Kangiella taiwanensis sp. nov. and *Kangiella marina* sp. nov., marine bacteria isolated from shallow coastal water

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Two Gram-negative, heterotrophic, aerobic, marine bacteria, designated strains KT1^T and KM1^T, were isolated from seawater samples collected from the shallow coastal regions of northern Taiwan. Cells grown in broth cultures were non-flagellated rods. NaCl was required for growth. Optimal growth occurred with 2–5 % NaCl, at 25–30 °C and at pH 8. They grew aerobically and were not capable of anaerobic growth by fermenting D-glucose or other carbohydrates. Q-8 was the only isoprenoid quinone. The major polar lipid detected in strain KT1^T was phosphatidylmonomethylethanolamine, whereas those detected in KM1^T were phosphatidylmonomethylethanolamine, phosphatidylmethylethanolamine and an unidentified phospholipid. Cellular fatty acids were nearly all iso-branched, with iso-C_{15:0} as the most abundant component (54.6–57.2 % of the total). Strains KT1^T and KM1^T had DNA G + C contents of 43.9 and 46.3 mol%, respectively. The two strains shared 98.1 % 16S rRNA gene sequence similarity; levels of similarity with the type strains of species of the genus *Kangiella* were 95.6–98.4 %. Data from the present taxonomic study conducted using a polyphasic approach revealed that the isolates could be classified as representatives of two novel species of the genus *Kangiella*, for which the names *Kangiella taiwanensis* sp. nov. (type strain KT1^T=BCRC 80330^T=JCM 17727^T) and *Kangiella marina* sp. nov. (type strain KM1^T=BCRC 80329^T=JCM 17728^T) are proposed.

The genus *Kangiella*, of the class *Gammaproteobacteria*, comprises marine, heterotrophic, Gram-negative rods that are non-motile and non-fermentative. The genus was originally established to accommodate two species, *Kangiella koreensis* (the type species) and *Kangiella aquimarina* (Yoon *et al.*, 2004). These species were recovered from tidal flat sediment of Daepo Beach, Yellow Sea, Korea (Yoon *et al.*, 2004). Three additional species have been described more recently: *Kangiella japonica* isolated from coastal seawater and sediment samples of the Sea of Japan, Russia (Romanenko *et al.*, 2010), *Kangiella spongicola* isolated from a marine sponge sample collected from the Florida Keys, Florida, USA (Ahn *et al.*, 2011) and *Kangiella geojedonensis* isolated from seawater of the South Sea, Korea (Yoon *et al.*, 2012). Species of the genus *Kangiella* are unusual in possessing a rather high content of iso-branched cellular fatty acids (>90 % of the total).

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains KT1^T and KM1^T are JN559389 and JN559388, respectively.

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

Two bacterial strains, designated KT1^T and KM1^T, were isolated from seawater samples collected in the shallow coastal regions of Bangbang and Shenao, respectively; the two sampling sites are located on the north coast of Taiwan. Data from the present taxonomic study conducted using a polyphasic approach support the establishment of two novel species of the genus *Kangiella* with KT1^T and KM1^T as the type strains.

Seawater samples were diluted 10-fold with sterile NaCl/Tris buffer (30 g NaCl and 0.24 g Tris base in 1 litre deionized water, pH 8.0). Aliquots (0.1 ml) of the decimally diluted samples (10⁻¹ to 10⁻³) were spread on polypeptone-yeast (PY) plate medium (Shieh *et al.*, 2000) in triplicate. The plates were incubated at 30 °C in the dark for 7 days under aerobic conditions. Individual colonies appearing on the plates were picked off and purified by successive streaking on PY plates. PY stab cultures of the isolates were maintained at 25 °C under aerobic conditions. Strains KT1^T and KM1^T, two of the isolates deposited in both the Bioresource Collection and Research Center (BCRC) and the Japan Collection of Microorganisms

(JCM) as lyophilized cultures, were used for the present study.

Physiological and morphological characterization of strains KT1^T and KM1^T, including growth and other phenotypic properties, were determined following previously established procedures (Jean *et al.*, 2006). Early stationary phase cells harvested from cultures grown in PY broth or on PY plate medium at 30 °C for 3 days under aerobic conditions were used for analyses of cellular fatty acids, isoprenoid quinones and DNA base composition according to the methods described by Shieh *et al.* (2008). However, the HPLC apparatus used for analysis of isoprenoid quinones was equipped with an Inertsil ODS-2 column (1.5 × 250 mm; GL Science) instead of the Waters model 5C₁₈-AR-II. Fatty acids in whole cells grown on PY plate medium were extracted, saponified and esterified, followed by GC analysis of fatty acid methyl esters according to the MIDI system (Sasser, 2001). The fatty acid methyl ester profile was identified by comparing with the database, 2005 library, supplied in the commercial software package Sherlock

Microbial Identification System version 6.0 (MIDI Inc.). Cells grown in marine agar 2216 (Difco) at 30 °C for 3 days under aerobic conditions were extracted, purified and analysed for polar lipids according to the methods described by Lin & Shieh (2006). However, two-dimensional thin-layer chromatograms were developed by using the solvent systems described by Collins & Jones (1980).

Cells grown in PY broth at 30 °C for 3 days were harvested by centrifugation. Extraction and purification of total genomic DNA from cells and PCR amplification of 16S rRNA genes were performed according to previously described methods (Jean *et al.*, 2006). Sequencing of 16S rRNA genes, multiple alignment and comparison of the resulting sequences with reference sequences available in GenBank, calculation of distance matrices for the aligned sequences, and reconstruction of phylogenetic trees based on the neighbour-joining, maximum-parsimony and maximum-likelihood methods were performed as described by Shieh *et al.* (2004) and Jean *et al.* (2006). Robustness of clusters was assessed by a bootstrap analysis of 1000 replications.

Fig. 1. Neighbour-joining tree showing the phylogenetic relationship between strains KT1^T and KM1^T and the type strains of recognized species of the genus *Kangiella*, together with other related taxa belonging to the class *Gammaproteobacteria*. GenBank accession numbers are given in parentheses. Bootstrap values (percentages of 1000 replicates) >50 % are shown at branch points. Bar, 2 nt substitutions per 100 nt.

Nearly complete 16S rRNA gene sequences were determined for strains KT1^T (1476 nt) and KM1^T (1490 nt). The two sequences were aligned and compared with those in GenBank. The two sequences shared 98.1 % similarity (28 differences out of 1476 nt positions). These 16S rRNA gene sequences showed the highest levels of similarity to those of the type strains of members of the genus *Kangiella* (95.6–98.4 %): *K. aquimarina* (95.6–96.0 %), *K. geojedonensis* (97.3–98.4 %), *K. japonica* (97.1–97.8 %), *K. koreensis* (96.0–96.6 %) and *K. spongicola* (97.2–98.4 %). No other bacterial species shared more than 90 % 16S rRNA gene sequence similarity with strains KT1^T and KM1^T. All the above values were lower than the re-evaluated threshold for species differentiation (98.7 %) as proposed by Stackebrandt & Ebers (2006). Phylogeny based on a neighbour-joining analysis of 16S rRNA gene sequences revealed that strains KT1^T and KM1^T were members of the class *Gammaproteobacteria* and that they formed a robust cluster (bootstrap value, 100 %) with their closest neighbours, the type strains of the five recognized *Kangiella* species (Fig. 1). The cluster could be further divided into two subclusters with bootstrap values of 99 and 100 %, respectively. However, relatively low bootstrap values (<65 %) were observed among the subcluster that consisted of strains KT1^T and KM1^T and the type strains of *K. geojedonensis*, *K. japonica* and *K. spongicola*. Similar results were obtained with the maximum-likelihood and maximum-parsimony algorithms (data not shown). The 16S rRNA gene-based phylogeny and sequence similarity data supported the assignment of strains KT1^T and KM1^T to two novel species of *Kangiella*.

Strains KT1^T and KM1^T contained iso-C_{15:0} as the most abundant cellular fatty acid (54.6–57.2 % of the total). Other cellular fatty acids comprising more than 3 % of the total were iso-C_{11:0} (3.8–4.4 %), iso-C_{17:0} (7.2–8.1 %), iso-C_{17:1ω9c} (7.5–11.0 %), iso-C_{11:0} 3-OH (8.4–9.5 %) and summed feature 1 [iso-C_{15:1} and/or C_{13:0} 3-OH (the two fatty acids could not be differentiated with the MIDI system); 4.6–6.7 % of the total fatty acids]. The fatty acid profiles were similar among the two isolates and the type strains of *Kangiella* species, but some qualitative and quantitative differences were observed (Table 1). Although the strains were not cultivated under the same conditions, such differences in the cellular fatty acids might serve to differentiate the two isolates from the type strains of recognized *Kangiella* species. Q-8 was the only isoprenoid quinone detected in strains KT1^T and KM1^T. All recognized *Kangiella* species were also reported as containing Q-8 as the predominant isoprenoid quinone. The polar lipids of strain KT1^T consisted of phosphatidylmonomethylethanolamine (major), phosphatidylethanolamine (minor) and two unidentified phospholipids (minor) (Fig. S1 in IJSEM Online). However, the polar lipids of KM1^T comprised three major components, phosphatidylmonomethylethanolamine, phosphatidylmethylethanolamine and an unidentified phospholipid (Fig. S1). No unidentified amino lipids were detected in the polar lipids of the new isolates

Table 1. Cellular fatty acid contents (%) of strains KT1^T and KM1^T and the type strains of recognized species of the genus *Kangiella*

Strains: 1, KT1^T (data from this study); 2, KM1^T (this study); 3, *K. geojedonensis* YCS-5^T (Yoon *et al.*, 2012); 4, *K. spongicola* A79^T (Ahn *et al.*, 2011); 5, *K. japonica* KMM 3899^T (Romanenko *et al.*, 2010); 6, *K. koreensis* SW-125^T (Yoon *et al.*, 2004); 7, *K. aquimarina* SW-154^T (Yoon *et al.*, 2004). –, Not detected/not reported; tr, trace amount detected (<1 %).

Fatty acid	1	2	3	4	5	6	7
C _{14:0}	1.1	1.1	–	–	–	–	–
C _{16:0}	1.2	2.2	3.0	4.0	3.9	1.1	1.6
iso-C _{11:0}	4.4	3.8	6.7	7.2	4.7	5.6	6.5
iso-C _{13:0}	tr	1.1	tr	tr	–	tr	tr
iso-C _{14:0}	tr	tr	–	–	–	–	–
iso-C _{15:0}	57.2	54.6	21.4	34.9	26.9	57.6	50.7
iso-C _{16:0}	1.5	tr	1.6	1.0	–	tr	1.0
iso-C _{17:0}	7.2	8.1	28.8	16.2	23.8	7.2	11.0
anteiso-C _{15:0}	tr	tr	–	tr	–	–	–
iso-C _{15:1} *	1.0	1.7	tr	tr	6.9	1.2	1.1
iso-C _{17:1ω9c}	7.5	11.0	10.9	10.5	12.7	8.6	7.0
iso-C _{11:0} 3-OH	9.5	8.4	13.0	15.4	13.6	10.5	11.7
iso-C _{15:0} 3-OH	–	–	tr	–	–	tr	tr
iso-C _{17:0} 3-OH	–	–	3.4	1.6	–	1.0	tr
Summed	6.7	4.6	5.2	5.2	–	3.2	4.0
feature 1†							
Summed	tr	1.1	tr	1.9	–	–	–
feature 3†							

*The double bond position is unknown.

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

when the analysis was performed by using ninhydrin as the staining reagent. Phosphatidylglycerol was found as one of the major phospholipids in both *K. koreensis* and *K. geojedonensis*. However, this phospholipid was not detected in strain KT1^T or in strain KM1^T (Table 2). The DNA G+C contents of strains KT1^T (43.9 mol%) and KM1^T (46.3 mol%) fell roughly within the range of values reported for recognized *Kangiella* species (44.0–47.0 mol%) (Table 2).

Strains KT1^T and KM1^T were mesophilic, halophilic, non-motile and Gram-negative. They produced circular, convex, light brown, opaque and non-luminescent colonies with entire edges. Cells of the strains grown in PY broth appeared to be straight and non-flagellated as revealed by transmission electron microscopy (not shown). The two strains were capable of anaerobic growth by nitrate reduction to nitrite but not by fermentation of D-glucose or other carbohydrates such as D-arabinose, L-arabinose, cellobiose, D-galactose, lactose, D-mannose, melibiose, sucrose, trehalose and D-xylose as substrates.

Table 2. Differential characteristics between strains KT1^T and KM1^T and the type strains of recognized species of the genus *Kangiella*

Strains: 1, KT1^T (data from this study); 2, KM1^T (this study); 3, *K. geojedonensis* YCS-5^T (Yoon *et al.*, 2012); 4, *K. spongicola* A79^T (Ahn *et al.*, 2011); 5, *K. japonica* KMM 3899^T (Romanenko *et al.*, 2010); 6, *K. koreensis* SW-125^T (Yoon *et al.*, 2004); 7, *K. aquimarina* SW-154^T (Yoon *et al.*, 2004). w, Weakly positive; ND, no data available.

Characteristic	1	2	3	4	5	6	7
Nitrate reduction	+	+	—	+	—	+	+
Anaerobic growth	+	+	—	+	—	+	+
Acid production from D-glucose	—	—	—	—	+	—	—
Hydrolysis of:							
Casein	—	+	+	+	+	+	+
Gelatin	+	+	+	—	+	+	+
Tween 80	+	—	+	+	+	+	+
Growth at/with:							
10 °C	—	—	+	+	+	+	+
42 °C	—	—	ND	+	+	+	+
45 °C	—	—	—	+	—	—	+
15 % NaCl	—	—	+	+	—	—	—
Temperature optimum (°C)	25–30	25–30	30	10–49	28–35	30–37	30–37
NaCl optimum (%)	2–5	2–5	2	2–10	2–3	2–3	2–3
API ZYM results							
Alkaline phosphatase	—	+	+	+	—	+	+
Valine arylamidase	+	+	—	+	+	+	+
Cystine arylamidase	+	+	—	—	—	—	—
Trypsin	+	w	—	—	—	+	+
α-Chymotrypsin	+	w	—	+	—	—	—
Acid phosphatase	—	—	+	+	—	—	+
Naphthol-AS-BI-phosphohydrolase	—	—	+	+	w	+	+
Polar lipids*							
Major	PME	PME, PDE, PL ₃	PG, AL	ND	ND	PG, PME, PE, AL	ND
Minor	PE, PL ₁ , PL ₂		PME, PE	ND	ND		ND
DNA G + C content (mol%)	43.9	46.3	47.0	44.9	45.8	44.0	44.0

*PME, phosphatidylmonomethylethanolamine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PDE, phosphatidyl dimethylethanolamine; PL₁, PL₂ and PL₃, three different unidentified phospholipids; AL, an unidentified aminolipid. Data for the polar lipids of *K. geojedonensis* YCS-5^T and *K. koreensis* SW-125^T are from Yoon *et al.* (2012).

Strains KT1^T and KM1^T shared many physiological and morphological characteristics. However, they could be differentiated from each other based on different reactions in tests for hydrolysis of casein and Tween 80 and activity of alkaline phosphatase, α-chymotrypsin and trypsin. The two strains could be distinguished from recognized *Kangiella* species based on the inability to grow at 10 °C, and presence of cystine arylamidase and absence of naphthol-AS-BI-phosphohydrolase in API ZYM tests. Additional characteristics useful for differentiating strains KT1^T and KM1^T from recognized *Kangiella* species are given in Table 2.

The polyphasic data from this study support the establishment of two novel *Kangiella* species to accommodate strains KT1^T and KM1^T, for which the names *Kangiella taiwanensis* sp. nov. and *Kangiella marina* sp. nov. are proposed, respectively.

Description of *Kangiella taiwanensis* sp. nov.

Kangiella taiwanensis (tai.wan.en/sis. N.L. fem. adj. *taiwanensis* pertaining to Taiwan, where the type strain was isolated).

Cells are non-motile, Gram-negative rods, 1.5–2.0 µm long and 0.5–1.0 µm wide. Colonies produced on PY plate medium at 30 °C for 3–7 days are circular (approx. 2–6 mm in diameter), light brown, opaque, convex and non-luminescent, with entire edges. Endospores are absent. Growth occurs at 15–40 °C, with optimum growth at 25–30 °C; no growth occurs at 4–10 or 42–45 °C. Sodium ions are required for growth; growth occurs in the presence of 0.5–12 % (w/v) NaCl, with optimum growth at 2–5 %, and no growth at 0 or 15 % NaCl. Grows at pH 7–10, but not at pH 5–6. Anaerobic growth occurs by nitrate reduction (to nitrite) but not by fermentation of D-glucose or other

carbohydrates as substrates. Oxidase- and catalase-positive. Indole is not produced from tryptophan. H₂S is not produced from thiosulfate. Hydrolyses gelatin, lecithin and Tween 80, but not aesculin, casein, chitin, DNA, starch or urea. In assays with the API ZYM system (bioMérieux), positive for cystine arylamidase, leucine arylamidase, valine arylamidase, esterase (C4), esterase lipase (C8), α -chymotrypsin and trypsin, but negative for α -fucosidase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, β -glucuronidase, α -mannosidase, lipase (C14), acid phosphatase, alkaline phosphatase, *N*-acetyl- β -glucosaminidase and naphthol-AS-BI-phosphohydrolase. Acid production is not observed with oxidation or fermentation of single carbon sources provided by the API 50CH system (bioMérieux) and Biolog GN2 microplates. Susceptible to ampicillin (10 μ g), cephalothin (30 μ g), chloramphenicol (30 μ g), colistin (10 μ g), erythromycin (15 μ g), nalidixic acid (30 μ g) and polymyxin B (300 U), and intermediately susceptible to penicillin G (10 U). Resistant to gentamicin (10 μ g), kanamycin (30 μ g), neomycin (30 μ g), oxacillin (1 μ g), streptomycin (10 μ g), tetracycline (30 μ g) and vancomycin (30 μ g). Q-8 is the only isoprenoid quinone. Phosphatidylmonomethylethanolamine is the major polar lipid. Major cellular fatty acids (>1 %) are iso-C_{15:0}, iso-C_{11:0} 3-OH, C_{17:1} ω 9c, iso-C_{17:0}, summed feature 1 (iso-C_{15:1} and/or C_{13:0} 3-OH), iso-C_{11:0}, C_{14:0}, C_{16:0}, iso-C_{16:0} and iso-C_{15:1}.

The type strain, KT1^T (=BCRC 80330^T=JCM 17727^T), was isolated from shallow coastal seawater of Bangbang, New Taipei City, Taiwan. The DNA G+C content of the type strain is 43.9 mol%.

Description of *Kangiella marina* sp. nov.

Kangiella marina (ma.ri'na. L. fem. adj. *marina* of the sea, marine).

Cells are non-motile, Gram-negative rods, 0.7–1.2 μ m long and 0.3–0.5 μ m wide. Colonies produced on PY plate medium at 30 °C for 3–7 days are circular (approx. 1–4 mm in diameter), light brown, opaque, convex and non-luminescent, with entire edges. Endospores are absent. Growth occurs at 15–40 °C, with optimum growth at 25–30 °C; no growth occurs at 4–10 or 42–45 °C. Sodium ions are required for growth; growth occurs in the presence of 1–8 % (w/v) NaCl, with optimum growth at 2–5 %, and no growth at 0 or 15 % NaCl. Grows at pH 7–9, but not at pH 5–6. Anaerobic growth occurs by reduction of nitrate to nitrite but not by fermentation of D-glucose or other carbohydrates as substrates. Oxidase- and catalase-positive. Indole is not produced from tryptophan. H₂S is not produced from thiosulfate. Hydrolyses casein, gelatin and lecithin (weakly positive), but not aesculin, chitin, DNA, starch, Tween 80 or urea. In assays with the API ZYM system (bioMérieux), positive for cystine arylamidase, leucine arylamidase, valine arylamidase, esterase (C4), esterase lipase (C8), α -chymotrypsin (weakly), alkaline phosphatase and trypsin (weakly), but negative for α -fucosidase,

α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, β -glucuronidase, α -mannosidase, lipase (C14), acid phosphatase, alkaline phosphatase, *N*-acetyl- β -glucosaminidase and naphthol-AS-BI-phosphohydrolase. Acid production is not observed with oxidation or fermentation of single carbon sources provided by the API 50CH system (bioMérieux) and Biolog GN2 microplates. Susceptible to ampicillin (10 μ g), cephalothin (30 μ g), chloramphenicol (30 μ g), colistin (10 μ g), erythromycin (15 μ g), nalidixic acid (30 μ g) and polymyxin B (300 U), and intermediately susceptible to penicillin G (10 U). Resistant to gentamicin (10 μ g), kanamycin (30 μ g), neomycin (30 μ g), oxacillin (1 μ g), streptomycin (10 μ g), tetracycline (30 μ g) and vancomycin (30 μ g). Q-8 is the only isoprenoid quinone. Major polar lipids are phosphatidylmonomethylethanolamine, phosphatidylmethylethanolamine and an unidentified phospholipid. Major cellular fatty acids (>1 %) are iso-C_{15:0}, C_{17:1} ω 9c, iso-C_{11:0} 3-OH, iso-C_{17:0}, summed feature 1 (iso-C_{15:1} and/or C_{13:0} 3-OH), iso-C_{11:0}, C_{14:0}, C_{16:0}, iso-C_{13:0}, iso-C_{15:1} and summed feature 3 (iso-C_{16:1} ω 7c and/or iso-C_{15:0} 2-OH).

The type strain, KM1^T (=BCRC 80329^T=JCM 17728^T), was isolated from shallow coastal seawater of Shenao, New Taipei City, Taiwan. The DNA G+C content of the type strain is 46.3 mol%.

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