Roseivirga ehrenbergii gen. nov., sp. nov., a novel marine bacterium of the phylum 'Bacteroidetes', isolated from the green alga *Ulva fenestrata*

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The taxonomic position of a novel marine bacterium isolated from the green alga *Ulva fenestrata* collected in the Sea of Japan was established. Cells of the strain studied, designated KMM 6017^T, were strictly aerobic, heterotrophic, pink-pigmented, non-motile by gliding, Gram-negative and oxidase-, catalase-, β -galactosidase- and alkaline phosphatase-positive. 16S rRNA gene sequence analysis indicated that the strain occupied a distinct lineage within the phylum 'Bacteroidetes' and formed a cluster with [*Flexibacter*] *tractuosus* and *Reichenbachia agariperforans*. The G+C content of the DNA of KMM 6017^T was 40.2 mol%. The major respiratory quinone was MK-7. The predominant fatty acids were i15:1, i15:0 and i17:0 3-OH (34.2, 24 and 7.7%, respectively). On the basis of phenotypic, chemotaxonomic, genotypic and phylogenetic characteristics, the novel bacterium was assigned to the genus *Roseivirga* gen. nov., as *Roseivirga ehrenbergii* gen. nov., sp. nov. The type strain is KMM 6017^T (=KCTC 12282^T=LMG 22567^T).

Bacteria of the phylum 'Bacteroidetes' are frequently found in marine ecosystems. The majority of recent novel species belonging to this phylum have been included in the family *Flavobacteriaceae* and contain menaquinone-6 (MK-6) as the only or main respiratory lipoquinone (Bernardet *et al.*, 1996, 2002). However, there are many other species of the phylum 'Bacteroidetes', isolated from different marine environments, that instead contain MK-7. For example, a rose-salmon-pigmented strain of the facultative anaerobic bacterium *Marinilabilia salmonicolor* biovar *agarivorans* (Suzuki *et al.*, 1999), formerly *Marinilabilia agarovorans* (Nakagawa & Yamasato, 1996), was found in a beach-mud sample collected from the Californian coast (Veldkamp, 1961). In addition, *Flammeovirga aprica*, formerly *Cytophaga aprica* (Reichenbach, 1989), and *Persicobacter diffluens*,

formerly *Cytophaga diffluens* (Reichenbach, 1989), were isolated from rocky sand in Hawaii and from beach mud near Mumbai, India, respectively (Lewin, 1969; Reichenbach, 1989; Nakagawa *et al.*, 1997). A number of marine bacteria isolated from different beach-sand samples, such as [*Flexibacter*] *aggregans*, '*Microscilla arenaria*', '*Microscilla furvescens*' and [*Flexibacter*] *tractuosus*, remain to be reclassified within new taxa (Bernardet *et al.*, 1996; Nakagawa *et al.*, 1997; Sly *et al.*, 1998). A single strain of the dark-orange-pigmented agarolytic bacterium *Reichenbachia agariperforans*, inhabiting coastal waters of the Sea of Japan, was described (Nedashkovskaya *et al.*, 2003b). Also, rose-pigmented representatives of the genera *Algoriphagus* and *Hongiella* have been recovered from different sources from saline lakes and marine environments (Bowman *et al.*, 2003; Yi & Chun, 2004; Nedashkovskaya *et al.*, 2004; Van Trappen *et al.*, 2004). The pink-coloured bacterium *Belliella baltica* was isolated from surface water of the central Baltic Sea (Brettar *et al.*, 2004).

We report here on the isolation and identification of a novel Gram-negative, aerobic, non-gliding and pink-pigmented marine bacterium isolated from a green alga. Based on a polyphasic taxonomic study of this bacterium, including phylogenetic, genotypic, chemotaxonomic and phenotypic

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Roseivirga ehrenbergii* KMM 6017^T is AY608410.

methods, the description of a novel genus *Roseivirga* is proposed.

Strain KMM 6017^T was isolated from the green alga *Ulva fenestrata* collected in Pallada Bay, Gulf of Peter the Great, Sea of Japan, during August 1999, and cultivated on marine agar 2216 at 28 °C and stored at -80 °C in marine broth supplemented with 20 % (v/v) glycerol.

Genomic DNA extraction, PCR and 16S rRNA gene sequencing followed the procedures of Kim *et al.* (1998). The 16S rRNA gene sequence data obtained were aligned with those of representative members of selected genera that belong to the family *Flavobacteriaceae* using PHYDIT version 3.2 (<http://plaza.snu.ac.kr/~jchun/phydit/>). Phylogenetic trees were inferred using suitable programs of the PHYLIP package (Felsenstein, 1993). Phylogenetic distances were calculated from the models of Kimura (1980) and the trees were constructed on the basis of the neighbour-joining algorithm (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1993) algorithms. Bootstrap analysis was performed with 1000 resampled datasets using the SEQBOOT and CONSENSE programs of the PHYLIP package.

Phylogenetic analysis of the almost-complete 16S rRNA gene sequence (1420 nt) revealed that strain KMM 6017^T forms a distinct lineage within the phylum 'Bacteroidetes' (Fig. 1). [*Flexibacter*] *tractuosus*, *Reichenbachia agariperforans*, *Hongiella mannitolivorans*, *Cyclobacterium marinum* and *Belliella baltica* were the closest phylogenetic relatives of KMM 6017^T (89.3, 87.2, 87, 86.4 and 88.8 % sequence similarity).

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences and generated by the neighbour-joining method showing the relationship between strain KMM 6017^T and other representative members of the *Flexibacteriaceae*. The same topology was also observed in the maximum-likelihood tree, except that KMM 6017^T was first clustered with *Reichenbachia agariperforans* KMM 3525^T, then with [*Flexibacter*] *tractuosus* ATCC 23168^T in the latter tree. Numbers at nodes indicate levels of bootstrap support (%) using 1000 resampled datasets. Bar, 0.1 substitutions per nucleotide position.

DNA was isolated following the method of Marmur (1961) and its G+C content was determined by the thermal denaturation method of Marmur & Doty (1962). G+C content of the DNA of strain KMM 6017^T was 40.2 mol%.

Analysis of fatty acid methyl esters was carried out according to the standard protocol of the Sherlock Microbial Identification System (Microbial ID). Cellular fatty acids comprising more than 1.0 % of the total were i13:0, i15:1, a15:1, i15:0, a15:0, i16:0, i15:0 3-OH, i16:0 3-OH, 16:0 3-OH, i17:0 3-OH and summed feature 3 (16:1 ω 7 and/or i15:0 2-OH) (3.2, 34.2, 1.8, 24, 4.5, 1.1, 3, 4.1, 1.6, 7.7 and 1.7 %, respectively). Isoprenoid quinones were extracted from lyophilized cells and analysed as described by Nedashkovskaya *et al.* (2003a). Menaquinones were identified by comparison with known quinones from reference strain *Reichenbachia agariperforans* KMM 3525^T. The major respiratory quinone was MK-7.

Testing for the following was done as described by Nedashkovskaya *et al.* (2003a, b): degradation of starch, casein, gelatin, cellulose, chitin, DNA, urea and alginic acids; oxidase and catalase activities; growth at different temperatures, NaCl concentrations and pH; production of flexirubin pigment; production of acid from carbohydrates; utilization of carbon sources; and susceptibility to antibiotics. Oxidative or fermentative utilization of glucose was determined on Hugh-Leifson medium modified for marine bacteria (Lemos *et al.*, 1985). Gram-staining reaction, β -galactosidase and alkaline phosphatase activities were tested according to the methods of Gerhardt *et al.* (1994). Gliding motility was determined as described by Bowman (2000).

Physiological, biochemical and morphological characteristics of strain KMM 6017^T are given under the species description and also in Table 1. Phenotypic examination demonstrated many common traits between the strain studied and its closest relatives, *R. agariperforans* and [*Flexibacter*] *tractuosus*. However, strain KMM 6017^T differs clearly from *R. agariperforans* by its inability to move by gliding, the presence of flexirubin pigments, growth at 37 °C, hydrolysis of agar and starch and higher DNA G+C content (Table 1). The algal isolate may be clearly differentiated from [*Flexibacter*] *tractuosus* by the lack of gliding motility, a requirement of Na⁺ for growth and its inability to form acid from carbohydrates (Table 1). Significant molecular and phenotypic differences between the strain studied and the type strains of *R. agariperforans* and [*Flexibacter*] *tractuosus* support the creation of a novel genus for strain KMM 6017^T. Based on the data presented here, we propose that strain KMM 6017^T should be placed in a novel genus and species, *Roseivirga ehrenbergii* gen. nov., sp. nov.

Description of *Roseivirga* gen. nov.

Roseivirga (Ro.se.i.vir'ga. L. adj. *roseus*, -a, -um pink-coloured; L. fem. n. *virga* rod; N. L. fem. n. *Roseivirga* pink-coloured rod).

Table 1. Differential characteristics of *Roseivirga ehrenbergii* gen. nov., sp. nov. and other related genera of the phylum 'Bacteroidetes'

Taxa: 1, *Roseivirga ehrenbergii* gen. nov., sp. nov.; 2, *Reichenbachia agariperforans*; 3, [*Flexibacter*] *tractuosus*; 4, *Cytophaga hutchinsonii*; 5, *Belliella baltica*; 6, *Algoriphagus*; 7, *Hongiella*; 8, *Cyclobacterium marinum*; 9, *Microscilla marina*; 10, *Sporocytophaga myxococcoides*; 11, *Flexibacter flexilis*. Data from Lewin & Lounsbury (1969), Reichenbach (1989), Raj & Maloy (1990), Bowman *et al.* (2003), Nedashkovskaya *et al.* (2003b, 2004), Brettar *et al.* (2004), Yi & Chun (2004), Yoon *et al.* (2004), Van Trappen *et al.* (2004) and this study. –, Negative; +, positive; V, variable; ND, not determined.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Oxidase/catalase	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	+/+	ND/+	+/-
Flexirubin	–	+	–	+	–	–	–	–	–	ND	+
Gliding motility	–	+	+	+	–	–	–	–	+	+	+
Na ⁺ requirement for growth	+	+	–	–	–	V	V	+	+	–	–
Growth at 37 °C	+	–	V	–	+	V	+	+	–	ND	+
Carbohydrate utilization	–	–	+	+	+	+	+	+	+	+	+
Hydrolysis of:											
Agar	–	+	–	–	–	V	–	–	–	–	–
DNA	+	+	ND	ND	+	V	+	–	ND	ND	ND
Starch	–	+	V	–	+	V	+	–	ND	ND	+
Cellulose hydrolysis											
Filter paper	–	–	ND	+	ND	–	–	–	–	+	ND
CM-cellulose	–	–	–	+	ND	–	–	–	–	+	–
Nitrate reduction	–	–	V	ND	+	V	V	–	–	ND	–
DNA G+C content (mol%)	40.2	44.5	35–40	39	35–36	35–42	38–43	34–38	42	36	40–43

Rod-shaped cells, non-motile by gliding. Gram-negative. Does not form endospores. Strictly aerobic. Produces non-diffusible carotenoid pigments. Chemo-organotrophs. Cytochrome oxidase-, catalase- and alkaline phosphatase-positive. The major respiratory quinone is MK-7. The main cellular fatty acids are straight-chain unsaturated and branched-chain unsaturated fatty acids i15:1, i15:0, a15:0 and i17:0 3-OH. 16S rRNA gene sequence analysis indicates that the genus *Roseivirga* is a member of the phylum 'Bacteroidetes'. The type species is *Roseivirga ehrenbergii*.

Description of *Roseivirga ehrenbergii* sp. nov.

Roseivirga ehrenbergii [eh.ren.ber'gi.i. N.L. gen. n. *ehrenbergii* of Ehrenberg, after the German biologist Christian Gottfried Ehrenberg (1795–1876), for his contribution to the development of microbiology].

Characteristics are as given for the genus. In addition, cells are 0.3–0.5 µm in width and 2.1–3.2 µm in length. On marine agar colonies are 2–4 mm in diameter, circular, shiny with entire edges and pink-pigmented. Growth is observed at 4–39 °C (optimum 23–25 °C). Growth occurs at 1–8 % NaCl. β-Galactosidase-positive. Decomposes gelatin, DNA and Tween 20. Does not degrade agar, casein, starch, urea, Tweens 40 or 80, cellulose (CM-cellulose 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, D-sucrose, L-rhamnose, DL-xylose, citrate, adonitol, dulcitol, glycerol, inositol or mannitol. Does not utilize L-arabinose, D-glucose, D-lactose, D-mannose, D-sucrose, mannitol,

inositol, sorbitol, malonate or citrate. Nitrate is not reduced. H₂S, indole and acetoin (Voges–Proskauer reaction) production are negative. Fatty acids accounting for more than 1.0 % of the total are i13:0 (3.2 %), i15:1 (34.2 %), a15:1 (1.8 %), i15:0 (24 %), a15:0 (4.5 %), i16:0 (1.1 %), i15:0 3-OH (3 %), i16:0 3-OH (1.6 %), i17:0 3-OH (7.7 %), summed feature 3 (1.7 %, 16:1ω7 and i15:0 2-OH), and unidentified fatty acids (10.9 %). The G+C content of the DNA is 40.2 mol%.

The type strain, KMM 6017^T (=KCTC 12282^T=LMG 22567^T), was isolated from the green alga *Ulva fenestrata* collected in Pallada Bay, Gulf of Peter the Great, Sea of Japan.

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