Flammeovirga kamogawensis sp. nov., isolated from coastal seawater in Japan

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Two strains of gliding, agarolytic bacteria, strains YS10^T and YML5, were isolated from coastal seawater off Kamogawa, Japan. Phylogenetic analysis based on 16S rRNA gene sequences showed that the novel isolates represent a separate lineage within the genus *Flammeovirga*. DNA–DNA hybridization values between these isolates and the type strains of species of the genus *Flammeovirga* were significantly lower than those accepted as threshold values for the phylogenetic definition of a species. Furthermore, some of the phenotypic characteristics indicate that the isolates differ from other *Flammeovirga* species. Based on these differences, it is suggested that the isolates represent a novel species, for which the name *Flammeovirga kamogawensis* sp. nov. is proposed. The type strain is YS10^T (=IAM 15451^T = NCIMB 14281^T).

The genus *Flammeovirga*, belonging to the family ‘*Flammeovirgaceae*’ (Garrity & Holt, 2001), was described by Nakagawa *et al.* (1997). At present, this genus consists of three species, *Flammeovirga aprica* (Nakagawa *et al.*, 1997), *Flammeovirga arenaria* and *Flammeovirga yaeyamensis* (Takahashi *et al.*, 2006).

In this study, the taxonomic positions of two novel strains, YS10^T and YML5, isolated from seawater collected from the Yoshiura coastline (Kamogawa, Chiba Prefecture, Japan) were determined. The sample (0.05 ml) was spread onto plates of SP5 agar [half-strength artificial seawater (ASW; full-strength ASW consists of 3 % NaCl (w/v), 0.07 % KCl (w/v), 1.08 % MgCl₂·6H₂O (w/v), 0.54 % MgSO₄·7H₂O (w/v) and 0.1 % CaCl₂·2H₂O (w/v)), 0.9 % Casitone (w/v), 0.1 % yeast extract (w/v) and 1.5 % agar (w/v)] and marine agar 2216 (MA; Difco) and incubated at 15 °C for a week. The novel agarolytic strains YS10^T and YML5 were purified and maintained at 25 °C on marine agar.

The 16S rRNA gene sequences were obtained by direct sequencing of PCR-amplified DNA, as described by Hosoya *et al.* (2006). The most similar sequences were obtained from the GenBank database using the BLAST program (Altschul *et al.*, 1990). Nucleotide substitution rates (K_{nuc} ; Kimura, 1980) were determined and a distance matrix tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) with the CLUSTAL_X program (version 1.83; Thompson *et al.*, 1997). Alignment gaps and unidentified

base positions were not taken into consideration in the calculation. Bootstrap analysis was based on 1000 trials.

The results of the phylogenetic analysis based on 16S rRNA gene sequences showed that strains YS10^T and YML5 fall into the genus *Flammeovirga* (Fig. 1). The highest 16S rRNA gene sequence similarity values were found with *F. aprica* (95.0 %), *F. arenaria* (95.7 %) and *F. yaeyamensis* (93.5 %). For analysis of genetic relatedness, DNA–DNA hybridization was carried out at 40 °C for 4 h and measured fluorometrically using the method of Ezaki *et al.* (1989). A high level of DNA–DNA relatedness (78–106 %) was found between strains YS10^T and YML5. The novel isolates showed relatively low DNA–DNA relatedness values with *F. aprica* IAM 14298^T (3.7–5.8 %), *F. arenaria* NBRC 15982^T (6.5–11.1 %) and *F. yaeyamensis* NBRC 100898^T (3.8–16.8 %). This is significantly lower than the value generally accepted as the threshold value for the phylogenetic definition of a species (Wayne *et al.*, 1987).

For determination of the G + C content, DNA was extracted using the method of Saito & Miura (1963). The DNA G + C content was determined according to the method of Mesbah *et al.* (1989). The DNA G + C content of the novel isolates was 32–33 mol%. The values obtained for the reference strains, *F. aprica* IAM 14298^T, *F. arenaria* NBRC 15982^T and *F. yaeyamensis* NBRC 100898^T, were 35.6 mol%, 32.7 mol% and 35.5 mol%, respectively.

The following physiological tests were performed. The respiratory quinone was analysed by the method of Komagata & Suzuki (1987). Growth at different temperatures (8–37 °C), salt tolerance, growth at different pH values, oxidase and catalase activities, degradation of DNA and alginate and hydrolysis of agar and carboxymethylcellulose

Abbreviation: ASW, artificial seawater.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequences of strains YS10^T (=IAM 15451^T = NCIMB 14281^T) and YML5 are AB251933 and AB251934, respectively.

Fig. 1. Neighbour-joining phylogenetic tree showing the position of *Flammeovirga kamogawensis* sp. nov. and related members of the phylum *Bacteroidetes* based on 16S rRNA gene sequence analysis. Bootstrap values greater than 500 are given at branch-points. Bar, 0.02 K_{nuc} .

were determined according to previously described procedures (Hosoya *et al.*, 2006). Degradation of starch was determined using methods described by Smibert & Krieg (1994). Degradation of Tweens (20, 40, 60 and 80) and L-tyrosine was determined by the methods described by Barrow & Feltham (1993). Acid production from carbon sources was assessed using modified O/F medium (Nogi *et al.*, 2004). Tests with the API ZYM and API 20E commercial systems (bioMérieux) were generally performed according to the manufacturer's instructions. The API ZYM tests were read after 4 h incubation at 37 °C and API 20E tests were read after 48 h incubation at 30 °C. Cells for inoculation onto the API test strips were suspended in half-strength ASW. Cell movement at colony edges was verified by using phase-contrast microscopy. For analysis of cellular fatty acids, cells were grown for 48 h at 30 °C on trypticase soy broth (BBL) containing 1.2% Gellan gum, supplemented with half-strength ASW. Cellular fatty acids were analysed by using the GC-based Microbial Identification system (MIDI).

Strains YS10^T and YML5 were Gram-negative, aerobic, non-sporulating, non-fruiting, gliding bacteria. The novel isolates formed reddish-orange colonies in the exponential growth phase, but turned white in the late stationary growth phase. Cells were long rods, 0.6–1.0 µm wide by 2.0–40 µm long. The phenotypic characteristics are given in the species description. Strains YS10^T and YML5 can be differentiated from species of the genus *Flammeovirga* by several characteristics (Table 1). Phenotypic differences between the novel isolates and *F. aprica* IAM 14298^T, *F. arenaria* NBRC 15982^T and *F. yaeyamensis* NBRC 100898^T were seen in the degradation of gelatin, activity of esterase (C4), chymotrypsin, α-galactosidase, β-glucuronidase and α-glucosidase and acid production from rhamnose and xylose.

On the basis of DNA–DNA hybridization, phylogenetic analysis and phenotypic characteristics, the isolates are proposed as representing a novel species of the genus *Flammeovirga*, for which the name *Flammeovirga kamogawensis* sp. nov. is proposed.

Description of *Flammeovirga kamogawensis* sp. nov.

Flammeovirga kamogawensis (kamo.ga.wen'sis. N.L. fem. adj. *kamogawensis* pertaining to Kamogawa, Japan, from where the organisms were isolated).

Table 1. Differential characteristics of strains YS10^T, YML5 and species of the genus *Flammeovirga*

Species: 1, *F. kamogawensis* sp. nov. (2 strains); 2, *F. aprica* IAM 14298^T; 3, *F. arenaria* NBRC 15982^T; 4, *F. yaeyamensis* NBRC 100898^T. +, Positive; –, negative.

Characteristic	1	2	3	4
Degradation of gelatin	+	–	–	+
Activities of:				
Chymotrypsin	–	+	+	–
Esterase (C4)	–	–	+	–
α-Galactosidase	–	–	–	+
α-Glucosidase	–	+	+	–
β-Glucuronidase	–	–	+	–
Acid produced from:				
Rhamnose	–	–	–	+
Xylose	–	+	–	+
DNA G + C content (mol%)	32–33	35.6	32.7	35.5

Table 2. Fatty acid profiles of species of the genus *Flammeovirga*

Species: 1, *F. kamogawensis* (2 strains); 2, *F. aprica* IAM 14298^T; 3, *F. arenaria* NBRC 15982^T; 4, *F. yaeyamensis* (5 strains; data from Takahashi *et al.*, 2006). Values are percentages (w/w) of total fatty acids. —, Not detected; tr, trace amount (<1.0 %).

Fatty acid	1	2	3	4
iso-13:0	1.9–2.5	3.1	2.5	1.2–1.5
13:1 AT 12-13	1.6–1.8	2.5	2.9	0.8–1.5
iso-14:0	4.3–4.8	4.4	5.0	tr
14:0	7.8–9.0	6.7	4.9	3.7–6.2
iso-15:0	21.2–23.0	31.0	25.0	30.5–54.3
anteiso-15:0	1.5–1.9	1.3	2.0	1.5–2.6
15:0	6.4–7.1	2.2	8.1	0.7–2.1
iso-16:1 H	0–3.2	—	1.1	tr
16:0 N alcohol	1.0–1.1	—	1.8	0.3–1.5
16:1 ω 5c	4.0–4.7	—	1.8	2.7–4.4
16:0	2.8–4.4	—	4.2	3.3–4.8
iso-15:0 3-OH	4.9–5.0	5.5	5.4	3.9–5.0
15:0 3-OH	2.2–2.6	2.1	2.8	tr
16:0 3-OH	12.0–14.6	4.1	7.7	8.4–10.5
iso-17:0 3-OH	—	—	—	2.0–2.4
20:4 ω 6c	11.4–12.8	24.7	14.4	15.0–23.2
18:0 3-OH	1.6–1.9	—	3.2	0–4.6
Summed feature 2*	1.1–2.7	1.4	—	tr

*Summed feature 2 contains 14:0 3-OH and/or 16:1 iso I.

Cells are Gram-negative, long rods, 0.6–1.0 μ m wide by 2.0–40 μ m long. Motile by gliding. Colonies are reddish-orange in the exponential growth phase and turn white in the late stationary growth phase. Cytochrome oxidase activity is variable. Catalase activity is positive. Growth occurs at 15–30 °C; the optimum growth temperature is 30 °C. No growth occurs at either 8 °C or 37 °C. The pH range for growth is 6.0–8.0. Growth occurs at NaCl concentrations of 2–4 % (w/v) on R2A agar. Degradation of agar, alginate, carboxymethylcellulose, DNA, gelatin, starch and Tweens (20, 40 and 60) is positive. Nitrate is reduced to nitrite. Positive for alkaline phosphatase, esterase lipase (C8), leucine arylamidase, valine arylamidase, cystine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α -glucosidase and *N*-acetyl- β -glucosaminidase. Acid is produced from cellobiose, galactose, glucose, lactose, maltose and mannose. Does not decompose citrate, Tween 80 or tyrosine. No production of acetoin, H₂S or indole. Negative in tests for arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, tryptophan deaminase, esterase (C4), lipase (C4), trypsin, chymotrypsin, α -galactosidase, β -galactosidase, β -glucuronidase, β -glucosidase, α -mannosidase and α -fucosidase activities. Does not produce acid from arabinose, dulcitol, fructose, glycerol, inositol, mannitol, raffinose, rhamnose, sorbitol, sucrose, trehalose or xylose. The respiratory quinone is MK-7. The

major fatty acids are iso-15:0, 16:0 3-OH and 20:4 ω 6c (Table 2). The DNA G + C content is 32–33 mol%.

The type strain, YS10^T (=IAM 15451^T=NCIMB 14281^T), was isolated from coastal seawater off Yoshiura in Kamogawa in Japan.

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