Fulvivirga kasyanovii gen. nov., sp. nov., a novel member of the phylum *Bacteroidetes* isolated from seawater in a mussel farm

Olga I. Nedashkovskaya,¹ Seung Bum Kim,² Dong Sung Shin,²
Irina A. Beleneva³ and Valery V. Mikhailov¹

Correspondence

Olga I. Nedashkovskaya
olganedashkovska@piboc.dvo.ru
olganedashkovska@yahoo.com

¹Pacific Institute of Bioorganic Chemistry of the Far-Eastern Branch of the Russian Academy of Sciences, Pr. 100 Let Vladivostoku 159, 690022, Vladivostok, Russia

²Department of Microbiology, School of Bioscience and Biotechnology, Chungnam National University, 220 Gung-dong, Yusong, Daejeon 305-764, Republic of Korea

³Institute of Marine Biology of the Far-Eastern Branch of the Russian Academy of Sciences, Pal'chevskogo St. 17, 690032, Vladivostok, Russia

A novel, strictly aerobic, heterotrophic, gliding, Gram-negative, oxidase-, catalase-, β -galactosidase- and alkaline phosphatase-positive marine bacterium, designated strain KMM 6220^T, was isolated from seawater and studied by using a polyphasic taxonomic approach. The DNA G + C content of strain KMM 6220^T was 59.9 mol%. The predominant fatty acids were iso-C_{15:1}, iso-C_{15:0}, iso-C_{15:0} 3-OH, iso-C_{17:0} 3-OH and C_{16:1} ω 7/iso-C_{15:0} 2-OH. Phylogenetic analysis based on 16S rRNA gene sequencing revealed that strain KMM 6220^T formed a cluster with the misclassified strains [*Flexibacter*] *aggregans* NBRC 15974 and [*Flexibacter*] *tractuosus* NBRC 16035 and with the type strains of *Reichenbachiella agariperforans* and *Roseivirga ehrenbergii* with levels of similarity of 95.9, 94.4, 92.0 and 91.8 %, respectively. On the basis of its phenotypic, chemotaxonomic, genotypic and phylogenetic characteristics, strain KMM 6220^T is considered to represent a novel species of a new genus in the phylum *Bacteroidetes*, for which the name *Fulvivirga kasyanovii* gen. nov., sp. nov. is proposed. The type strain of the type species is KMM 6220^T (= CCTCC AB 206119^T = KCTC 12832^T).

Bacteria belonging to the phylum *Bacteroidetes* and sharing menaquinone 7 (MK-7) as a main respiratory quinone have attracted the attention of microbiologists in recent years. Nakagawa *et al.* (2002) conducted the complete 16S rRNA gene sequence-based phylogenetic analysis of NBRC (National Institute of Technology and Evaluation Biological Resource Center, Tokyo, Japan) strains previously placed in the genera *Flexibacter*, *Microscilla* and *Flexithrix* on the basis of their phenotypic properties. They proposed restricting the genera *Flexibacter* and *Microscilla*, known by their extreme heterogeneity, to the type species *Flexibacter flexilis* and *Microscilla marina*, respectively. Nakagawa *et al.* (2002) suggested that other species of these genera be reclassified as new taxa based on their genomic and phenotypic characteristics. Indeed, Kämpfer *et al.* (2006) proposed the transfer of several species of the genus *Flexibacter*, [*Flexibacter*] *sancti*, [*Flexibacter*] *filiformis* and [*Flexibacter*] *japonensis*, to the genus *Chitinophaga* based on phylogenetic, genomic and phenotypic evidence. In addition, the species '*Microscilla arenaria*' was recently

placed in the genus *Flammeovirga* as *Flammeovirga arenaria* (Takahashi *et al.*, 2006). Moreover, members of the genus '*Taxeobacter*' erected by Reichenbach (1992) (name not validly published) were reclassified as species of the genus *Hymenobacter*, the closest phylogenetic relative of '*Taxeobacter*' (Buczolits *et al.*, 2006).

Here, we report the isolation of a novel bacterium of the phylum *Bacteroidetes* isolated from seawater and its identification, based on a polyphasic approach, as a representative of a new genus.

Strain KMM 6220^T was isolated from a seawater sample collected from a mussel farm located in a lagoon of Nha Trang Bay, South China Sea, Viet Nam, in January 2005. After primary isolation by direct plating on medium containing 0.5 % (w/v) Bacto peptone (Difco), 0.2 % (w/v) casein hydrolysate (Merck), 0.2 % (w/v) Bacto yeast extract (Difco), 0.1 % (w/v) glucose, 0.02 % (w/v) KH₂PO₄, 0.005 % (w/v) MgSO₄ and 1.5 % (w/v) Bacto agar (Difco) in 50 % (v/v) natural seawater and 50 % (v/v) distilled water, the strain was cultivated on marine agar (Difco) at 25 °C for 48 h and stored at –80 °C in marine broth (Difco) supplemented with 20 % (v/v) glycerol.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Fulvivirga kasyanovii* sp. nov. KMM 6220^T is DQ836305.

Genomic DNA extraction, PCR and 16S rRNA gene sequencing were carried out as described by Kim *et al.* (1998). Sequence data obtained were aligned with those of representative members of the phylum *Bacteroidetes* using PHYDIT version 3.2 (<http://plaza.snu.ac.kr/~jchun/phydit/>). Phylogenetic trees were inferred by using suitable programs from the PHYLIP package (Felsenstein, 1993). Phylogenetic distances were calculated with the Kimura two-parameter model (Kimura, 1980) and trees were constructed on the basis of the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and maximum-likelihood (Felsenstein, 1993) algorithms. Bootstrap analysis was performed with 1000 resampled datasets by using the SEQBOOT and CONSENSE programs of the PHYLIP package.

Phylogenetic analysis of the almost-complete 16S rRNA gene sequences revealed that strain KMM 6220^T formed a distinct branch within the phylum *Bacteroidetes* (Fig. 1). Its nearest neighbours were [*Flexibacter*] *aggregans* IFO 15974, [*Flexibacter*] *tractuosus* IFO 16035, *Reichenbachiella agariperforans* KMM 3525^T and *Roseivirga ehrenbergii* KMM 6017^T with 16S rRNA gene sequence similarities of 95.9, 94.4, 92.0 and 91.8 %, respectively. Other close relatives were the misclassified species [*Flexibacter*] *tractuosus* ATCC 23168^T, '*Microscilla sericea*' ATCC 23182 and '*Microscilla furvescens*' ATCC 23129 with levels of similarity of 89–89.3 %.

DNA was isolated according to the method of Marmur (1961) and its G + C content was determined by using the thermal denaturation method (Marmur & Doty, 1962). The DNA G + C content of strain KMM 6220^T was 59.9 mol%.

Analysis of fatty acid methyl esters was carried out according to the standard protocol of the Sherlock Microbial Identification System (MIDI Inc.). The predominant cellular fatty acids of strain KMM 6220^T were iso-C_{15:0} (31.2 %), iso-C_{17:0} 3-OH (23.7 %), iso-C_{15:0} 3-OH (7.0 %), iso-C_{15:1} G (5.1 %) and summed feature 3 (24.7 %; comprising C_{16:1}ω7c and/or iso-C_{15:0} 2-OH). Isoprenoid quinones were extracted from lyophilized cells and analysed as described by Nedashkovskaya *et al.* (2004a). Menaquinones were identified by comparison with known quinones from the reference strain *Reichenbachiella agariperforans* KMM 3525^T. The main isoprenoid quinone of the novel isolate was MK-7.

Physiological and biochemical properties of strain KMM 6220^T were examined as described by Nedashkovskaya *et al.* (2004a, b, 2005c).

Cells of strain KMM 6220^T were Gram-negative, chemo-organotrophic, motile by gliding and formed irregular, smooth and yellow–brownish colonies on marine agar. The main physiological and biochemical characteristics are given in Table 1 and in the genus and species descriptions below. Strain KMM 6220^T differed from its closest recognized relatives, which include species of the genera

Fig. 1. Neighbour-joining phylogenetic tree based on the 16S rRNA gene sequences of strain KMM 6220^T and related members of the family *Flexibacteraceae*. Asterisks indicate branches that were also recovered with the maximum-likelihood and maximum-parsimony algorithms. Numbers at nodes indicate bootstrap values above 50 %. Bar, 0.1 substitutions per nucleotide position.

Reichenbachiella and *Roseivirga*, by the production of caseinase and a higher DNA G + C content (Table 1). Strain KMM 6220^T could also be distinguished from *Reichenbachiella agariperforans* by its inability to form acid from carbohydrates. Phenotypic characteristics such as production of flexirubin-type pigments, NaCl requirement for growth and hydrolysis of agar also discriminated the novel isolate from *Reichenbachiella agariperforans* KMM 3525^T (Table 1). The presence of amylase and production of hydrogen sulfide differentiated strain KMM 6220^T from recognized species of the genus *Roseivirga*.

Consequently, the molecular distinctiveness together with differential phenotypic characteristics indicates that strain KMM 6220^T represents a novel species of a new genus within the phylum *Bacteroidetes*, for which the name *Fulvivirga kasyanovii* gen. nov., sp. nov. is proposed.

Table 1. Characteristics that differentiate strain KMM 6220^T from related genera of the phylum *Bacteroidetes*

Taxa: 1, strain KMM 6220^T; 2, *Reichenbachiella*; 3, *Roseivirga*. Reference data are from Lau *et al.* (2006), Nedashkovskaya *et al.* (2003, 2005a, b, c) and Yoon *et al.* (2005). +, Positive; –, negative; v, variable.

Characteristic	1	2	3
Cell size (µm)	0.2–0.3 × 2.1–2.5	0.5–0.7 × 5.0–15.0	0.2–0.5 × 2.1–4.0
Flexirubin-type pigments	–	+	v
Production of hydrogen sulfide	+	–	–
Salinity range for growth (% w/v)	0–10	1–6	0–16
Temperature range for growth (°C)	12–44	4–35	4–44
Acid from carbohydrates	–	+	–
Hydrolysis of:			
Agar	–	+	–
Casein	+	–	–
Gelatin	+	+	v
Starch	+	+	–
DNA G + C content (mol%)	59.9	44.5	40–44

Description of *Fulvivirga* gen. nov.

Fulvivirga (Ful.vi.vir'ga. L. adj. *fulvus* yellow–brownish; L. fem. n. *virga* rod; N.L. fem. n. *fulvivirga* a yellow–brownish-coloured rod).

Cells are strictly aerobic, Gram-negative rods. Do not form endospores. Produce non-diffusible and/or diffusible pigments. Chemo-organotrophic. Cytochrome oxidase-, catalase- and alkaline phosphatase-positive. The predominant cellular fatty acids are branched-chain saturated and unsaturated and straight-chain unsaturated, iso-C_{15:0}, iso-C_{15:1}, iso-C_{15:0} 3-OH, iso-C_{17:0} 3-OH and summed feature 3 (comprising C_{16:1ω7} and/or iso-C_{15:0} 2-OH fatty acids). The main respiratory quinone is MK-7. 16S rRNA gene sequence analysis indicates that the genus *Fulvivirga* is a member of the phylum *Bacteroidetes*. The type species is *Fulvivirga kasyanovii*.

Description of *Fulvivirga kasyanovii* sp. nov.

Fulvivirga kasyanovii [ka.sy.a'no.vi.i. N.L. gen. n. *kasyanovii* of Kasyanov, in honour of Vladimir L. Kasyanov (1940–2005), the famous Russian marine biologist, for his contributions to the development of marine biology and microbiology in the Far East].

The main characteristics are the same as those given for the genus. In addition, cells range from 0.2 to 0.3 µm in width and from 2.3 to 2.5 µm in length and are motile by means of gliding. On marine agar, colonies are 2–3 mm in diameter, irregular, shiny and yellow–brownish-coloured after cultivation for 1–2 days. Weakly diffusible black pigment is also produced after prolonged incubation (4–7 days) on marine agar. Growth is observed at 14–44 °C, with an optimal temperature for growth of 35–37 °C. Growth occurs with 0–10 % (w/v) NaCl, with optimum growth with 2–3 %

(w/v) NaCl. Flexirubin-type pigments are not produced. Decomposes casein, gelatin, starch, elastin, DNA and Tweens 40 and 80. Does not degrade agar, Tween 20, cellulose (carboxymethylcellulose and filter paper) or chitin. Does not form acid from L-arabinose, D-cellobiose, L-fucose, D-galactose, D-glucose, D-lactose, D-maltose, D-melibiose, L-raffinose, L-rhamnose, L-sorbose, D-sucrose, DL-xylose, N-acetylglucosamine, citrate, adonitol, dulcitol, glycerol, inositol or mannitol. Nitrate is not reduced. Hydrogen sulfide is produced, but indole is not. Susceptible to carbenicillin, doxycycline and tetracycline. Resistant to ampicillin, benzylpenicillin, chloramphenicol, erythromycin, gentamicin, kanamycin, lincomycin, neomycin, oleandomycin, polymixin B and streptomycin. Fatty acids accounting for more than 1 % of the total acid content are iso-C_{15:0} (31.2 %), summed feature 3 (24.7 %; comprising C_{16:1ω7c} and/or iso-C_{15:0} 2-OH), iso-C_{17:0} 3-OH (23.7 %), iso-C_{15:0} 3-OH (7.0 %), iso-C_{15:1} G (5.1 %), C_{15:0} (2.9 %), C_{16:1ω5c} (2.5 %) and C_{16:0} (1.2 %). The DNA G + C content of the type strain is 59.9 mol%.

The type strain, KMM 6220^T (=CCTCC AB 206119^T = KCTC 12832^T), was isolated from a seawater sample collected from a mussel farm located in a lagoon of Nha Trang Bay, South China Sea, Viet Nam.

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