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*'

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A marine bacterium, designated strain GW14-5^T, capable of degrading high-molecular-mass polycyclic aromatic hydrocarbons was isolated from the sediments of Gwangyang Bay, Republic of Korea, after enrichment culture for 2 years with a mixture of benzo[a]pyrene and pyrene. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the isolate forms a phyletic lineage that is distinct from the seven known orders within the '*Alphaproteobacteria*'. 16S rRNA gene sequence similarity of strain GW14-5^T to all recognized bacterial species was not greater than 92%. The dominant fatty acids of the isolate were i-17:1 (46.2%), i-15:0 (15.1%) and i-17:0 (12.6%). The major respiratory quinone was MK-5, and the DNA G+C content was 39.3 mol%. Cells of strain GW14-5^T were Gram-negative, motile, catalase-positive, oxidase-positive and weakly halophilic. Glucose, *N*-acetylglucosamine and maltose were utilized as sole carbon sources. The strain was positive for β -glucosidase activity. Optimal growth of strain GW14-5^T was at pH 7.0 and 37–40 °C and required the presence of 2% (w/v) NaCl. On the basis of this evidence, strain GW14-5^T represents a novel genus and species in the '*Alphaproteobacteria*' for which the name *Kordiimonas gwangyangensis* gen. nov., sp. nov. is proposed. The novel order *Kordiimonadales* is proposed for the distinct phyletic line represented by the genus *Kordiimonas*. The type strain is GW14-5^T (=KCCM 42021^T = JCM 12864^T).

The '*Alphaproteobacteria*' is one of the five classes of the phylum *Proteobacteria* (Garrity & Holt, 2001). The second edition of *Bergey's Manual of Systematic Bacteriology* proposed that the class '*Alphaproteobacteria*' should be divided into six orders: *Caulobacteriales*, '*Rhizobiales*', '*Rhodobacterales*', *Rhodospirillales*, *Rickettsiales* and '*Sphingomonadales*' (Garrity & Holt, 2001). In the last few years, 24 new genera, and the novel order '*Parvularculales*', have been reported in the '*Alphaproteobacteria*' [Cho & Giovannoni, 2003; refer also to the NCBI taxonomy homepage (<http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi>) and articles in this journal (<http://ij.sgmjournals.org>)]. More than half of these new genera were isolated from marine environments, and most members could survive in oligotrophic environments.

In the present study, the taxonomic identification of a novel marine bacterium, strain GW14-5^T, belonging to the

'*Alphaproteobacteria*' is described; on the basis of these results, the strain is considered to represent a novel species and genus, *Kordiimonas gwangyangensis* gen. nov., sp. nov.

Strain GW14-5^T was isolated from marine sediments collected in the Gwangyang Bay of the South Sea, Republic of Korea. About 1 g sediment was enriched with 100 p.p.m. each of pyrene and benzo[a]pyrene (BaP) in MM2 liquid medium [18 mM (NH₄)₂SO₄, 1 μ M FeSO₄·7H₂O and 100 μ l 1 M KH₂PO₄/Na₂HPO₄ buffer solution in 1 litre of aged sea water, pH 7.2] for 2 years (a long-term enrichment method) at a temperature of 10–30 °C. Then, 100 μ l slurry was plated onto marine agar 2216 (MA; Difco) after serial dilution. A tiny, translucent creamy white colony with morphology different from that of the other colonies growing on this medium was isolated and designated GW14-5^T. Strain GW14-5^T was cultivated for 2–3 days at 30 °C on MA for morphological and biochemical characterization.

The degradation rate of BaP by strain GW14-5^T was determined by the fluorimetry method (Zhang *et al.*, 2004). Approximately 40 p.p.b. BaP and 1% 2-hydroxypropyl- β -cyclodextrin were solubilized in MM2 liquid medium,

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Abbreviations: BaP, benzo[a]pyrene; PAH, polycyclic aromatic hydrocarbon.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GW14-5^T is AY682384.

Fig. 1. Rate of degradation of benzo[a]pyrene (BaP) by strain GW14-5^T. The degradation rate was determined by the fluorimetry method (excitation at 293 nm, emission at 406 nm). The y-axis represents the relative intensity of fluorescence, which was measured depending upon the concentration of remaining BaP (180=40 p.p.b. BaP). ▲, Uninoculated control; ●, microcosm with strain GW14-5^T.

and strain GW14-5^T was inoculated into this mixture. The relative intensity of fluorescence (excitation at 293 nm, emission at 406 nm) was measured after 1, 3, 4 and 10 days incubation. The fluorescence of BaP in the culture medium was significantly decreased during the period of incubation compared with that of the non-inoculated control (Fig. 1). The degradation rates of six different mixed polycyclic aromatic hydrocarbons (PAHs) (fluorene, phenanthrene, anthracene, pyrene, chrysene and BaP) were estimated

using the GC method described by Sohn *et al.* (2004) with 5 p.p.m. of each PAH compound at 30 °C. Based on triplicate samples, the degradation rates of the compounds relative to that of the non-inoculated control were: fluorene, 42.1 ± 19.2 %; phenanthrene, 20.8 ± 16.7 %; anthracene, 13.9 ± 14.3 %; pyrene, 18.1 ± 11.2 %; and BaP, 28.0 ± 4.8 %. Chrysene was not degraded within 1 week of incubation.

Unless stated otherwise, physiological characterization was conducted according to the methods of Sohn *et al.* (2004). The Gram reaction was determined using the Bio-Rad Gram-staining kit, and motility was determined with an optical microscope (Zeiss Axioplan) by the hanging drop technique (Skerman, 1967). Cell size and morphology after dehydration were examined with a scanning electron microscope (model S-4300; Hitachi) at 10 kV. Growth temperature was tested over the range 12–48 °C at 4 °C intervals in marine broth 2216 (MB; Difco) in a temperature gradient incubator (TVS126MA; Advantec). Bacterial suspensions for the physiological and biochemical characterizations using an API 20NE test (bioMérieux) and a Microlog GN2 plate (Biolog) were prepared in a 2 % sea salt (Sigma) solution. The physiological, biochemical and morphological characteristics of strain GW14-5^T are given below and in Table 1.

The profile of cellular fatty acid methyl esters was determined according to the method of Bae *et al.* (2005). The dominant fatty acids in strain GW14-5^T were i-17:1 (46.2 %), i-15:0 (15.1 %) and i-17:0 (12.6 %). Strain

Table 1. Phenotypic characteristics differentiating strain GW14-5^T from other members of the ‘*Alphaproteobacteria*’

Strains: 1, GW14-5^T; 2, *Parvularcula bermudensis* HTCC2503^T; 3, *Parvibaculum lavamentivorans* DSM 13023^T; 4, *Caulobacter vibrioides* DSM 9893^T; 5, *Zymomonas mobilis* ATCC 10988^T; 6, *Paracoccus denitrificans* ATCC 17741^T; 7, *Rhizobium leguminosarum* ATCC 10004^T; 8, *Rickettsia prowazekii* ATCC VR-142^T; 9, *Azospirillum lipoferum* (characteristics represent all strains of *Azospirillum lipoferum*, including the type strain, ATCC 29707^T). Data are from Cho & Giovannoni (2003), Jordan (1984), Kocur (1984), Krieg & Döbereiner (1984), Poindexter (1989), Schleheck *et al.* (2004), Swings & De Ley (1984) and Weiss & Moulder (1984). +, Positive; –, negative; w, weakly positive; D, 11–89 % of the strains are positive.

Characteristic	1	2	3	4	5	6	7	8	9
Cell size (µm)	0.25 × 1.3–1.4	0.8 × 1.0	0.2 × 0.8	0.4–0.6 × 1–2	1.0–1.4 × 2.0–6.0	0.5–0.9 × 0.9–1.2	0.5–0.9 × 1.2–3.0	0.3–0.5 × 0.8–2.0	1.0 × 2.1–3.8
Colony size (mm)	2–3	0.3–0.8			1–2	2–3	2–4	0.5–1.5	
Growth temperature (°C)*	17–44 (37–41)	10–37 (30)		10–35 (20–25)	15–38 (30)	5–37 (30)	4–38	(35)	(35–37)
Growth pH*	6–8.5 (7)	6–8.5 (8)		6–9 (7)	3.5–7.5	(7)	4.5–9.0	(7)	(7)
NaCl range for growth (%)*	0.5–4 (2)	0.75–25 (3)			0–1	0–5			≤ 3
DNA G+C content (mol%)	39.3	60.8	64.5	64–65	47.5–49.5	64–67	59–63	29–30	69–70
O ₂ requirement	+	+	+	+	–	–	+	+	–
Oxidase/catalase activity	+ / w	+ / –	+ / +		– / +	+ / +			+ / D
Nitrate reduction	–				–	+			+

*Values in parentheses are the optimum range.

Table 2. Cellular fatty acid composition (%) of strain GW14-5^T and other members of the 'Alphaproteobacteria'

Strains: 1, GW14-5^T; 2, *Parvularcula bermudensis* HTCC2503^T; 3, *Parvibaculum lavamentivorans* DSM 13023^T; 4, *Novosphingobium pentaromativorans* US6-1^T; 5, *Caulobacter vibrioides* DSM 9893^T; 6, *Paracoccus haeundensis* KCCM 10460^T. Data are from Cho & Giovannoni (2003), Lee *et al.* (2004), Poindexter (1989), Schleheck *et al.* (2004) and Sohn *et al.* (2004). No fatty acid composition data are available for members of the orders 'Rhizobiales', 'Rhodospirillales' and 'Rickettsiales'. Fatty acids that represented <1.0 % were omitted.

Fatty acid	1	2	3	4	5	6
Saturated fatty acids						
10:0 3-OH						2.1
12:0 2-OH		5.2				
14:0		2.9				
14:0 2-OH and/or 3-OH			6.3	19.7	3.9	1.5
15:0	1.9					
i-15:0	15.1					
16:0	2.7	8.6	7.7*	3.5*	1.1	
17:0	2.0					
i-17:0	12.6					
a-17:0	3.5					
18:0		4.0	9.7		1.3	7.8
Unsaturated fatty acids						
12:1 5c						2.0
i-15:1	1.4					
16:1†	1.0		1.4‡	8.9		
i-17:1	46.2			2.0		
18:1§	4.2	79.3	53.2	63.9	88.8	84.5
Remainder						
17:0 cyclo	2.6					
19:0 cyclo			22.7¶			
Unidentified, etc.	4.5				3.3	

*Includes 16:0 2-OH and/or 3-OH fatty acids.

†Contained the following fatty acids: 16:1ω5c and/or 16:1ω7c.

‡Includes 16:1 3-OH fatty acids.

§Contained one or more of the following isomers: 18:1ω7c, 18:1ω5c, 18:1ω9t and/or 18:1ω12t (*cis* and *trans* isomer are indicated by the suffixes *c* and *t*, respectively).

||Includes 18:1 2-OH fatty acids.

¶Includes 19:0 2-OH fatty acids.

GW14-5^T also contained other straight-chain saturated fatty acids (7.3 %), straight-chain unsaturated fatty acids (5.4 %) and a cyclo-fatty acid (2.6 %) (Table 2).

The DNA G + C content was 39.3 mol%, as determined by HPLC according to the method of Stackebrandt & Liesack (1993), using a Symmetry reversed-phase C18 column (Waters). The major respiratory quinone was determined to be MK-5 by HPLC analysis according to the method of Collins (1985).

Genomic DNA extraction, PCR and sequencing of the 16S rRNA gene were conducted using the procedures described in Sohn *et al.* (2004). Preliminary sequence BLAST analysis and subsequent phylogenetic analyses indicated that strain GW14-5^T belonged to the 'Alphaproteobacteria' and that its closest recognized relative was *Blastochloris sulfovirdis* (92.0 % 16S rRNA gene sequence similarity). However, 16S rRNA gene sequence similarity to *B. sulfovirdis* decreased to 89.3 % after manual alignment and trimming of the sequence; hence this species was excluded from the phylogenetic tree (Fig. 2). Other species belonging to the 'Rhizobiales', such as *Rhodobium orientis* (91.5 %), *Methylosinus trichosporium* (91.4 %), *Blastochloris viridis* (90.9 %), *Phyllobacterium myrsinacearum* (90.8 %) and *Parvibaculum lavamentivorans* (90.6 %), as well as members of the 'Rhodobacterales', such as *Paracoccus alkaliphilus* (91.0 %) and *Amaricoccus macauensis* (90.4 %), also showed less than 92 % 16S rRNA gene sequence similarity. Therefore, the 16S rRNA gene sequence of strain GW14-5^T was aligned with representative sequences, in the NCBI and RDP databases, of organisms that belong to the seven different orders of the 'Alphaproteobacteria' (Cho & Giovannoni, 2003). The program PHYDIT version 3.2 (<http://plaza.snu.ac.kr/~jchun/phydit/>) was used for this analysis. Phylogenetic distances were calculated from the models of Jukes & Cantor (1969), and the tree was constructed on the basis of the neighbour-joining method (Saitou & Nei, 1987). The resultant phylogenetic tree indicates that strain GW14-5^T forms a phyletic line distinct from all seven orders of the 'Alphaproteobacteria' (Fig. 2). Furthermore, a specific relationship of the strain to these orders was not seen in the maximum-parsimony analysis. On the basis of the phylogenetic analysis, this novel strain appears to represent a novel order of the 'Alphaproteobacteria'.

Another interesting point is the phylogenetic status of *Parvibaculum lavamentivorans*. This strain was considered to be a member of the order 'Rhizobiales' (<http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi>). However, it forms a phyletic lineage with *Parvularcula bermudensis* in the present study (Fig. 2), indicating that *Parvibaculum lavamentivorans* should be reclassified as a member of the 'Parvularculales' or another new order instead of 'Rhizobiales'.

Strain GW14-5^T was clearly distinct, both phenotypically and ecologically, from other members of the 'Alphaproteobacteria'. Many strains belonging to the 'Sphingomonadales', 'Rhodospirillales' and 'Rhizobiales' contain photosynthetic pigments, but strain GW14-5^T does not. It does not associate with plant roots, in contrast to *Mesorhizobium loti*. It has a relatively low DNA G + C ratio similar to *Rickettsia prowazekii* (Weiss & Moulder, 1984) but does not require a host organism. The major fatty acid of members of the 'Alphaproteobacteria', such as *Parvularcula bermudensis* (Cho & Giovannoni, 2003), *Parvibaculum lavamentivorans* (Schleheck *et al.*, 2004), *Roseobacter litoralis* (Labrenz *et al.*, 1999), *Phyllobacterium myrsinacearum* (Mergaert *et al.*,

Fig. 2. Rooted neighbour-joining tree based on nearly complete 16S rRNA gene sequences (1261 unambiguously aligned bases) showing relationships between strain GW14-5^T and representatives of the 'Alphaproteobacteria'. Bootstrap values > 50 % of 1000 resamples are shown. Closed circles at nodes indicate recovered nodes with > 50 % bootstrap value in a maximum-parsimony tree. Bar, 0.05 nucleotide substitutions per nucleotide position.

2002), *Paracoccus haeundensis* (Lee *et al.*, 2004) and *Novosphingobium pentaromativorans* (Sohn *et al.*, 2004), is octadecenoic acid (18:1) (Table 2). That of strain GW14-1^T is iso-heptadecenoic acid (i-17:1).

The results of our morphological, physiological and chemotaxonomic investigations together with the phylogenetic analysis revealed that strain GW14-5^T is clearly distinct from other members of the 'Alphaproteobacteria'. The strain represents a novel species and genus in the 'Alphaproteobacteria', for which the name *Kordiimonas gwangyangensis* gen. nov., sp. nov. is proposed. The new order *Kordiimonadales* is proposed for the distinct phyletic line represented by the genus *Kordiimonas*.

Description of *Kordiimonadales* ord. nov.

Kordiimonadales (Kordii.mo.na.da'les. N.L. fem. n. *Kordiimonas* type genus of the order; -ales ending to denote an order; N.L. fem. n. *Kordiimonadales* the order of *Kordiimonas*).

The description is the same as for the genus *Kordiimonas*. The type genus is *Kordiimonas*.

Description of *Kordiimonas* gen. nov.

Kordiimonas (Kordii.mo'nas. N.L. fem. n. *Kordia* arbitrary name derived from the abbreviation KORDI, which stands for Korea Ocean Research and Development Institute; L. fem. n. *monas* a monad, unit; N.L. fem. n. *Kordiimonas* a micro-organism described by scientists working at KORDI).

Cells are Gram-negative, motile and rod-like in form with a length of 1.3–1.4 µm and width of 0.25 µm. Oxidase and catalase are produced. It is obligately aerobic and weakly halophilic. It has mesophilic characteristics for growth temperature and grows optimally at neutral pH. The dominant fatty acids are branched. The respiratory quinone is MK-5. Phylogenetically, the genus forms the novel order *Kordiimonadales* of the 'Alphaproteobacteria'. The type species is *Kordiimonas gwangyangensis*.

Description of *Kordiimonas gwangyangensis* sp. nov.

Kordiimonas gwangyangensis (gwang.yang.en'sis. N.L. fem. adj. *gwangyangensis* from Gwangyang Bay, the geographical origin of the strain).

In addition to the characteristics reported for the genus, the cells form tiny colonies on MA that are circular, translucent, creamy white and grow up to 2–3 mm in diameter after 1 week. Cells are able to grow between 17 and 44 °C and optimally between 37 and 40 °C. No growth is observed below 16 °C or above 48 °C. Growth occurs at pH 6.0–8.5 and optimally at pH 7.0. The optimal salt concentration for growth is 2%, and the organism has difficulty growing at NaCl concentrations above 4% or below 0.5%. It does not reduce nitrate to nitrite. The DNA G+C content is 39.3 mol%. Positive results are obtained for β -glucosidase and for the assimilation of glucose, *N*-acetylglucosamine and maltose. All other tests in API 20NE were negative. Based on the Microlog system, the strain can oxidize α -cyclodextrin, dextrin, Tweens 40 and 80, *N*-acetylglucosamine, cellobiose, α -D-glucose, maltose, D-trehalose, methyl pyruvate, β -hydroxybutyric acid, L-alanyl glycine, L-aspartic acid, L-glutamic acid, glycyl-L-glutamic acid, L-threonine, putrescine and glucose 1-phosphate. The dominant fatty acids are i-17:1 (46.2%), i-15:0 (15.1%) and i-17:0 (12.6%).

The type strain, GW14-5^T (=KCCM 42021^T=JCM 12864^T), was isolated from the sediments of Gwangyang Bay, Republic of Korea.

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