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TAXONOMIC DESCRIPTION

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Kordiimonas pumila sp. nov., isolated from coastal sediment

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Abstract

A novel Gram-stain-negative, translucent-white, aerobic, motile and rod-shaped strain, designated N18^T, was isolated from a coastal sediment sample collected in Zhoushan, Zhejiang Province, China. 16S rRNA gene similarity analysis revealed that strain N18^T demonstrated highest similarity to the genus *Kordiimonas* (95.3–97.2 %). Phylogenetic analysis of 16S rRNA gene sequence showed that strain N18^T represented a distinct lineage in the clade consisting of the genus *Kordiimonas*. Strain N18^T was found to grow at 10–37 °C (optimum 28 °C), pH 6.0–8.0 (optimum 7.0) and with 1.0–4.0 % (w/v) NaCl (optimum 2.5 %). The G+C content of the genomic DNA was 55.3 mol%. The major cellular fatty acids were identified as summed feature 3 (comprising iso-$C_{15:0}$ 2-OH/$C_{16:1}\omega 7c$), iso-$C_{17:1}\omega 9c$ and iso-$C_{15:0}$. The polar lipid profile of N18^T consisted of phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol, an unidentified glycolipid, an unidentified aminoglycolipid, an unidentified aminophospholipid and five unidentified lipids. The respiratory quinone was Q-10. Based on chemotaxonomic, morphological and physiological properties, strain N18^T could be distinguished from its closest phylogenetic neighbours. Thus, we propose *Kordiimonas pumila* sp. nov., the type strain is N18^T (=MCCC 1K03436^T=KCTC 62164^T).

The genus *Kordiimonas*, a member of the order *Kordiimonadales* in the class *Alphaproteobacteria*, was first proposed by Kwon *et al.* and currently consists of six species including *Kordiimonas aestuarii* [1], *Kordiimonas aquimaris* [2], *Kordiimonas gwangyangensis* [3], *Kordiimonas lacus* [4], *Kordiimonas lipolytica* [5] and *Kordiimonas sediminis* [6], which are isolated from marine environments, such as marine sediments and seawater. Cells of members of the genus *Kordiimonas* are Gram-stain-negative, motile, rod-shaped and aerobic. They are oxidase- and catalase-positive. In chemotaxonomic characterizations of the genus *Kordiimonas*, Q-10 is the predominant quinone, phosphatidylglycerol and an unidentified glycolipid are the major components of the polar lipids, and iso-$C_{17:1}\omega 9c$, iso-$C_{15:0}$ and summed feature 3 (comprising iso-$C_{15:0}$ 2-OH/$C_{16:1}\omega 7c$) are detected as major cellular fatty acids [2].

Strain N18^T was isolated from a sediment sample collected from the coast (122.3956° N, 29.9771° E) of Zhoushan, PR China in January 2016. Approximately 10 g sediment sample was inoculated aseptically on marine agar 2216 (MA; BD) at 30 °C by using 1 : 10 serial dilution plating methods. After 4 days of cultivation, one translucent-white colony, designated strain N18^T, was picked and purified by repeated restreaking. Purity was confirmed by the uniformity of cell morphology. The isolate was routinely cultured on marine broth 2216 (MB) medium and maintained at −80 °C with 20 % (v/v) glycerol. Two strains, *K. aquimaris* JCM 16665^T and *K. gwangyangensis* JCM 12864^T were selected as reference strains for polyphasic taxonomic study. Unless otherwise stated, the reference strains *K. aquimaris* JCM 16665^T and *K. gwangyangensis* JCM 12864^T were cultured under the same conditions as strain N18^T.

Cell morphology and motility were observed by optical microscope (BX40; Olympus) and transmission electron microscopy (JEM-1230; JEOL), performed as described by Han *et al.* [7]. The temperature range for growth was determined by incubating the strain at 4, 10, 15, 20, 25, 28, 30, 35, 37, 40, 42, 45 and 50 °C. The pH range for growth was determined in MB adjusted to pH 5–9 (in 0.5 pH unit intervals), using appropriate buffers with the concentration of 30 mM, including MES (pH 5.5–6.5), PIPES (pH 6.5–7.5), Tricine buffer (pH7.5–8.5) and CAPSO (pH 9.0–10.0). After autoclaving, evaluation of the pH values revealed only minor changes. A modified MB was used for NaCl tolerance tests, in which NaCl was omitted (0 %) or added at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 6.0, 8.0, 10.0, 12.0, 13.0 and 15.0 % (w/v) final concentration.

Catalase and Oxidase activities were tested according to Kovacs *et al.* [8], respectively. Gram reaction was tested by the Gram-staining method [9]. H_2S production tests and hydrolysis of casein were assayed according to the method of Zhang

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Abbreviations: HPLC, high-performance liquid chromatography; MA, marine agar; MB, marine broth.

The 16S rRNA gene sequence of strain N18^T has been deposited in GenBank under the accession number MF099898.

Two supplementary figures are available with the online version of this article.

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et al. [10]. Hydrolysis of Tweens 20, 40, 60 and 80 were performed on plates to observe if a transparent circle or aureola appeared according to Sun *et al.* [11]. Anaerobic growth was determined with the AnaeroPack (Mitsubishi) with fumarate (20 mM), nitrate (20 mM) or nitrite (10 mM) used as a potential electron acceptor, as described by Pan *et al.* [12]. Acid production was tested by using API 50CH (bioMérieux) strips. For the inoculation of API 50CH strips, modified oxidative–fermentative medium [13] was used to suspend the cells. API 50CH strips were read after 24 h and 48 h. Additional physiological characteristics and enzyme activities were tested with API 20NE and API ZYM strips (bioMérieux), which were observed after 24 h and 4 h, respectively, according to the manufacturer's instructions. GN2 MicroPlates (Biolog) were used to detect the utilization of organic substrates according to the manufacturer's instructions.

After incubation in MA at 28 °C for 48 h, cells at exponential phase of strain N18^T, *K. aquimaris* JCM 16665^T and *K. gwangyangensis* JCM 12864^T were freeze-dried and used for fatty acid, G+C content, polar lipid and isoprenoid quinone analyses. Fatty acids were extracted as described by Kuykendall *et al.* [14] and analysed according to the standard protocol of the Microbial Identification System (MIDI;

Table 1. Differential characteristics between strain N18^T and its closest phylogenetic relatives

Stains: 1, strain N18^T; 2, *K. aquimaris* JCM 16665^T; 3, *K. gwangyangensis* JCM 12864^T. All strains were motile, strictly aerobic, positive for oxidase and catalase activities, hydrolysis of casein, leucyl-arylaminase, valyl-arylaminase and naphthol-AS-BI-phosphohydrolase activities (API ZYM), acid production from aesculin and potassium gluconate (API 50CH) and utilization of α-cyclodextrin, cellobiose, α-D-glucose, inositol, keto α-glutaric acid, L-asparagnie, L-aspartic acid, glycyl-L-glutamic acid, L-leucine, L-phenylalanine, L-proline and L-threonine (Biolog GN2). +, Positive; −, negative; W, weakly positive; ND, no data available.

Characteristic	1	2	3
Cell size (width×length; μm)	0.4–0.7×1.0–2.9	0.6–0.9×0.7–1.2*	ND*
Temperature range for growth (optimum) (°C)	15–40 (28)	10.5–35*(20)*	17–44*(37–40)*
pH range for growth (optimum)	6.0–8.0 (7.0)	6.0–8.0*(7.0)*	6.0–8.5 (7.0)*
NaCl tolerance (optimum) (%, w/v)	1–4 (2.5)	0–13*(3–3.5)*	0.5–4*(2)*
Hydrolysis of:			
Tween 20	+	−	+
Tween 40	−	W	+
Tween 80	−	W	+
H_2S production	W	−	+
API ZYM tests:			
Chymotrypsin	−	W	W
N-acetyl glucosaminase	−	−	+
Hydrolysis of (API 20NE tests):			
Aesculin	+	−	+
Gelatin	+	−	−
N-Methyl-β-galactose	−	+	−
Acid production from:			
M-Inositol, trehalose	+	−	+
N-Acetyl glucosamine, lyxose, D-mannose, methyl α-D-mannopyranoside	−	−	+
Potassium gluconate	−	+	−
Utilization of:			
Tween 40, Tween 80, D-alanine, L-glutamic acid, L-ornithine, L-serine, γ-amino butyric acid	−	+	+
N-acetyl glucosamine, gentiobiose, maltose, trehalose, L-alanyl-glycine, L-histidine	+	−	+
N-acetyl-D-galactosamine, D-arabitol, putrescine	+	+	−
Dextrin, methyl pyruvate, β-hydroxy butyric acid, glycerol	−	−	+
D-Mannose, citric acid, γ-hydroxy, malonic acid, succinic acid, succinamic acid, L-alaninamide, L-alanine	−	+	−
Lactose, β-D-glucoside, raffinose, sucrose, hydroxy-L-proline, L-pyroglutamic acid, uridine	+	−	−
Polar lipids†	DPG, PG, PE, GL2, L1–5, AGL1, APL	DPG, PG, PE, AL, L6, L7, AGL1, AGL2	DPG, PG, PE, AL, GL1, GL3–5, L1, L3, L5
DNA G+C content (mol%)	55.3	50.3*	55.6–58*

*Data from Yang *et al.* [2] or Kwon *et al.* [3].

†DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; GL, unidentified glycolipid; AGL, unidentified aminoglycolipid; APL, unidentified aminophospholipid; AL, unidentified aminolipid; L, unidentified lipid.

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Microbial ID) and library (RTSBA6 6.21). Isoprenoid quinones were extracted as described by Komagata and Suzuki [15] and analysed by LC-MS (Agilent) [16], using reversed-phase high-performance liquid chromatography (HPLC) [15]. The polar lipids were extracted by two-dimensional thin-layer chromatography on silica gel plates (10×10 cm; Merck 5554) [17] and separated using standard procedures [17, 18].

Genomic DNA was extracted using a Quick Bacteria Genomic DNA Extraction kit (DongSheng Biotech). The primer pair 27F (5′-AGAGTTTGATCCTGGCTCAG-3′)/1492R (5′-GGTTACCTTGTTACGACTT- 3′) was used to amplify the 16S rRNA gene sequence by PCR and the products were cloned into pMD19-T vector (Takara) for sequencing [19]. The almost-complete 16S rRNA gene sequence of strain N18^T was subjected to pairwise sequence alignment by the EzTaxon-e server [20]. Phylogenetic trees were reconstructed by the neighbour-joining [21], maximum-likelihood [22] and maximum-parsimony methods [23] with the MEGA7 program package [24]. One thousand replications were set as the basis of bootstrap analyses for all trees. By using the algorithm described by Kimura, evolutionary distances were calculated with the MEGA 7 program package [24] using the two-parameter model [25] for the neighbour-joining method. The DNA G+C content was determined by means of reversed-phase HPLC according to Mesbah and Whitman [26].

Cells of the strain N18^T were Gram-stain-negative, rod-shaped and motile by means of polar single flagellum (Fig. S1, available in the online version of this article), which was in accordance with the description of the genus *Kordiimonas* [5]. Strain N18^T grew at 10–37 °C (optimum 28 °C), pH 6.0–8.0 (optimum 7.0) and with 1.0–4.0 % (w/v) NaCl (optimum 2.5 %) and could be distinguished from the two reference strains according to optimal temperature for growth (Table 1). All strains were similar in that they could not utilize glycogen, adonitol, i-arabinose, D-erythritol, L-fructose, D-fucose, lactulose, D-mannitol, melibiose, D-psicose, L-rhamnose, D-sorbitol, turanose, xylitol, mono-methyl-succinate, acetic acid, cis-aconitic acid, citric acid, formic acid, D-galactonic acid lactone, D-galacturonic acid, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α-hydroxy butyric acid, p-hydroxy phenylacetic acid, itaconic acid, α-keto butyric acid, α-keto valeric acid, D,L-lactic acid, propionic acid, quinic acid, saccharic acid, sebacic acid, bromo succinic acid, glucuronamide, glycyl-L-asparticacid, D-serine, D,L-carnitine, urocanic acid, inosine, thymidine, phenylethylamine, 2-aminoethanol, 2,3-butanediol, α-glycerol phosphate, glucose-1-phosphate or glucose-6-phosphate. However, some characteristics were found that could be used discriminate strain N18^T from the reference strains. Strain N18^T could hydrolyse gelatin and utilize lactose, methyl β-D-glucoside, raffinose, sucrose, hydroxyl-L-proline, L-pyroglutamic acid and uridine as sole carbon, nitrogen and energy source, while *K. aquimaris* JCM 16665^T and *K. gwangyangensis* JCM 12864^T could not. In contrast, strain N18^T could not hydrolyse Tween 40 and Tween 80 or utilize D-alanine, L-glutamic acid, L-ornithine, L-serine or γ-amino butyric acid, for which *K. aquimaris* JCM 16665^T and *K. gwangyangensis* JCM 12864^T were positive. Detailed results of physiological and biochemical tests are exhibited in the species description. Differential characteristics between strain N18^T, *K. aquimaris* JCM 16665^T and *K. gwangyangensis* JCM 12864^T are summarized in Table 1.

The sole respiratory quinone of the strain N18^T was detected as ubiquinone Q-10, which was in accordance with the genus description [5]. The polar lipid profile of strain N18^T consisted of phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol, an unidentified glycolipid (GL2), an unidentified aminoglycolipid (AGL1), an unidentified aminophospholipid and five unidentified lipids (L1–5) (Fig. S2). The major cellular fatty acids of strain N18^T were summed feature 3 (comprising iso-$C_{15:0}$ 2-OH/$C_{16:1}\omega7c$, 28.5 %), iso-$C_{17:1}\omega9c$ (24.2 %), iso-$C_{15:0}$ (13.0 %) (Table 2).

An almost-complete 16S rRNA gene sequence (1460 nt) was determined for strain N18^T. On the basis of 16S rRNA gene sequence similarity, strain N18^T was affiliated with the genus *Kordiimonas*. According to the EzTaxon service

Table 2. Fatty acid contents (%) of strain N18^T, *K. aquimaris* JCM 16665^T, *K. gwangyangensis* JCM 12864^T

Strains: 1, strain N18^T; 2, *K. aquimaris* JCM 16665^T; 3, *K. gwangyangensis* JCM 12864^T. All strains were grown under identical conditions on MA for 5 days at 30 °C. –, Not detected; TR, trace (<0.5 %). Fatty acids for which amounts were lower than 0.5 % among the three strains were omitted. (All data were from this study.)

Fatty acid	1	2	3
Saturated:			
iso-$C_{15:0}$	13.0	17.8	16.9
iso-$C_{16:0}$	0.7	TR	–
iso-$C_{17:0}$	5.2	9.5	13.4
$C_{14:0}$	0.5	0.7	–
$C_{16:0}$	8.2	4.5	4.4
$C_{17:0}$	0.9	1.4	2.2
$C_{18:0}$	4.0	3.1	0.5
Unsaturated:			
iso-$C_{15:1}$ F	1.3	2.0	0.7
iso-$C_{15:1}\omega8c$	1.1	1.1	TR
iso-$C_{16:1}$ H	0.8	TR	–
iso-$C_{17:1}\omega9c$	24.2	28.8	37.9
$C_{17:1}\omega6c$	3.3	1.8	2.3
$C_{17:1}\omega8c$	3.3	4.4	3.8
Hydroxy:			
iso-$C_{17:0}$ 3-OH	–	2.0	1.0
$C_{18:0}$ 2-OH	0.6	–	–
Summed feature 3*	28.5	18.6	11.7
Summed feature 8*	4.4	1.8	3.8

*Summed features represent groups of two or three fatty acids that could not be separated by gas–liquid chromatography with the MIDI system. Summed feature 3 contained iso-$C_{15:0}$ 2-OH/$C_{16:1}\omega7c$ and summed feature 8 contained $C_{18:1}\omega6c$/iso-$C_{18:0}\omega7c$.

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results, strain N18^T showed a high 16S rRNA gene sequence similarity with respect to *K. aquimaris* JCM 16665^T (97.2 %), and exhibited less than 97.0 % sequence similarity to other type strains of the species of the genus *Kordiimonas*. The phylogenetic trees reconstructed with neighbour-joining method showed that strain N18^T constituted an independent clade within the genus *Kordiimonas*, which was separate from other taxa with validly published names and formed a single cluster (Fig. 1). The DNA G+C content of strain N18^T was 55.3 mol% (HPLC), which was close to members of genus *Kordiimonas* (50–58 mol%) [2].

The phylogenetic analysis was also supported by phenotypic data (detailed differences are shown in Table 1). Cell morphology and characteristics, such as being Gram-stain-negative, aerobic, catalase-positive, oxidase-positive and weakly halophilic, were similar to the description of the genus *Kordiimonas* according to Wu *et al.* [5]. The numerous similarities shared by strain N18^T and the type strains in this study are as follows. Strain N18^T had moderate values of DNA G+C content between 50–58 mol% [2]. Three major fatty acids (>10 %), including iso-$C_{15:0}$, iso-$C_{17:1}\omega 9c$ and summed feature 3 (comprising iso-$C_{15:0}$ 2-OH/$C_{16:1}\omega 7c$), were the same as in strain *K. aquimaris* JCM 16665^T and similar to strain *K. gwangyangensis* JCM 12864^T except for a lower content of iso-$C_{17:0}$. The sole respiratory quinone (Q-10) was identical to strain *K. aquimaris* JCM 16665^T and strain *K. gwangyangensis* JCM 12864^T. Major polar lipids including phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol and an unidentified lipid were detected in all strains. However, there were still some difference in other components. Firstly, strain N18^T had an unidentified glycolipid and five unidentified lipids, while *K. gwangyangensis* JCM 12864^T has four and three, respectively, none were revealed in *K. aquimaris* JCM 16665^T. Secondly, an unidentified aminoglycolipid exists in strain N18^T and *K. aquimaris* JCM 16665^T, but not in *K. gwangyangensis* JCM 12864^T. Thirdly, an unidentified aminophospholipid was detected in strain N18^T, while an unidentified aminoglycolipid was only found in *K. aquimaris* JCM 16665^T. Moreover, an unidentified aminolipid was observed in the reference strains but not in strain N18^T. The differential phenotypic characteristics of strain N18^T and the reference strains are displayed in Table 1.

On the basis of the phylogenetic, genomic, chemotaxonomic and phenotypic characteristics described above, we suggest that the isolate represents a new species within the genus *Kordiimonas*, for which the name *Kordiimonas pumila* sp. nov. is proposed.

DESCRIPTION OF *KORDIIMONAS PUMILA* SP. NOV.

Kordiimonas pumila (pu′mi.la. L. fem. adj. *pumila*, referring to the tiny colonies formed by this organism).

Cells are Gram-stain-negative, rod-shaped, approximately 1.0–2.9 µm long and 0.4–0.7 µm wide, motile by means of a

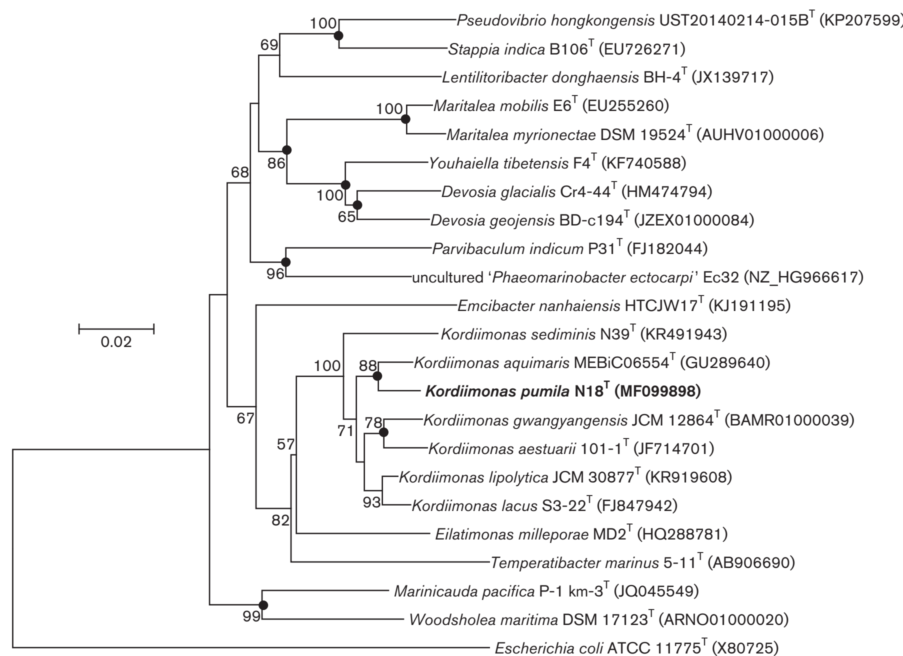

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationships of strain N18^T and related species. Bootstrap values are based on 1000 replicates, shown at branching points. Only bootstrap values above 50 % are shown. Filled circles indicate that the corresponding nodes were also recovered in both the maximum-likelihood and the maximum-parsimony trees. Bar, 0.02 substitutions per nucleotide position. *E. coli* ATCC 11775^T was used as an outgroup.

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polar single flagellum. Colonies are 0.2–0.8 mm in diameter after being incubated for 48 h on MA plates at 28 °C, obviously convex, circular, translucent and smooth. The NaCl concentration range for growth is 1–4 % (w/v) and optimal growth occurs at 2.5 % (w/v). The temperature range for growth is 10–37 °C and the optimal growth temperature is 28 °C. Growth occurs at pH 6.0–8.0, while the optimum is 7.0. No growth is detected in anaerobic conditions by anaerobic respiration with fumarate (20 mM), nitrate (20 mM) or nitrite (10 mM) as electron acceptors. Positive for oxidase, catalase and H_2S production (from thiosulfate L-cysteine). Negative for nitrate or nitrite reduction. Tween 20 and Tween 80 can be hydrolysed, while casein and Tween 40 can only be hydrolysed weakly. Growth using the following substrates (but not other substrates in the strip) as the sole carbon source is observed: α-cyclodextrin, *N*-acetyl-D-galactosamine, *N*-acetyl glucosamine, D-arabitol, cellobiose, gentiobiose, α-D-glucose, m-inositol, lactose, maltose, methyl β-D-glucoside, raffinose, sucrose, trehalose, keto α-glutaric acid, L-alanyl-glycine, L-asparagine, L-aspartic acid, glycyl-L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-phenylalanine, L-proline, L-pyroglutamic acid, L-threonine, uridine and putrescine. The polar lipid profile consists of phosphatidylglycerol, phosphatidylethanolamine, diphosphatidylglycerol, two unidentified glycolipids, an unidentified aminoglycolipid, an unidentified aminophospholipid and five unidentified lipids. The major cellular fatty acids are iso-$C_{15:0}$, iso-$C_{17:1}\omega9c$ and summed feature 3 (comprising iso-$C_{15:0}$ 2-OH/$C_{16:1}\omega7c$). The sole respiratory quinone is ubiquinone Q-10.

The type strain, N18^T (=MCCC 1K03436^T=KCTC 62164^T), was isolated from coastal sediment collected in Zhoushan, PR China. The DNA G+C content of the type strain is 55.3 mol% (determined by HPLC).

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Conflicts of interest

The authors declare that there are no conflicts of interest.

References

1. Math RK, Jeong SH, Jin HM, Park MS, Kim JM *et al. Kordiimonas aestuarii* sp. nov., a marine bacterium isolated from a tidal flat. *Int J Syst Evol Microbiol* 2012;62:3049–3054.
2. Yang SH, Kim MR, Seo HS, Lee SH, Lee JH *et al.* Description of *Kordiimonas aquimaris* sp. nov., isolated from seawater, and emended descriptions of the genus *Kordiimonas* Kwon *et al*. 2005 emend. Xu *et al*. 2011 and of its existing species. *Int J Syst Evol Microbiol* 2013;63:298–302.
3. Kwon KK, Lee HS, Yang SH, Kim SJ. *Kordiimonas gwangyangensis* gen. nov., sp. nov., a marine bacterium isolated from marine sediments that forms a distinct phyletic lineage (*Kordiimonadales* ord. nov.) in the *'Alphaproteobacteria'*. *Int J Syst Evol Microbiol* 2005;55: 2033–2037.
4. Xu XW, Huo YY, Bai XD, Wang CS, Oren A *et al. Kordiimonas lacus* sp. nov., isolated from a ballast water tank, and emended description of the genus *Kordiimonas*. *Int J Syst Evol Microbiol* 2011;61: 422–426.
5. Wu YH, Meng FX, Jian SL, Wang CS, Tohty D. *Kordiimonas lipolytica* sp. nov., isolated from seawater. *Int J Syst Evol Microbiol* 2016;66:2198–2204.
6. Zhang HX, Zhao JX, Chen GJ, du ZJ. *Kordiimonas sediminis* sp. nov., isolated from a sea cucumber culture pond. *Antonie van Leeuwenhoek* 2016;109:705–711.
7. Han SB, Su Y, Hu J, Wang RJ, Sun C *et al. Terasakiella brassicae* sp. nov., isolated from the wastewater of a pickle-processing factory, and emended descriptions of *Terasakiella pusilla* and the genus *Terasakiella*. *Int J Syst Evol Microbiol* 2016;66:1807–1812.
8. Kovacs N. Identification of *Pseudomonas pyocyanea* by the oxidase reaction. *Nature* 1956;178:703.
9. Sun C, Huo YY, Liu JJ, Pan J, Qi YZ *et al. Thalassomonas eurytherma* sp. nov., a marine proteobacterium. *Int J Syst Evol Microbiol* 2014;64:2079–2083.
10. Zhang WY, Huo YY, Zhang XQ, Zhu XF, Wu M. Halolamina salifodinae sp. nov. and *Halolamina salina* sp. nov., two extremely halophilic archaea isolated from a salt mine. *Int J Syst Evol Microbiol* 2013;63:4380–4385.
11. Sun C, Pan J, Zhang X, Su Y, Wu M. *Pseudoroseovarius zhejiangensis* gen. nov., sp. nov., a novel alpha-pro. *Antonie Van Leeuwenhoek* 2015;108:291–299.
12. Pan J, Sun C, Zhang XQ, Huo YY, Zhu XF *et al. Paracoccus sediminis* sp. nov., isolated from Pacific Ocean marine sediment. *Int J Syst Evol Microbiol* 2014;64:2512–2516.
13. Leifson E. Determination of carbohydrate metabolism of marine bacteria. *J Bacteriol* 1963;85:1183–1184.
14. Kuykendall LD, Roy MA, O'Neill JJ, Devine TE. Fatty acids, antibiotic resistance, and deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Bacteriol* 1988;38:358–361.
15. Komagata K, Suzuki KI. 4 Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* 1988;19:161–207.
16. Tindall BJ, Sikorski J, Smibert RA, Krieg NR. *Chapter 15: Phenotypic Characterization and the Principles of Comparative Systematics*. Washington, DC: American Society of Microbiology; 2007.
17. Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M *et al.* An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 1984;2: 233–241.
18. Fang MX, Zhang WW, Zhang YZ, Tan HQ, Zhang XQ *et al. Brassicibacter mesophilus* gen. nov., sp. nov., a strictly anaerobic bacterium isolated from food industry wastewater. *Int J Syst Evol Microbiol* 2012;62:3018–3023.
19. Xu XW, Wu YH, Zhou Z, Wang CS, Zhou YG *et al. Halomonas saccharevitans* sp. nov., *Halomonas arcis* sp. nov. and *Halomonas subterranea* sp. nov., halophilic bacteria isolated from hypersaline environments of China. *Int J Syst Evol Microbiol* 2007;57:1619–1624.
20. Kim OS, Cho YJ, Lee K, Yoon SH, Kim M *et al.* Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 2012;62:716–721.
21. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406.
22. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 1981;17:368–376.
23. Fitch WM. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* 1971;20:406–416.
24. Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.

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25. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.
26. Mesbah M, Premachandran U, Whitman WB. Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* 1989;39:159–167.

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