Kangiella japonica sp. nov., isolated from a marine environment

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Three Gram-negative, aerobic, halophilic, non-motile, yellowish-pigmented bacteria, designated KMM 3896, KMM 3897 and KMM 3899T, were isolated from coastal seawater and sediment samples of the Sea of Japan, Russia. The isolates were shown to belong to the same species on the basis of 16S rRNA gene sequence similarities (99.6–99.9 %) and DNA–DNA relatedness (73–98 %). Phylogenetic analysis of 16S rRNA gene sequences demonstrated that the isolates formed a subcluster within the genus Kangiella in the class Gammaproteobacteria. 16S rRNA gene sequence similarities between strain KMM 3899T and its closest phylogenetic neighbours, Kangiella koreensis SW-125T and Kangiella aquimarina SW-154T, were 96.6 and 95.5 %, respectively. On the basis of phenotypic differences and phylogenetic distances, it is proposed that strains KMM 3896, KMM 3897 and KMM 3899T are assigned to a novel species of the genus Kangiella, Kangiella japonica sp. nov. The type strain is KMM 3899T (= NRIC 0764T = JCM 16211T).

The genus Kangiella was described by Yoon et al. (2004) and comprises two species, Kangiella koreensis and Kangiella aquimarina, which were isolated from a tidal flat sediment sample of Daepo Beach, Yellow Sea, Korea. Here, we describe three Gram-negative, aerobic bacteria, isolated as described previously (Romanenko et al., 2004) from offshore samples of the Sea of Japan, Russia (42° 44.348' N 133° 14.490' E): strains KMM 3896 and KMM 3897 from seawater and strain KMM 3899T from seawater and sediment KMM 3899T from sediment.

Routine bacterial cultivation was performed in aerobic conditions on marine 2216 agar (MA), in marine broth (MB) and on seawater medium [SWM; containing 5.0 g peptone, 2.5 g yeast extract, 1.0 g glucose, 0.2 g K2HPO4, 0.05 g MgSO4, 15.0 g agar, 1 l natural seawater/distilled water (75:25)]. Motility was observed by the hanging drop method as described by Gerhardt et al. (1994). Phenotypic properties were tested according to the standard methods described by Smibert & Krieg (1994). The oxidation/fermentation medium of Leifson (1963) for marine bacteria was used to test acid production from carbohydrates with 1 % (w/v) of each compound. Growth at different temperatures and pH and resistance to antibiotics were studied as described previously (Romanenko et al., 2004, 2005). Growth with 0–20 % NaCl was examined on SWM prepared with artificial seawater (Lyman & Fleming, 1940). Biochemical tests were performed using API 20E and API ZYM test kits (bioMérieux) according to the manufacturer’s instructions, except that cells were suspended in artificial seawater. For comparative fatty acid analysis, strains KMM 3896, KMM 3897 and KMM 3899T were cultivated on MA at 30 °C for 7 days and the fatty acids were extracted using chloroform/methanol, as described by Bligh & Dyer (1959). Fatty acid methyl esters (FAMES) were obtained by alkaline methanolyis (NaOH/methanol, 15:85), extracted with hexane and analysed by GLC-MS using a gas chromatograph (model 6890) equipped with a 5 % phenyl-methyl-siloxane capillary column (HP5 MS; 30 m × 250 μm × 0.25 μm) and connected to a mass spectrometer (model 5973; all from Hewlett Packard). DNA–DNA hybridization was performed using the fluorometric method of Ezaki et al. (1989).

16S rRNA gene sequences (1511–1514 nt) were determined for strains KMM 3896, KMM 3897 and KMM 3899T as described by Shida et al. (1997) and compared with sequences retrieved from the GenBank database using the FASTA program (Pearson & Lipman, 1988). Phylogenetic analysis was performed using the MEGA4 software (Tamura

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains KMM 3896, KMM 3897 and KMM 3899T are AB505049–AB505051, respectively.

A maximum-parsimony phylogenetic tree based on 16S rRNA gene sequences is available with the online version of this paper.

The International Journal of Systematic and Evolutionary Microbiology (2010), 60, 2583–2586 DOI 10.1099/ijs.0.017087-0

017087 © 2010 IUMS Printed in Great Britain 2583

On: Tue, 12 Feb 2019 21:13:20
et al., 2007) after multiple alignment using CLUSTAL X (version 1.83; Thompson et al., 1997). Phylogenetic trees were reconstructed using the neighbour-joining and maximum-parsimony methods and distances were calculated according to the Kimura two-parameter model. The robustness of phylogenetic trees was estimated by bootstrap analysis of 1000 replicates.

Phylogenetic analysis of the nearly complete 16S rRNA gene sequences showed that the isolates were phylogenetically closely related. For strain KMM 3896, 16S rRNA gene sequence similarity with strain KMM 3899T was 99.9 % and with strain KMM 3897 was 99.6 %. In both the neighbour-joining and the maximum-parsimony trees (Fig. 1 and Supplementary Fig. S1, available in IJSEM Online), the isolates were placed in a cluster that clustered with the genus Kangiella. Strain KMM 3899T exhibited the highest 16S rRNA gene sequence similarities with K. koreensis SW-125T (96.6 %) and K. aquimarina SW-154T (95.5 %). All of the 16S rRNA gene sequence similarities between the isolates and the type strains of the genus Kangiella were significantly lower than the 97 % threshold value proposed by Stackebrandt & Goebel (1994) (subsequently re-evaluated to 98.7 %; Stackebrandt & Ebers, 2006), which indicated that the isolates could be assigned to the genus Kangiella in a novel species.

DNA–DNA relatedness between strains KMM 3899T and KMM 3896 was 98 %; lower values were obtained between strains KMM 3899T and KMM 3897 and between strains KMM 3897 and KMM 3896 (73 and 78 %, respectively). According to the 70 % threshold proposed by Wayne et al. (1987) for the discrimination of species using DNA–DNA relatedness, the results confirmed that the three isolates belonged to the same species.

The physiological, biochemical and chemotaxonomic characteristics of the isolates are given in Tables 1 and 2 and the species description. The fatty acid compositions of the isolates were similar to each other and contained iso-C15:0 as a major fatty acid (Table 2). These results closely agree with data reported for the genus Kangiella (Yoon et al., 2004), although there were differences between the isolates and the recognized type strains, for example the proportions of C16:0 and iso-C17:0 and the presence/absence of iso-C15:0 3-OH, iso-C16:0 and iso-C17:0 3-OH. The DNA G+C content of strains KMM 3896, KMM 3897 and KMM 3899T was determined to be 43.8–45.8 mol%, which is close to those reported for the genus Kangiella (44 mol%; Table 1).

Phenotypically, the characteristics of strains KMM 3896, KMM 3897 and KMM 3899T matched those of the genus Kangiella (Table 1). The isolates were Gram-negative, aerobic, oxidase- and catalase-positive, non-fermentative, halophilic, yellowish-pigmented, non-motile rod-shaped micro-organisms. The isolates could be distinguished from their closest relatives by their negative reaction for trypsin and from K. aquimarina KCTC 12183T by their temperature range for growth. Strains KMM 3899T and KMM 3896 were phenotypically similar to each other; however, some characteristics that differentiated strain KMM 3897 from the other isolates were found (Table 1).

On the basis of the phenotypic, chemotaxonomic and phylogenetic analysis, it is proposed to assign strains KMM 3899T, KMM 3896 and KMM 3897 to the genus Kangiella as representatives of a novel species, Kangiella japonica sp. nov., with KMM 3899T as the type strain.

Description of Kangiella japonica sp. nov.

Kangiella japonica (ja.po’ni.ca. N.L. fem. adj. japonica Japanese, pertaining to the Sea of Japan, from which strains of the species were first isolated).

Gram-negative, aerobic, oxidase- and catalase-positive, non-fermentative, halophilic, non-motile rods, 2.5–3.5 μm in length. On MA, forms slightly yellow, transparent, smooth and shiny colonies with regular edges, 2–3 mm in diameter. Grows with 0.5–13 % NaCl (optimum 2–3 % NaCl); does not grow with 0 or 14 % NaCl. Grows at 4 and 39–42 °C (optimum 28–35 °C); growth at 42 °C is strain-dependent and no growth is observed above 42 °C. Grows at pH 5.5–11.0 (optimum 8.0–10.0). Positive for hydrolysis of casein, Tweens 20, 40 and 80 and L-tyrosine; on L-tyrosine-containing medium, produces melanin-like pigments and clearance zones. Negative for degradation of starch, chitin, agar and CM-cellulose and production of acid from L-arabinose, cellobiose, D-galactose, D-glucose, lactose, maltose, D-mannitol, D-mannose, L-rhamnose, D-sorbitol, sucrose and D-xylose. Hydrolysis of gelatin and

![Fig. 1.](image-url)
DNA and production of H$_2$S are strain-dependent; the type strain is negative for gelatin hydrolysis and negative for DNA hydrolysis and H$_2$S production. With API 20E, negative for PNPG test, H$_2$S production under anaerobic conditions, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, urease, tryptophan deaminase, utilization of citrate and production of indole and acetoin (Voges–Proskauer reaction) and oxidation/fermentation of amygdalin, L-arabinose, D-glucose, inositol, D-mannitol, melibiose, L-rhamnose, D-sorbitol and sucrose. With API ZYM, positive for esterase lipase (C8), leucine arylamidase and valine arylamidase; negative for cystine arylamidase, trypsin, x-chymotrypsin, N-acetyl-β-glucosaminidase, α-fucosidase, α- and β-galactosidase, α- and β-glucosidase, β-gluconoridase and α-mannosidase. Production of alkaline phosphatase, esterase (C4), lipase (C14), acid phosphatase and naphthol-AS-BI-phosphohydrolase is strain-dependent; the type strain is susceptible to penicillin (20 U) and cephalzin (30) and resistant to cephazolin. The major isoprenoid quinone is Q-8. The predominant fatty acids are iso-C$_{15:0}$ 3-OH and iso-C$_{17:0}$. The DNA G+C content of the type strain is 45.8 mol% ($T_m$).

The type strain, KMM 3899$^T$ (=NRIC 0764$^T$ =JCM 16211$^T$), was isolated from an offshore sediment of the Sea of Japan, Russia.

Acknowledgements

This study was supported by the Federal Agency for Science and Innovations of the Russian Federation (state contract no. 02.518.11.7169) and the Presidium of the Far-Eastern Branch of Russian Academy of Sciences for 'Search of marine heterotrophic bacteria biodiversity’ (grant no. 09-III-A-06_227).

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