Aliikangiella marina gen. nov., sp. nov., a marine bacterium from the culture broth of Picochlorum sp. 122, and proposal of Kangiellaceae fam. nov. in the order Oceanospirillales

Guanghua Wang,1 Mingxing Tang,1,2 Huanlian Wu,1 Shikun Dai,1 Tao Li,1 Chenghao Chen,1,2 Hui He,1 Jiewei Fan,1 Wenzhou Xiang1 and Xiang Li1

1Key Laboratory of Tropical Marine Bio-resources and Ecology (LMB), Guangdong Key Laboratory of Marine Materia Medica (LMMM-GD), South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, PR China
2University of Chinese Academy of Sciences, Beijing 100049, PR China

A Gram-stain-negative, non-motile, non-spore-forming, long rod-shaped bacterium, designated strain GYP-15T, was isolated from the culture broth of a marine microalga, Picochlorum sp. 122. Phylogenetic analyses revealed that strain GYP-15T shared 90.6 % 16S rRNA gene sequence similarity with its closest relative, Kangiella aquimarina KCTC 12183T, and represents a distinct phylogenetic lineage in a robust clade consisting of GYP-15T and members of the genera Kangiella and Pleionea in the order Oceanospirillales. Chemotaxonomic and physiological characteristics, including major cellular fatty acids, NaCl tolerance and pattern of carbon source utilization, could also readily distinguish strain GYP-15T from all established genera and species. Thus, it is concluded that strain GYP-15T represents a novel species of a new genus, for which the name Aliikangiella marina gen. nov., sp. nov. is proposed. The type strain of Aliikangiella marina is GYP-15T (=MCCC 1K01163T=KCTC 4266T). Based on phylogenetic results, 16S rRNA gene signature nucleotide pattern and some physiological characteristics, the three genera Kangiella, Pleionea and Aliikangiella are proposed to make up a novel family, Kangiellaceae fam. nov., in the order Oceanospirillales.

The family Alcanivoracaceae was established with the only genus Alcanivorax by Golyshin et al. (2005), and comprises species capable of degradation of petroleum-derived compounds as their main carbon source (Silveira & Thompson, 2014). Recently, the genus Kangiella was assigned to this family based on molecular analyses of 16S rRNA gene sequences (Silveira & Thompson, 2014). However, phylogenetic results of the latest The All-Species Living Tree (Release LTPs119, Munoz et al., 2011) and species identification in the genera Kangiella and Pleionea do not support this assignment (Yoon et al., 2004, 2012; Romanenko et al., 2010; Ahn et al., 2011; Jean et al., 2012; Fagervold et al., 2013; Lee et al., 2013; Kim et al., 2015; Xu et al., 2015), so the exact taxonomic standings of these Kangiella-related organisms are uncertain (Fagervold et al., 2013). In this study, strain GYP-15T, phylogenetically close to the genera Kangiella and Pleionea, was isolated from the culture broth of a marine microalga, Picochlorum sp. 122, and is proposed to represent a novel species of a new genus (Aliikangiella) in the order Oceanospirillales. Finally, these three genera (Kangiella, Pleionea and Aliikangiella) are proposed to make up a new family, Kangiellaceae fam. nov., in the order Oceanospirillales on the basis of their phylogenetic, chemotaxonomic and physiological characteristics.

Picochlorum sp. 122 was isolated from the India Ocean, and cultivated outdoors in filtered natural seawater (collected from the coast of Sanya at 18.30° N 109.32° E, in December 2013) emended with (per litre) 1 g urea, 1 g NaHCO3, 8 mg Na2HPO4 and 5 mg FeSO4·7H2O. The culture broth of Picochlorum sp. 122 was collected at the late exponential phase, stored and transported at room temperature. Strain GYP-15T was isolated from this culture broth by series dilution on a GYP plate (2 g tryptone, 1 g yeast extract, 3 ml glycerol, 18 mg agar powder and 1 litre aged seawater, pH 8.0, with autoclaving at 121°C for 15 min). Subsequent growth experiments were performed aerobically on marine agar 2216 (MA; BD) or in marine broth 2216 (MB; BD) in the dark at 30°C. Bacterial stocks were stored at −70°C in sterile aged seawater supplemented with

The GenBank/EMBL accession number for the 16S rRNA gene sequence of strain GYP-15T is KR078283.

One supplementary table and five supplementary figures are available with the online Supplementary Material.
20 % (v/v) glycerol. For comparative studies, reference strains _Kangiella koreensis_ KCTC 12182^T_, _Kangiella aquimarina_ KCTC 12183^T_ and _Pleione mediterranea_ DSM 25350^T_ were obtained from the Korean Collection for type Cultures (KCTC) and the German Collection of Microorganisms and Cell Cultures (DSMZ). These bacteria were grown on MA or in MB.

The 16S rRNA gene of strain GYP-15^T_ was obtained by using PCR amplification with the universal primers 27F and 1492R (Lane, 1991) and sequenced by Shanghai Majorbio Biopharm Technology. 16S rRNA gene sequence similarities were determined in the GenBank and EzTaxon-e (Kim et al., 2012) databases by using the BLAST tool (Altschul et al., 1990) and Global Alignment (Myers & Miller, 1988). Alignment of 16S rRNA gene sequences was performed using the SINA software package (Pruesse et al. 2012) in the SILVA rRNA database. Phylogenetic trees were reconstructed using the maximum-likelihood (Felsenstein, 1981), neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Swofford, 1993) algorithms in the software package _MEGA_ version 5.0 (Tamura et al., 2011). The phylogenetic distance matrices were estimated by using Kimura’s two-parameter model (Kimura, 1980). The topology of the phylogenetic trees was evaluated by using the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

The nearly complete 16S rRNA gene sequence (1491 bases) of strain GYP-15^T_ was obtained. Results of 16S rRNA gene sequence BLAST comparisons revealed that strain GYP-15^T_ was related most closely to _K. aquimarina_ KCTC 12183^T_ with a similarity of 90.63 %. This low sequence similarity suggested that strain GYP-15^T_ may represent a novel species of a new genus (Yarza et al., 2014). Phylogenetic analysis based on the maximum-likelihood algorithm indicated that strain GYP-15^T_ formed a distinct monophyletic lineage in a robust clade consisting of strain GYP-15^T_, and members of the genera _Kangiella_ and _Pleione_ ( _Kangiella_ group) in the order _Oceanospirillales_ (Fig. 1; see also Fig. S1 in the online Supplementary Material), and this tree topology was also supported by the neighbour-joining and maximum-parsimony algorithms (Figs S2 and S3). Phylogenetic analyses also indicated that _Alcanivorax_, the type genus of the family _Alcanivoracaceae_, does not form a monophyly with the _Kangiella_ group (Figs 1, S2 and S3), which contrasts with the results of Silveira & Thompson (2014). The 16S rRNA gene signature nucleotides of the _Kangiella_ group were found to consist of characteristic nucleotides at positions 103(T), 134(A), 152(G), 169(T), 235(T), 511–513(C/CTC), 538–540(GAG), 589(A), 650(T), 658(A), 681(A), 719(T), 748(T) and 1152(A) ( _Escherichia coli_ 16S rRNA gene sequence numbering). This signature nucleotide profile readily distinguished members of the _Kangiella_ group from those of the genus _Alcanivorax_ and all other defined families in the order _Oceanospirillales_ (Table 1). Therefore, the genera _Kangiella_, _Pleionea_ and the newly proposed genus herein should be assigned to a new family separate from _Alcanivoracaceae_ in the order _Oceanospirillales_.

Cellular morphology and size were determined by using transmission electron microscopy (Hitachi TEM System-H7650). Cell mobility was tested by optical microscopy (Olympus BX53) using the hanging drop technique (Bernardet et al., 2002). The Gram reaction was determined as described by Gerhardt et al. (1994). Catalase activity was determined by observing bubble production in a 3 % (v/v) hydrogen peroxide solution and oxidase activity was determined by using oxidase test strips (Huankai). NaCl requirement and tolerance were tested at 30 °C for 4 days in reconstituted MB with NaCl concentrations ranging from 0 to 15 %, specifically 0, 0.25, 0.5, 1, 2, 3, 5, 6, 8, 10, 13 and 15 % (w/v). Growth at different pH was tested in MB at 30 °C for 4 days emended with different buffers (at 0.5 pH unit intervals: pH 5–8, 0.1 M K_2HPO_4_K,HPO_4; pH 8.5–10, 0.1 M NaHCO_3Na_2CO_3; pH 10.5–11, 0.1 M Na_2CO_3NaOH). Optimal growth temperature was determined on MA after 4 days of growth at 4, 10, 15, 22, 28, 37, 40 and 45 °C. The ability to form endospores and hydrolisis of starch, casein, chitin, gelatin and Tweens 20, 40 and 80 were tested as described by Dong & Cai (2001). Utilization of sole carbon sources was assayed by addition of trehalose, sucrose, d-glucose, d-mannose, d-xylene, lactose, myo-inositol, nicotinic acid, citric acid, L-malic acid, maleic acid, L-alanine, L-glycine, L-glutamate, L-arginine, L-histidine, L-methionine and glycerol to sterile aged natural seawater, respectively. For alkane degradation, 0.25 % n-dodecane was added to bacterial culture broth (MB amended with 0.5 % Tween 20), and after 7 days of incubation with shaking at 30 °C in the dark, the alkane residue was extracted in n-hexane and analysed by GC (Shimadzu 2014C). Additional carbohydrate metabolism was tested using the API 20NE and Biolog GEN III MicroPlate systems according to the manufacturer’s protocols except that cells were suspended in sterile aged natural seawater. Anaerobic growth was determined by using the nitrate reduction hole of the API 20NE system, which was sealed with mineral oil. Other phenotypic characteristics were tested using standard procedures (Tindall et al., 2007).

Cells of strain GYP-15^T_ were Gram-stain-negative, non-motile, non-spore-forming, aerobic long rods (Fig. S4). Colonies on MA were pale tyre-like circles 1–3 mm in diameter after 3 days of incubation at 30 °C, which then became smooth, transparent and pale yellow–green after extended periods of incubation. NaCl was essential for growth, although this isolate was less halotolerant than members of the genera _Pleionea_ (Fagervold et al., 2013) and _Kangiella_ (Kim et al., 2013). There was no decrease in n-dodecane content after incubation, so strain GYP-15^T_, _K. koreensis_ KCTC 12182^T_, _K. aquimarina_ KCTC 12183^T_ and _P. mediterranea_ DSM 25350^T_ appear to have no n-alkane-degrading ability. Only L-arginine was used as sole carbon source. Other cultural, physiological and biochemical properties of strain GYP-15^T_ are listed in Table 2 and in the genus and species descriptions.
Biomass of strain GYP-15\textsuperscript{T} and reference strains for cellular fatty acid analysis was harvested from MA plates grown at 30°C at late exponential phase (3 days). The fatty acid composition was analysed by GC (Agilent G6890N) and components were identified by using the Sherlock Microbial Identification System (Version 6.0) according to the manufacturer’s instructions. Respiratory lipoquinones were extracted as described by Collins (1994) and analysed using reversed-phase HPLC (Komagata & Suzuki, 1987). Polar lipids were extracted as described by Kamekura (1993) and identified by spraying with ethanoic molybdophosphoric acid, molybdenum blue and ninhydrin after two-dimensional TLC (Tindall, 1990). The G+C content of the genomic DNA was determined by using the HPLC method (Mesbah et al., 1989).

The major cellular fatty acids (>5 % of the total) detected in strain GYP-15\textsuperscript{T} were iso-C\textsubscript{15\,}:\,0, iso-C\textsubscript{17\,}:\,0\textsubscript{9c}/C\textsubscript{16\,}:\,0 10-methyl, iso-C\textsubscript{17\,}:\,0, iso-C\textsubscript{16\,}:\,0, C\textsubscript{16\,}:\,0\textsubscript{7c}/C\textsubscript{16\,}:\,0\textsubscript{6c} and iso-C\textsubscript{11\,}:\,0 3-OH (Table S1). Strain GYP-15\textsuperscript{T} and P. mediterranea DSM 25350\textsuperscript{T} (Fagervold et al., 2013) share a high amount of iso-C\textsubscript{17\,}:\,0\textsubscript{9c}/C\textsubscript{16\,}:\,0 10-methyl (>21 %) in addition to iso-C\textsubscript{15\,}:\,0, while members of the genus Kangiella are characterized by a dominance of iso-C\textsubscript{15\,}:\,0 (~50 %, Xu et al., 2015). Strain GYP-15\textsuperscript{T} contained a higher proportion of C\textsubscript{16\,}:\,0\textsubscript{7c}/C\textsubscript{16\,}:\,0\textsubscript{6c} than P. mediterranea DSM 25350\textsuperscript{T}. Meanwhile, members of

**Fig. 1.** Maximum-likelihood phylogenetic tree based on 1491 positions of the 16S rRNA gene sequence of strain GYP-15\textsuperscript{T} and related Gammaproteobacteria. Numbers at nodes indicate percentages of 1000 bootstrap resamplings; only values above 50 % are shown. Bar, 0.05 substitutions per nucleotide position.
the genus *Alcanivorax* are characterized by a dominance of C$_{16}:0$, C$_{18}:1 \omega 7c/\omega 6c$, cyclo fatty acids and C$_{16}:1 \omega 7c/\omega 6c$ (Lai et al., 2013; Rahul et al., 2014; Kwon et al., 2015). Thus, the cellular fatty acid profile not only distinguishes strain GYP-15$^T$ from members of the genera *Alcanivorax*, *Kangiella* and *Pleionea*, but also distinguishes the *Kangiella* group from the genus *Alcanivorax*. Polar lipids in strain GYP-15$^T$ included diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, an unidentified amino phospholipid, an unidentified amino lipid and four unknown lipids (Fig. S5). The presence of phosphatidylethanolamine, phosphatidylglycerol and unidentified amino lipid (near the phosphatidylethanolamine spot) is in line with data for *P. mediterranea* DSM 25350$^T$ (Fagervold et al., 2013), but no diphosphatidylglycerol was detected in *P. mediterranea* DSM 25350$^T$ (Fagervold et al., 2013). An amino phospholipid was detected in GYP-15$^T$ and *P. mediterranea* DSM 25350$^T$ (Fagervold et al., 2013), but not found in members of the genus *Kangiella* (Yoon et al., 2012; Xu et al., 2015). Phosphatidylmonomethylethanolamine was found in some species of the genus *Kangiella* (Yoon et al., 2012; Xu et al., 2015), but was not detected in *P. mediterranea* DSM 25350$^T$ (Fagervold et al., 2013) or strain GYP-15$^T$. The predominant respiratory lipoquinone was ubiquinone-8 (Q-8), which is also the major isoprenoid quinone of members of the genus *Kangiella* and *Pleionea* (Table 2).

In summary, strain GYP-15$^T$ differs from members of the genera *Kangiella* and *Pleionea*, and also members of the genus *Alcanivorax*, in phylogenetic, chemotaxonomic and numerous metabolic characteristics listed in Table 2. Thus, it is suggested that strain GYP-15$^T$ represents a novel species of a new genus, for which the name *Aliikangiella marina* gen. nov., sp. nov. is proposed. On the basis of phylogenetic distance, and differences in major cellular fatty acids and alkane degradation ability of the *Kangiella* group from members of the genus *Alcanivorax* (Table 2), the *Kangiella* group (genera *Kangiella*, *Pleionea* and *Aliikangiella*) is proposed to make up a new family, *Kangiellaceae* fam. nov., in the order *Oceanospirillales*; the type genus of the family is *Kangiella*.

**Description of *Aliikangiella* gen. nov.**

*Aliikangiella* (A.li.i.kan.gi.el’la. L. pronoun. alius other, another; N.L. dim. fem. n. *Kangiella*, the name of a bacterial genus; N.L. fem. n. *Aliikangiella* the other *Kangiella*).

Cells are Gram-stain-negative, non-motile, non-spore-forming rods. Catalase- and oxidase-positive. Negative for urease. Nitrate reduction is weakly positive under aerobic conditions, or negative under anaerobic conditions. NaCl is required for growth. The respiratory lipoquinone is Q-8. The major fatty acids (>10%) are iso-C$_{15}:0$, iso-C$_{17}:1 \omega 6c$, C$_{16}:1 \omega 7c/\omega 6c$, 10-methyl and iso-C$_{17}:0$. The major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and an unidentified amino phospholipid.
The type species is *Aliikangiella marina*.

**Description of Aliikangiella marina sp. nov.**

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

The description is as for the genus with the following additional properties. Cells are usually 0.44–0.67 μm wide and 3.42–5.2 μm long. Colonies are pale tyre-like circles on MA. Growth occurs at pH 6.0–9.0, at 15–37°C and 3.42–5.2% NaCl range for growth (w/v), and optimally at pH 7.0–8.0, at 30°C and 2–12% (w/v) NaCl. Cells cannot degrade n-dodecane. Gelatin, casein, starch, Tweens 20 and 40 are hydrolysed. Production of H2S does not occur. Protease and β-glucosidase are positive, while indole production, D-glucose fermentation, arginine dihydrolase, urease and β-galactosidase are negative. Utilization of D-glucose, L-arabinose, D-mannose, D-mannitol, N-acetylglucosamine, maltose, potassium gluconate, capric acid, adipic acid, malic acid, trisodium citrate and phenylacetic acid is negative. Only L-arginine is used as sole carbon source. In Biolog Gen III microplates, cellobiose, gentiobiose, sucrose, turanose, stachyose, raffinose, α-lactose, melibiose, D-salicin, N-acetyl-D-glucosamine, N-acetyl-β-D-mannosamine, N-acetyl-D-galactosamine, N-acetyl neuraminic acid, D-glucose, D-mannose, D-fructose, L-fucose, L-rhamnose, D-sorbitol, D-mannitol, D-arabitol, myo-inositol, glycerol, glucuronamide, mucic acid, quinic acid, D-saccharic acid, citric acid, α-ketoglutaric acid, D-malic acid, L-malic acid and acetooacetic acid are oxidized.

The type strain, GYP-15*T* (=MCCC 1K01163*T* = KCTC 42267*T*), was isolated from the culture broth of a marine microalga, *Picocloruma* sp. 122. The DNA G+C content of the type strain is 44.7 mol%.

**Description of Kangiellaceae fam. nov.**

*Kangiellaceae* (Kan.gi.el.la.cae’ae. N.L. dim. fem. n. *Kangiella*, the name of a bacterial genus; suff. -aceae ending to denote the name of a family; N.L. fem. pl. n. *Kangiellaceae* the *Kangiella* family).

The family is a member of the order *Oceanospirillales* and encompasses Gram-stain-negative bacteria retrieved from marine environments. The family comprises the genera *Kangiella*, *Plionea* and *Aliikangiella*. The G+C content of the genomic DNA is 43.7–48.9 mol%. The major fatty acids are iso-C15:0 and iso-C17:0 3-OH, C16:1ω9c/C16:0 10-methyl. The 16S rRNA gene signature nucleotides include 103(T), 134(A), 152(G), 169(T), 235(T), 511–513(CTC), 538–540(GAG), 65–72(CTG), 152(G/A) 169(T) 235(T). The 16S rRNA gene signature nucleotides include 103(T), 134(A), 152(G), 169(T), 235(T), 511–513(CTC), 538–540(GAG), 65–72(CTG), 152(G/A) 169(T) 235(T).
589(A), 650(T), 658(A), 681(A), 719(T), 748(T) and 1152(A) (E. coli numbering). The type genus is Kangiella.

Acknowledgements

We thank the reviewers of the manuscript for their contribution to this work and would like to acknowledge editorial support. This research was supported by the Public Science and Technology Research Funds Projects of Ocean (201305018-3), National Natural Science Foundation of China (Nos. 41206136 and 41230962), Guangdong Province and Chinese Academy of Science cooperation Foundation (2012B091100276), Funds for marine renewable energy (GHME-2011SW04) and Guangdong Ocean Innovative Demonstration Area of Economic Development Project (SZHY2012-B01-003).

References


http://ijs.microbiologyresearch.org

Downloaded from www.microbiologyresearch.org by
IP: 54.70.40.11
On: Tue, 11 Dec 2018 09:08:23

Alikangiella marina gen. nov., sp. nov.


