Ferrimonas pelagia sp. nov., isolated from seawater

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A Gram-stain-negative bacterium, designated strain CBA4601^T, was isolated from a seawater sample obtained off the coast of Jeju Island, Korea. The organism grew in the presence of 0–4 % (w/v) NaCl and at 20–35 °C and pH 7.0–9.0, with optimal growth in 2 % NaCl, and at 25 °C and pH 8.0. Phylogenetic trees based on 16S rRNA gene sequences showed that strain CBA4601^T was related to the genus *Ferrimonas* within the class *Gammaproteobacteria*. 16S rRNA gene sequence similarity between strain CBA4601^T and *Ferrimonas marina* A4D-4^T, the most closely related species, was 96.9 %. The G+C content of the genomic DNA from strain CBA4601^T was 54.2 mol%, and the isoprenoid quinones menaquinone 7 (MK-7), ubiquinone 7 (Q-7) and ubiquinone 8 (Q-8) were detected. The major fatty acids were $C_{17:1}\omega8c$, $C_{18:1}\omega9c$ and $C_{16:0}$, and the major polar lipids were phosphatidylethanolamine, phosphatidylglycerol and an unidentified ninhydrin-positive phospholipid. On the basis of this taxonomic study using a polyphasic approach, strain CBA4601^T represents a novel species of the genus *Ferrimonas*, for which the name *Ferrimonas pelagia* sp. nov. is proposed. The type strain is CBA4601^T (=KACC 16695^T=KCTC 32029^T=JCM 18401^T).

The genus *Ferrimonas*, affiliated with the family *Ferrimonadaceae* in the class *Gammaproteobacteria*, was first proposed by Rosselló-Mora *et al.* (1995). This genus includes, at the time of writing, *Ferrimonas marina* (Katsuta *et al.*, 2005), *F. balearica* (Rosselló-Mora *et al.*, 1995), *F. senticii* (Campbell *et al.*, 2007), *F. kyonanensis* and *F. futtsuensis* (Nakagawa *et al.*, 2006), as well as *F. sediminum* (Ji *et al.*, 2013). Members of the genus *Ferrimonas* are Gram-negative, short, straight rod-shaped cells and facultative anaerobes. In the present study, strain CBA4601^T was evaluated as representing a novel species belonging to the genus *Ferrimonas*, based on phenotypic, phylogenetic, genotypic and chemotaxonomic analyses.

A novel strain, designated CBA4601^T, was isolated from marine water obtained off the coast of Jeju Island, Korea, using a dilution-plating technique on marine agar 2216 medium (MA; Difco). *F. balearica* DSM 9799^T (=PAT^T), *F. futtsuensis* DSM 18154^T (=FUT3661^T), *F. marina* DSM 16917^T (=A4D-4^T) and *F. senticii* DSM 18821^T (=P2S11^T) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH and used for comparative purposes. Cell morphology was observed by scanning transmission electron microscopy (SUPRA VP55; Carl Zeiss) and light microscopy (BA210; Motic). The flagellum was visualized by transmission electron microscopy. Gram staining was performed by the non-staining method described by Buck (1982), using a Gram staining kit (bioMérieux). Growth at different temperatures (4, 10, 15, 20, 35, 40, 45 and 50 °C) and pH (5.0–11.0) was assessed using marine broth medium (MB; Difco). pH was adjusted by adding the following buffers as needed: 10 mM MES for pH 5.0 and 6.0; 10 mM Bistris propane for pH 7.0, 8.0 and 9.0; and 10 mM CAPS for pH 10.0 and 11.0. NaCl tolerance (0.0, 1.0, 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0 %, w/v) was tested using a medium containing all of the constituents of MB except NaCl and supplemented

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

with the appropriate concentrations of NaCl. Growth under anoxic conditions was determined on MA in a Coy anaerobic chamber. Available electron acceptors were identified using 3 mM Fe (III) or selenite in a minimal medium supplemented with 5 mM galactose, glucose and mannose. Both the degradation of starch and cellulose on MA, and H_2S production on Kligler iron agar medium were followed as described by Tindall *et al.* (2007). Enzyme activities and substrate utilization were determined using API ZYM and API 20NE test strips (bioMérieux) according to the manufacturer's instructions. The assimilation of sole carbon sources was tested using API 20NE and AUX medium supplemented with 0.1 % yeast extract. Catalase and oxidase activities were measured using a 3 % (v/v) hydrogen peroxide solution and 1 % (w/v) tetramethyl-*p*-phenylenediamine (bioMérieux), respectively. Strain CBA4601^T was Gram-stain-negative, catalase-positive and oxidase-negative. Colonies of strain CBA4601^T grown on MA were pale yellow, circular, smooth and opaque. The rod-shaped cells (0.5–1.3 μm wide and 1.0–2.0 μm long) were motile by means of a single flagellum (Fig. S1, available in IJSEM Online). Optimal growth of strain CBA4601^T was obtained at 25 °C and pH 8.0, in medium containing 2 % (w/v) NaCl. A detailed description is given in the species description below. A comparison of the characteristics of CBA4601^T with those of its close relatives in the genus *Ferrimonas* is provided in Table 1.

Chromosomal DNA was extracted using a DNA extraction kit (iNtRON Biotechnology). The 16S rRNA gene was amplified by PCR, using the AccPower PCR PreMix (Bioneer) and the bacteria-specific primer set 8F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-AAGGAGGTGATCCAGCCGC-3′). 16S rRNA gene sequencing was performed as described previously (Roh *et al.*, 2008). The identification of phylogenetic neighbours and the calculation of pairwise sequence similarities were carried out using EzTaxon-e (Kim *et al.*, 2012). Phylogenetic relationships between closely related species were determined using MEGA 5.0 (Tamura *et al.*, 2011). A bootstrap analysis to evaluate the stability of phylogenetic trees was performed by obtaining a consensus tree based on 1000 randomly generated trees. The results showed that strain CBA4601^T was closely related to members of the genus *Ferrimonas* (listed in order of highest 16S rRNA gene sequence similarity): *F. marina* A4D-4^T (96.9 %), *F. balearica* PAT^T (95.6 %), *F. senticii* P2S11^T (94.6 %), *F. kyonanensis* Asr22-7^T (92.7 %), *F. futtsuensis* FUT3661^T (92.3 %) and *F. sediminum* JYr13^T (90.0 %). Phylogenetic trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1981) methods. The phylogenetic trees based on 16S rRNA gene sequences showed that strain CBA4601^T formed a tight phyletic lineage with the type strains of species of the genus *Ferrimonas*, regardless of the applied tree-reconstruction algorithm (Fig. 1).

The quinones of strain CBA4601^T grown on MA were analysed by LC-MS using a HPLC system (UltiMate 3000; Dionex) coupled to a diode array detector and a single quadropole mass spectrometer (HCT Ion-Trap MS; Bruker). Three isoprenoid quinones, menaquinone 7 (MK-7, 56.9 %), ubiquinone 7 (Q-7, 21.7 %) and ubiquinone 8 (Q-8, 21.5 %), were identified. Quinone analysis also showed that the quinones of strain CBA4601^T were identical to those of other members of the genus *Ferrimonas* (Campbell *et al.*, 2007; Ji *et al.*, 2013; Katsuta *et al.*, 2005; Nakagawa *et al.*, 2006). Cellular fatty acid composition was analysed according to the instructions provided in the Sherlock Microbial Identification System (MIDI), using strain CBA4601^T and the four reference strains cultivated under the same conditions on MA at

Table 1. Differential characteristics between strain CBA4601^T and the type strains of phylogenetically related species of the genus *Ferrimonas*

Strains: 1, CBA4601^T (data from this study); 2, *F. marina* A4D-4^T (Katsuta *et al.*, 2005); 3, *F. balearica* PAT^T (Rosselló-Mora *et al.*, 1995); 4, *F. senticii* P2S11^T (Campbell *et al.*, 2007); 5, *F. futtsuensis* FUT3661^T (Nakagawa *et al.*, 2006). Based on data obtained from this study, all strains were negative for the degradation of starch and cellulose; lipase (C14), cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activities (based on API ZYM); indole production and D-glucose fermentation (API 20NE). All strains were positive for activities of alkaline phosphatase, naphthol-AS-BI-phosphohydrolase (API ZYM) and β-glucosidase (aesculin hydrolysis) (API 20NE). −, Negative; +, positive.

Characteristic	1	2	3	4	5
Motility	+	+	+	−	+
Optimal temperature (°C)	25	25–30	37*	20–37	30
Growth at 37 °C	−	+	+*	+	−
Growth with NaCl (%):					
0	+	−	−	−	−
5.5	−	−	−	+	−
Fe(III) reduction	−	−	+	−	+
Production of H_2S*	−	−	+	+	+
API ZYM results*:					
Esterase (C4)	−	+	+	+	+
Esterase lipase (C8)	−	+	+	+	+
Leucine arylamidase	−	+	+	+	−
Valine arylamidase	−	−	−	+	−
Acid phosphatase	+	+	+	−	+
α-Glucosidase	−	−	−	+	−
API 20NE results*:					
Reduction of nitrates to nitrites	−	+	+	−	+
L-Arginine dihydrolase	+	−	−	−	−
Urease	+	−	−	−	−
Protease (gelatin hydrolysis)	+	−	−	−	−
β-Galactosidase (PNPG hydrolysis)	−	+	−	+	−
DNA G+C content (mol%)	54.2	60–61	54	54.9	58.1

*Data from this study using reference strains from DSMZ.

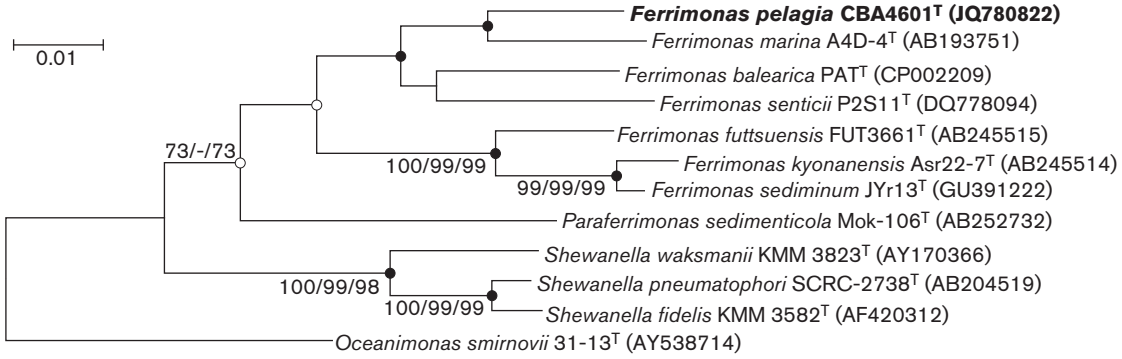

Fig. 1. Phylogenetic tree derived from the 16S rRNA gene sequences of strain CBA4601^T and the type strains of closely related species, based on the neighbour-joining (NJ) algorithm. Filled circles represent nodes also recovered by the maximum-parsimony (MP) and maximum-likelihood (ML) methods. Open circles indicate nodes also recovered either by the MP or the ML method. Numbers at nodes indicate bootstrap values (>70 %) as calculated on the basis of NJ/MP/ML probabilities, expressed as percentages of 1000 replications. *Oceanimonas smirnovii* 31-13^T served as an outgroup. Bar, 0.01 accumulated changes per nucleotide.

25 °C for 4 days. Fatty acids were analysed by GC (6890; Hewlett Packard) and identified using the Microbial Identification software package (Sasser, 1990) based on the TSBA6 database. The detailed fatty acid compositions of the novel strain and of the reference strains are shown in Table 2. The fatty acid pattern of strain CBA4601^T was similar to that of the reference strains, supporting the affiliation of strain CBA4601^T with the genus *Ferrimonas*. However, the fatty acid composition of strain CBA4601^T differed from those of the reference strains in the proportions of certain minor fatty acids (Table 2). Polar lipids of strain CBA4601^T and *F. balearica* DSM 9799^T, the type species of the genus *Ferrimonas*, were extracted according to the method of Xin *et al.* (2000), separated by two-dimensional TLC on a silica gel glass plate (Merck), and detected by spraying the plate with 5 % ethanolic molybdophosphoric acid (for total polar lipids), ninhydrin (aminolipids), Zinzadze reagent (phospholipids) and α-naphthol-sulfuric acid (glycolipids), according to the method of Minnikin *et al.* (1984). The designations of all lipid spots were given according to Khan & Harayama (2007). The major polar lipids of strain CBA4601^T and *F. balearica* DSM 9799^T comprised phosphatidylethanolamine, phosphatidylglycerol and an unidentified ninhydrin-positive phospholipid (Fig. S2), in agreement with those reported for members of the genus *Ferrimonas* (Khan & Harayama, 2007).

DNA–DNA hybridization was performed using photobiotin-labelled DNA probes and microwell plates, as described by Ezaki *et al.* (1989). The DNA–DNA relatedness value between strain CBA4601^T and *F. marina* DSM 16917^T was 27.7 %. It has been shown that two strains with 16S rRNA gene sequence similarity values of less than 97.0 % and DNA–DNA hybridization values of less than 70 % represent different species (Stackebrandt & Goebel, 1994, Wayne *et al.*, 1987). The genomic DNA G+C content was determined according to a fluorimetric method using SYBR Green and a real-time PCR thermocycler (Gonzalez & Saiz-Jimenez, 2002). The DNA G+C content of strain CBA4601^T was 54.2 mol%, which is in the range reported for recognized species of the genus *Ferrimonas* (54–61 mol%) (Katsuta *et al.*, 2005; Rossello-Mora *et al.*, 1995).

The results from the physiological and biochemical tests and from the phylogenetic, chemotaxonomic and genomic analyses indicated genotypic and phenotypic differences between strain CBA4601^T and other members of the genus *Ferrimonas*. Thus, based on this taxonomic study using a polyphasic approach, strain CBA4601^T represents a novel species of the genus *Ferrimonas*, for which the name *Ferrimonas pelagia* sp. nov. is proposed.

Description of *Ferrimonas pelagia* sp. nov.

Ferrimonas pelagia (pe.la′gi.a. L. fem. adj. *pelagia* of or belonging to the sea).

Cells are Gram-stain-negative, motile by a single flagellum, rod-shaped (0.5–1.3 μm wide and 1.0–2.0 μm long), and form round, yellow colonies with a diameter of 1–2 mm after 7 days incubation on MA at 20 °C. Growth occurs in medium containing 0–4 % NaCl, at 20–35 °C and pH 7.0–9.0, with optimal growth in 2 % (w/v) NaCl, at 25 °C and pH 8.0. Catalase-positive but oxidase-negative. Degradation of starch and cellulose, production of H_2S, and reduction of Fe (III) and selenite do not occur, but anaerobic growth on MA does occur. According to API ZYM test strips, positive for alkaline phosphatase, acid phosphatase, and naphthol-AS-BI-phosphohydrolase activities, but negative for esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase

Table 2. Comparison of the fatty acid content (%) of strain CBA4601^T and closely related species of the genus *Ferrimonas*

Strains: 1, CBA4601^T; 2, *F. marina* DSM 16917^T; 3, *F. balearica* DSM 9799^T; 4, *F. senticii* DSM 18821^T; 5, *F. futtsuensis* DSM 18154^T. Data are from this study. Values are percentages of the total fatty acids. tr, Trace (<1.0 %); −, not detected.

Fatty acid	1	2	3	4	5
$C_{10:0}$	1.6	1.1	tr	1.6	1.3
$C_{11:0}$	tr	tr	tr	tr	tr
iso-$C_{11:0}$	tr	tr	tr	−	tr
iso-$C_{11:0}$ 3-OH	−	−	tr	−	−
$C_{11:0}$ 2-OH	tr	tr	tr	tr	tr
$C_{11:0}$ 3-OH	1.1	1.1	1.3	2.2	2.5
iso-$C_{13:0}$ 3-OH	−	−	−	−	7.4
iso-$C_{13:0}$	2.8	3.5	6.3	tr	3.7
$C_{13:0}$	2.3	1.5	3.0	4.3	2.1
$C_{12:0}$	−	6.0	−	−	−
iso-$C_{12:0}$ 3-OH	tr	1.3	1.3	tr	1.2
$C_{12:0}$ 3-OH	3.3	3.4	2.6	2.1	3.6
iso-$C_{14:0}$ 3-OH	−	tr	tr	−	tr
iso-$C_{14:0}$ E	−	−		−	tr
iso-$C_{14:0}$	1.1	3.7	2.3	tr	2.5
$C_{14:0}$	1.5	1.0	tr	tr	tr
iso-$C_{13:0}$ 3-OH	4.6	5.8	7.1	1.2	7.3
iso-$C_{15:0}$	8.9	13.3	15.6	1.7	7.4
iso-$C_{15:0}$ F	−	−	tr	−	−
anteiso-$C_{15:0}$	−	tr	tr	−	tr
$C_{15:1}\omega8c$	1.7	tr	2.0	2.7	1.2
$C_{15:1}\omega6c$	tr	−	tr	tr	tr
$C_{16:1}\omega7c$ alcohol	−	−	−	−	2.6
$C_{16:1}\omega9c$	6.1	2.8	4.7	3.3	3.3
$C_{16:0}$ N alcohol	−	−	tr	−	tr
iso-$C_{16:0}$	−	1.4	tr	tr	tr
$C_{16:0}$	11.0	6.7	6.3	11.8	8.1
$C_{15:0}$ 3-OH	−	−	tr	−	−
iso-$C_{15:0}$ 3-OH	tr	tr	1.6	−	−
10-methyl $C_{17:0}$	−	tr	tr	−	−
iso-$C_{17:0}$	1.5	1.1	tr	−	tr
$C_{17:1}\omega8c$	18.5	12.3	16.9	29.2	21.6
$C_{17:1}\omega6c$	tr	1.1	1.1	4.0	1.9
$C_{17:0}$	2.0	1.5	2.7	8.8	3.0
$C_{18:1}\omega9c$	11.4	11.5	6.0	4.1	6.0
$C_{18:0}$	3.1	1.6	1.5	3.8	1.8
Summed features*					
1	2.8	2.5	3.6	9.4	4.4
2	2.3	2.9	1.9	tr	tr
3	6.2	3.4	2.8	1.9	5.0
8	3.2	6.2	2.2	2.1	3.6
9	tr	tr	−	−	−

*Summed features are groups of two or three fatty acids that cannot be separated by GC with the MIDI system. Summed feature 1 comprises iso-$C_{15:1}$ H and/or $C_{13:0}$ 3-OH; summed feature 2 comprises $C_{12:0}$ aldehyde and/or unknown fatty acids; summed feature 3 comprises $C_{16:1}\omega7c$ and/or $C_{16:1}\omega6c$; summed feature 8 comprises $C_{18:1}\omega7c$ and/or $C_{18:1}\omega6c$; summed feature 9 comprises iso-$C_{17:1}\omega9c$ and/or 10-methyl $C_{16:0}$.

and α-fucosidase activities. According to API 20NE test strips, positive for L-arginine dihydrolase, urease, β-glucosidase (aesculin hydrolysis) and protease (gelatin hydrolysis) activities, and for assimilation of L-arabinose, D-mannose, D-mannitol, *N*-acetylglucosamine, maltose, potassium gluconate, capric acid, adipic acid and phenylacetic acid, but negative for the reduction of nitrates to nitrites and nitrogen, for indole production, D-glucose fermentation and β-galactosidase (PNPG hydrolysis) activity, and for assimilation of D-glucose, L-malate and trisodium citrate. The major isoprenoid quinones are MK-7, Q-7 and Q-8. The predominant fatty acids are $C_{17:1}\omega8c$, $C_{18:1}\omega9c$ and $C_{16:0}$. The major polar lipids are phosphatidylethanolamine, phosphatidylglycerol and an unknown ninhydrin-positive phospholipid.

The type strain, CBA4601^T (=KACC 16695^T=KCTC 32029^T=JCM 18401^T), was isolated from marine water off the coast of Jeju Island, Korea. The genomic DNA G+C content of strain CBA4601^T is 54.2 mol%.

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