Kordiimonas lacus sp. nov., isolated from a ballast water tank, and emended description of the genus Kordiimonas

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A Gram-negative, motile, rod-shaped bacterial strain, designated S3-22T, was isolated from a sediment sample collected from a ballast water tank of a commercial ship and subjected to a polyphasic taxonomic characterization. The isolate formed small, light-yellow, semi-translucent and circular colonies on solid complex media. The strain was oxidase- and catalase-positive and metabolized a large number of carbon sources. Chemotaxonomic analysis showed ubiquinone Q-10 as predominant respiratory quinone, phosphatidylglycerol and an unidentified glycolipid as major polar lipids and iso-C17:1ω9c, iso-C15:0 ω7c and/or iso-C19:0 ω7c as major fatty acids and the hydroxy fatty acids iso-C17:0 3-OH and C16:0 3-OH. The genomic DNA G+C content was 54.9 mol%. 16S rRNA gene sequence analysis revealed that the isolate has 96.1 % similarity to the type strain of Kordiimonas gwangyangensis, the sole described species within the order Kordiimonadales, and less than 91.0 % similarity to other recognized species. On the basis of phenotypic and genotypic data, strain S3-22T represents a novel species of the genus Kordiimonas, for which the name Kordiimonas lacus sp. nov. is proposed, with the type strain S3-22T (=CGMCC 1.9109T =JCM 16261T). An emended description of the genus Kordiimonas is also presented.

Ballast water is essential for ships to maintain stability and safety at sea when sailing without cargo. When the water is discharged, sediments and biofilm from the ship’s ballast water tanks can release invasive aquatic species to coastal regions. Micro-organisms have been largely ignored in ballast water research (Drake et al., 2007). The interesting environment of ballast water tanks has rarely been explored for the presence of novel species, and little is known about the potential significance of ship-mediated transfer of these micro-organisms. Here we present a polyphasic study describing a novel Kordiimonas strain isolated from a sediment sample collected from a ship’s ballast water tank. The genus Kordiimonas, belonging to the order Kordiimonadales within the class Alphaproteobacteria, was proposed by Kwon et al. (2005). At the time of writing, Kordiimonas gwangyangensis was the sole described species within the order Kordiimonadales.

The sediment sample was collected from the ballast water tank of a commercial ship arriving at Zhoushan, Zhejiang province, China, in November 2007, and was stored in darkness at 4 °C until micro-organisms were isolated in February 2008. Approximately 100 mg sediment subsample was incubated for 24 h in ZoBell medium, which contained (per l distilled water) 19.45 g NaCl, 8.8 g MgCl2, 3.24 g Na2SO4, 1.8 g CaCl2, 0.55 g KCl, 0.16 g NaHCO3, 0.1 g ferric citrate pentahydrate, 0.08 g KBr, 34 mg SrCl2, 22 mg H3BO3, 4.0 mg Na2SiO3, 2.4 mg NaF, 1.6 mg NH4NO3, 8.0 mg Na2PO4, 5.0 g peptone (BD) and 1.0 g yeast extract (BD), pH 7.4 (ZoBell, 1941). The liquid was plated on ZoBell agar plates using a tenfold dilution series.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain S3-22T is FJ847942.

Four supplementary figures and a supplementary table are available with the online version of this paper.
After 3 days of aerobic inoculation at 30 °C, a light-yellow colony, designated S3-22T, was picked. The strain was purified by repeated restreaking; purity was confirmed by the uniformity of colony morphology. Unless otherwise stated, strain S3-22T was maintained on marine agar 2216 (MA; BD) at 35 °C.

Optimal conditions for growth were determined by using marine broth 2216 (MB) with different NaCl concentrations (0, 0.5, 1.0, 1.5, 2.0, 3.0, 5.0, 7.5, 10.0, 15.0 and 20.0 %, w/v). The pH range for growth was determined by adding MES (pH 5.0–6.0), PIPES (pH 6.5–7.0), Tricine (pH 7.5–8.5) or CAPSO (pH 9.0–10.5) to MB at concentrations of 25 mM. The temperature range for growth was determined by incubating at 4, 10, 15, 18, 25, 30, 37, 43 and 48 °C. Cell motility and morphology were examined by optical microscopy (BX40; Olympus) and transmission electron microscopy (JEM-1230; JEOL).

Single-carbon-source assimilation tests were performed using basal medium (BM). The corresponding filter-sterilized sugar (0.2 %), alcohol (0.2 %), organic acid (0.1 %) or amino acid (0.1 %) was added to liquid medium. The BM (Mikhailov et al., 2006) contained (1−1 distilled water) 1.0 g NH₄Cl, 0.044 g K₂HPO₄, 0.028 g FeSO₄·7H₂O, 500 ml artificial seawater and 100 ml Tris/HCl (1 M, pH 7.5). The artificial seawater contained (1−1 distilled water) 23.4 g NaCl, 24.6 g MgSO₄·7H₂O, 1.5 g KCl and 2.9 g CaCl₂. Acid production was tested using modified marine oxidation-fermentation (MOF) medium supplemented with 1.0 % sugars or alcohols (Leifson, 1963; Xu et al., 2008). Sensitivity to antimicrobial agents was determined on MA for at least 3 days. Oxidase and catalase activities, H₂S production, nitrate reduction and the ability to hydrolyse agar, casein, gelatin, starch and Tween 20, 40, 60 and 80 were assessed according to Dong & Cai (2001). Additional enzyme activities and biochemical characteristics were determined using API 20E, API 20 NE and API ZYM kits (bioMérieux).

Quinones were extracted with chloroform/methanol (2:1, v/v) from freeze-dried cells. The extracts were filtered and evaporated to near dryness and then redissolved in chloroform/methanol (2:1, v/v). The fraction was analysed by heating at 150 °C for 5 min. Glycolipids were detected by spraying the plate with 0.5 % (w/v) x-naphthol in methanol/water (1:1) and then with sulfuric acid/ethanol (1:1) following by heating at 120 °C for 5 min; phospholipids were detected with Zinzadze reagent. Genomic DNA was obtained by using the method described by Marmur (1961). Purified DNA was hydrolysed with P1 nuclease and nucleotides were dephosphorylated with calf intestine alkaline phosphatase; the DNA G+C content of the resulting deoxyribonucleosides was determined by reversed-phase HPLC and calculated from the ratio of deoxyguanosine (dG) and thymidine (dT) (Mesbah & Whitman, 1989).

The 16S rRNA gene was amplified and analysed as described previously (Xu et al., 2007). PCR products were cloned into vector pMD 19-T (TaKaRa) and then sequenced to determine the almost-complete sequence of the 16S rRNA gene. The sequence was compared with closely related sequences of reference organisms from the FASTA and EzTaxon service (Chun et al., 2007). Similar sequences were aligned using the PHYDIT 3.1 program (Jeon et al., 2005). Phylogenetic trees were constructed by the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods with the MEGA 4 program package (Tamura et al., 2007) and the maximum-likelihood method (Felsenstein, 1981) with the PHYLIP 3.6a3 software package. Evolutionary distances were calculated according to the algorithm of Kimura’s two-parameter model (Kimura, 1980) for the neighbour-joining method.

Cells of strain S3-22T were Gram-negative rods, approx. 0.5–0.8 μm wide and 3.0–6.0 μm long. Cells were motile by means of flagella (Supplementary Fig. S1, available in IJSEM Online). Strain S3-22T was able to hydrolyse aesculins, gelatin, starch and Tween 20, 40, 60 and 80 and to utilize more than 20 carbon sources including glycerol, glucose and L-glutamine. The results indicated that the isolate could grow well in seawater containing mixed soluble organic materials. Phenotypic characteristics of strain S3-22T are given in the species description. A comparison of the phenotypic properties of strain S3-22T and K. gwangyangensis JCM 12864T is shown in Table 1.

The almost-complete 16S rRNA gene sequence (1419 nt) of strain S3-22T showed the highest sequence similarity (96.1 %) to that of the type strain of K. gwangyangensis and less than 91.0 % sequence similarity to sequences from other recognized species. The 16S rRNA gene sequence divergence between strain S3-22T and the type strain of K. gwangyangensis exceeded 3 %, an accepted value for the distinction of different genomic species (Stackebrandt & Goebel, 1994). Phylogenetic analysis based on 16S rRNA gene sequences showed that strain S3-22T formed a clade adjacent to the type strain of K. gwangyangensis with a high bootstrap resampling value (100 % by the neighbour-joining method) (Fig. 1). Trees based on the maximum-parsimony and maximum-likelihood methods are available as Supplementary
Table 1. Differentiating characteristics between strain S3-22T and K. gwangyangensis JCM 12864T

Data were obtained in this study under identical growth conditions. Both strains are positive for catalase, oxidase and hydrolysis of starch and Tween 80 and negative for arginine dihydrolase, lysine and ornithine decarboxylases, tryptophan deaminase and urease. Neither and Tween 80 and negative for arginine dihydrolase, lysine and ornithine decarboxylases, tryptophan deaminase and urease. Both strains are positive for catalase, oxidase and hydrolysis of starch.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Strain S3-22T</th>
<th>K. gwangyangensis JCM 12864T</th>
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<tr>
<td>Utilization of:</td>
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<tr>
<td>Gluconate</td>
<td>+</td>
<td>−</td>
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<tr>
<td>Malonate</td>
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<td>−</td>
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<tr>
<td>Mannose</td>
<td>−</td>
<td>+</td>
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<tr>
<td>D-Phenylalanine</td>
<td>−</td>
<td>+</td>
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<tr>
<td>Acid production from mannose</td>
<td>−</td>
<td>+</td>
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<td>Sensitivity to (μg per disc):</td>
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<tr>
<td>Nitrofurantoin (300)</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Tobramycin (10)</td>
<td>−</td>
<td>+</td>
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<tr>
<td>API ZYM results</td>
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<tr>
<td>Cystine arylamidase</td>
<td>−</td>
<td>+</td>
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<tr>
<td>Lipase (C14)</td>
<td>−</td>
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<tr>
<td>α-Mannosidase</td>
<td>−</td>
<td>+</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>54.9</td>
<td>55.6*</td>
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</table>

*Kwon et al. (2005) reported a value of 39.3 mol%.

Figs S2 and S3. Glycolipid analysis indicated that strain S3-22T and K. gwangyangensis JCM 12864T contained the same four glycolipids (GL1–GL4) but that the latter possessed an additional glycolipid (GL5) (Supplementary Fig. S4). Phospholipid analysis revealed that both contained large amounts of phosphatidyglycerol (identification based on location on the TLC plate, the presence of phosphate and the absence of sugar moieties). This characteristic is shared with members of the genera Caulobacter, Brevundimonas, Maricaulis and Hyphomonas, which belong to the order Caulobacterales, the closest neighbour of the order Kordiimonadales in phylogenetic trees (Abraham et al., 1999). An in-depth study of the structure of the polar lipids found in the genus Kordiimonas is recommended in view of the diversity of lipids in phylogenetically related groups (Abraham et al., 1997, 1999; Poin dexter, 2005), but is outside the scope of this paper. The major fatty acids of strain S3-22T were iso-C_{17:0} 3-OH, iso-C_{15:0} 2-OH, C_{16:0}, C_{16:0} 3-OH, and C_{18:1} 107c, with hydroxy fatty acids iso-C_{17:0} 3-OH and C_{16:0} 3-OH. Branched fatty acids are seldom found in alphaproteobacteria, but high contents of iso- and anteiso-branched fatty acids were reported in K. gwangyangensis (Kwon et al., 2005) and in Croceccoccus marinus (Xu et al., 2009). The content of iso-branched fatty acids of strain S3-22T (56.3%) was less than that of K. gwangyangensis JCM 12864T (68.1%) (Supplementary Table S1). Strain S3-22T and K. gwangyangensis JCM 12864T could be differentiated by different reactions in tests for utilization of gluconate, malonate, mannose and D-phenylalanine as sole carbon and energy sources. Further differences included formation of acid from mannose (negative for strain S3-22T), sensitivity to nitrofurantoin and tobramycin for strain S3-22T and several enzyme activities (Table 1).

Ubiquinone Q-10 is found in most members of the Alphaproteobacteria (Dadhwal et al., 2009). LC-MS analysis revealed that the predominant quinone of strain S3-22T and K. gwangyangensis JCM 12864T was Q-10. The data from the control strains, Escherichia coli K-12 (Q-8) and Zangella mobilis CGMCC 1.7002T (Q-10), were in agreement with data from the literature. The G+C content of DNA of strain S3-22T was 54.9 ± 0.3 mol%, as determined by HPLC. This value is much higher than that reported for K. gwangyangensis (39.3 mol%; Kwon et al., 2005). We therefore tested K. gwangyangensis JCM 12864T as well, and found a value of 55.6 ± 0.3 mol%. E. coli K-12, Piscibacillus halophilus DSM 21622T, Oceanobacillus oncornychni subsp. incaldenensis DSM 16557T and Sphingomonas melonis DSM 14444T, included as controls, gave values of 47.6, 38.7, 35.5 and 67.1 mol%, respectively, being close to values reported in the literature. We therefore conclude that the respiratory quinone profile and the G+C content published previously for K. gwangyangensis may be incorrect.

Based on the phenotypic differentiation (Table 1) and genotypic data presented above, we consider that strain S3-22T represents a novel species of the genus Kordiimonas, for which the name Kordiimonas lacus sp. nov. is proposed.

Emended description of Kordiimonas Kwon et al. 2005

In addition to the characteristics reported by Kwon et al. (2005), the following properties are observed. The G+C content of the DNA varies between 54 and 56 mol%. The predominant quinone is Q-10. The polar lipid profile consists of the major components phosphatidyglycerol and an unidentified glycolipid and trace amounts of three unidentified glycolipids. The major fatty acids are iso-C_{17:0} 3-OH, iso-C_{15:0} 2-OH, and C_{16:1} 107c and/or iso-C_{15:0} 2-OH.

Description of Kordiimonas lacus sp. nov.

Kordiimonas lacus (la’cus. L. gen. n. lacus of a lake or any large body of water, of a tank, referring to the isolation of the type strain from a ballast water tank).
Gram-negative and motile by means of flagella. Cells are straight to slightly curved rods (0.5–0.8 μm wide and 3.0–6.0 μm long) with rounded ends. Colonies on MA are 1–2 mm in diameter, smooth, circular with regular borders and light-yellow after 72 h. Growth is observed at 0.5–2 mm in diameter, smooth, circular with regular borders and light-yellow after 72 h. Growth is observed at 0.5–2 mm in diameter, smooth, circular with regular borders and light-yellow after 72 h. Growth is observed at 0.5–2 mm in diameter, smooth, circular with regular borders and light-yellow after 72 h. Growth is observed at 0.5–2 mm in diameter, smooth, circular with regular borders and light-yellow after 72 h.

The type strain, S3-22T (CGMCC 1.9109T = JCM 16261T), was isolated from a sediment sample collected from a ballast water tank. The type strain is 54.9 mol% (HPLC).

**References**


**Acknowledgements**

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**Fig. 1.** Neighbour-joining tree based on 16S rRNA gene sequences, showing the phylogenetic relationships of strain S3-22T and related taxa. Bootstrap values are percentages based on 1000 replicates; only values >70% are shown. Bar, 0.01 substitutions per nucleotide position. Filled circles indicate nodes recovered with bootstrap values >70% in the maximum-parsimony tree. Trees based on the maximum-parsimony and maximum-likelihood methods are available as Supplementary Figs S2 and S3.


