15:0} (Table 2). The major polar lipids detected in strain YCS-5^T were phosphatidylglycerol and an unidentified aminolipid (Supplementary Fig. S1, available in IJSEM Online). This polar lipid profile was similar to that of *K. koreensis* SW-125^T although phosphatidylethanolamine and phosphatidylmonomethylethanolamine, which were detected as major components in *K. koreensis* SW-125^T, were minor components in strain YCS-5^T. The DNA G+C content of strain YCS-5^T was 47 mol%. The results obtained from chemotaxonomic analyses, which showed that the isolate shared similar properties to *Kangiella* species, were in agreement with the results of phylogenetic analysis, i.e. that strain YCS-5^T belongs to the genus *Kangiella* (Yoon *et al.*, 2004; Romanenko *et al.*, 2010).

Strain YCS-5^T exhibited a mean DNA–DNA relatedness value of 12 % to *K. japonica* JCM 16211^T. Strain YCS-5^T was distinguishable from *Kangiella* species by differences in several phenotypic characteristics, most of which were determined under the same conditions and using the same methods (Table 1). The phylogenetic and genetic distinctiveness of strain YCS-5^T, together with its differential phenotypic properties, are sufficient to show that this strain is separate from other *Kangiella* species (Stackebrandt & Goebel, 1994; Wayne *et al.*, 1987). Therefore, on the basis of the phenotypic, chemotaxonomic and phylogenetic data, strain YCS-5^T is considered to represent a novel species of the genus *Kangiella*, for which the name *Kangiella geojedonensis* sp. nov. is proposed.

Description of *Kangiella geojedonensis* sp. nov.

Kangiella geojedonensis (geo.je.do.nen'sis. N.L. fem. adj. *geojedonensis* pertaining to Geojedo, the Korean island where the type strain was isolated).

Cells are Gram-stain-negative, non-spore-forming, non-flagellated rods (0.2–0.4 × 0.8–6.5 μm). Colonies on MA are circular to irregular, flat, smooth, glistening, greyish-yellow in colour and 3.0–5.0 mm in diameter after incubation for 7 days at 30 °C. Optimal growth occurs at 30 °C; growth occurs at 10 and 40 °C, but not at 4 or 45 °C. Optimal pH for growth is between 7.0 and 7.5; growth occurs at pH 5.5, but not at pH 5.0. Optimal growth occurs in the presence of 2 % (w/v) NaCl; growth occurs at 0.5–16.0 % (w/v) NaCl. Mg²⁺ ions are required for growth. Growth does not occur under anaerobic conditions on MA or on MA supplemented with nitrate. Catalase- and oxidase-positive. Nitrate reduction is negative. Casein, gelatin, tyrosine, and Tweens 20, 40, 60 and 80 are hydrolysed, but aesculin, hypoxanthine, starch and xanthine are not. Acid is not produced from L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, *myo*-inositol, lactose, maltose, D-mannitol, D-mannose, melezitose,

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the positions of *Kangiella geojedonensis* YCS-5^T and strains of other *Kangiella* species and some other related taxa. Bootstrap values (expressed as percentages of 1000 replications) >50 % are shown at branching points. Filled circles indicate that the corresponding nodes were also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms. Open circles indicate that the corresponding nodes were also recovered in the tree generated with the maximum-likelihood algorithm. *Escherichia coli* ATCC 11775^T (accession no. X80725) was used as an outgroup. Bar, 0.01 substitutions per nucleotide position.

Table 2. Cellular fatty acid compositions (%) of *Kangiella geojedonensis* YCS-5^T and the type strains of three *Kangiella* species

Strains: 1, *K. geojedonensis* YCS-5^T; 2, *K. koreensis* SW-125^T; 3, *K. aquimarina* SW-154^T; 4, *K. japonica* KMM 3899^T. Data for reference strains are from Yoon *et al.* (2004) and Romanenko *et al.* (2010). Fatty acids that represented <0.5% in all strains were omitted. —, Not detected or <0.5%.

Fatty acid	1	2	3	4
Straight-chain fatty acid				
C _{16:0}	3.0	1.1	1.6	3.9
Branched fatty acid				
iso-C _{11:0}	6.7	5.6	6.5	4.7
iso-C _{13:0}	0.3	0.4	0.6	—
iso-C _{15:0}	21.4	57.6	50.7	26.9
iso-C _{15:1}	0.5	1.2	1.1	6.9
iso-C _{16:0}	1.6	0.7	1.0	—
iso-C _{17:0}	28.8	7.2	11.0	23.8
iso-C _{17:1} ω9c	10.9	8.6	7.0	12.7
Hydroxy fatty acid				
iso-C _{11:0} 3-OH	13.0	10.5	11.7	13.6
iso-C _{15:0} 3-OH	0.9	0.9	0.7	—
iso-C _{17:0} 3-OH	3.4	1.0	0.6	—
Summed features*				
1	5.2	3.2	4.0	—
3	0.9	—	—	—
Unknown fatty acid†				
ECL 13.565	0.8	—	—	—

*Summed features represent groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 1 contained C_{13:0} 3-OH and/or iso-C_{15:1}. Summed feature 3 contained C_{16:1}ω7c and/or iso-C_{15:0} 2-OH.

†ECL, Equivalent chain-length.

melibiose, raffinose, L-rhamnose, D-ribose, D-sorbitol, sucrose, trehalose or D-xylose. The predominant ubiquinone is Q-8. The major fatty acids (>10% of total fatty acids) are iso-C_{17:0}, iso-C_{15:0}, iso-C_{11:0} 3-OH and iso-C_{17:1}ω9c. The major polar lipids are phosphatidylglycerol and an unidentified aminolipid. Other phenotypic properties are shown in Table 1.

The type strain, YCS-5^T (=KCTC 23420^T=CCUG 60526^T), was isolated from seawater off Geoje in the South Sea, Korea. The DNA G+C content of the type strain is 47 mol% (HPLC).

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