Arenibacter troitsensis sp. nov., isolated from marine bottom sediment

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A novel marine, heterotrophic, aerobic, pigmented, non-motile bacterium was isolated from a bottom sediment sample collected from Troitsa Bay in the Gulf of Peter the Great, Sea of Japan, during June 2000. 16S rDNA sequence analysis revealed that this bacterium was a member of the family *Flavobacteriaceae*. On the basis of phenotypic, chemotaxonomic, genotypic and phylogenetic analyses, the bacterium was shown to belong to a novel species of the genus *Arenibacter*, for which the name *Arenibacter troitsensis* sp. nov. is proposed. The type strain is KMM 3674^T (=JCM 11736^T).

The genus *Arenibacter* was established to accommodate Gram-negative, strictly aerobic, heterotrophic, dark-orange pigmented, non-motile marine bacteria that belong to the *Cytophaga-Flavobacterium-Bacteroides* (CFB) phylum (Ivanova *et al.*, 2001). Strains of the single species in this genus, *Arenibacter latericius*, were isolated from different sources: bottom sediments, the brown alga *Chorda filum* and the holothurian *Apostichopus japonicus*. These bacteria have been found in the South China Sea (Indian Ocean), Okhotsk Sea and Sea of Japan (Pacific Ocean). Such a wide distribution of representatives of the genus *Arenibacter* in different marine environments may be explained by their broad spectrum of adaptation to habitat conditions. Phylogenetically, the genus *Arenibacter* forms a cluster with the recently described genera *Zobellia* (Barbeyron *et al.*, 2001) and *Vitellibacter* (Nedashkovskaya *et al.*, 2003) and marine psychrophile ACAM 210.

In June 2000, we isolated an unknown bacterium (strain KMM 3674^T) from a marine bottom sediment sample collected at a depth of 3 m in Troitsa Bay in the Gulf of Peter

the Great, Sea of Japan. Polyphasic taxonomic study of the phenotypic, chemotaxonomic and genotypic characteristics and phylogenetic position of strain KMM 3674^T cultured on Marine Agar 2216 (Difco) that is presented in this work indicates that this isolate is a member of the family *Flavobacteriaceae* and belongs to a novel species of the genus *Arenibacter*, for which the name *Arenibacter troitsensis* sp. nov. is proposed.

Flexirubin pigments were determined by the method of Fautz & Reichenbach (1980). Degradation of alginic acids (1%, w/v) and agar (1.5%, w/v), growth at different temperatures, NaCl concentrations or pH, production of acid from carbohydrates and hydrolysis of starch, casein, chitin, gelatin, cellulose (CM-cellulose and filter paper), DNA and urea were carried out according to Smibert & Krieg (1994). To examine carbon source utilization, a medium that contained 0.2 g NaNO₃, 0.2 g NH₄Cl, 0.05 g yeast extract (Difco) and 0.4% (w/v) carbon source (1 artificial sea water)⁻¹ was used. Carbon sources tested were arabinose, glucose, lactose, mannose, sucrose, inositol, sorbitol, mannitol, fumarate, citrate and malonate. Susceptibility to antibiotics was examined by the plate diffusion method. Discs were impregnated with the following antibiotics: ampicillin (10 µg), benzylpenicillin (10 µg), carbenicillin (100 µg), gentamicin (10 µg), kanamycin (30 µg), lincomycin (15 µg), neomycin (30 µg), oleandomycin (15 µg), polymyxin B (300 U), streptomycin (10 µg) and tetracycline (30 µg).

To determine whole-cell fatty acid profiles, bacteria were grown at 28 °C for 48 h on Marine Agar 2216 (Difco).

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Abbreviations: CFB, *Cytophaga-Flavobacterium-Bacteroides*; JCM, Japan Collection of Microorganisms, Institute of Physical and Chemical Research (RIKEN), Wako, Japan; KMM, Collection of Marine Microorganisms of the Pacific Institute of Bioorganic Chemistry of the Far-Eastern Branch of the Russian Academy of Sciences, Vladivostok, Russia.

The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of *Arenibacter troitsensis* KMM 3674^T is AB080771.

Analysis of fatty acid methyl esters was performed by GLC [30 m × 0.25 mm Supelcowax 10 column (Sigma), 205 °C] as described by Svetashev *et al.* (1995). Isoprenoid quinones were extracted and analysed by the method of Nakagawa & Yamasato (1993). Genomic DNA was isolated by the method of Marmur (1961) and the G+C content of the DNA was determined by the thermal denaturation method (Marmur & Doty, 1962). The 16S rDNA sequence of strain KMM 3674^T was determined by PCR amplification and direct sequencing (Hiraishi, 1992), using conditions and reagents that were described previously (Suzuki *et al.*, 2001). The determined sequence was added to an alignment based on a secondary-structure model, maintained by the small-subunit rRNA database (Van de Peer *et al.*, 2000), by using the profile alignment program of the CLUSTAL W software (Thomson *et al.*, 1994). Evolutionary distances were computed with the DNADIST program in the PHYLIP 3.572 package (Felsenstein, 1995) by using the Kimura two-parameter model (Kimura, 1980) and a phylogenetic tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987). To evaluate the tree, bootstrap analysis with 1000 sample replications was performed with the SEQBOOT and CONSENSE programs in the PHYLIP 3.572 package.

Strain KMM 3674^T was Gram-negative, chemo-organotrophic with a respiratory type of metabolism, non-motile, non-spore-forming and occurred as single flexible rods, 0.4–0.7 µm wide and 3–5 µm long. On Marine Agar 2216, colonies were round with entire edges, 1–3 mm in diameter, dark orange, low convex and shiny. The bacterium was oxidase-, catalase- and alkaline phosphatase-positive and required Na⁺ for growth (Table 1). Growth occurred in media that contained 1–6 % NaCl. The temperature range for growth was 10–42 °C and optimum growth occurred at 30 °C. The pH range for growth was 5.5–10.0 and optimum growth occurred between pH 7.5 and 8.5. No flexirubin pigments were formed. Gelatin and Tween 40 were degraded. Agar, starch, alginate, DNA, cellulose (CM-cellulose and filter paper), chitin, casein, urea, Tween 20, Tween 60 and Tween 80 were not hydrolysed. The organism produced H₂S but did not produce indole or acetoin (Voges-Proskauer reaction) and formed no acid from arabinose, galactose, glucose, lactose, maltose, melibiose, rhamnose, sucrose, xylose, adonitol, dulcitol, inositol or mannitol. The bacterium utilized arabinose, glucose, lactose, mannose and sucrose. Utilization of inositol, mannitol, sorbitol, citrate, fumarate and malonate as sole carbon sources was not observed. Nitrate reduction was positive. The strain was susceptible to oleandomycin, lincomycin and tetracycline and resistant to ampicillin, benzylpenicillin, carbenicillin, kanamycin, streptomycin, gentamicin, neomycin and polymyxin B. Predominant cellular fatty acids were straight-chain saturated and monounsaturated or branched-chain saturated and unsaturated fatty acids, namely C_{15:0} (29.0 %), C_{16:1ω7} (9.6 %), i-C_{15:0} (8.5 %), i-C_{15:1} (18.1 %) and i-C_{17:1} (5.8 %). The major isoprenoid quinone was MK-6. The G+C content

Table 1. Phenotypic characteristics of *Arenibacter troitsensis* KMM 3674^T

Species: 1, *Arenibacter troitsensis* KMM 3674^T; 2, *Arenibacter latericius* (n=5). All strains were positive for: oxidase, catalase and alkaline phosphatase production; Na⁺ requirement for growth; nitrate reduction; growth at 1–6 % NaCl; growth at 10–42 °C; susceptibility to lincomycin and oleandomycin. All strains were negative for: gliding motility; requirement for organic growth factors; flexirubin pigments; indole and acetoin production; degradation of agar, starch, alginic acids, casein, cellulose (CM-cellulose and filter paper) and Tween-80; acid production from arabinose, sucrose, adonitol, dulcitol, inositol, malate, fumarate and citrate; susceptibility to kanamycin, benzylpenicillin, streptomycin, gentamicin, neomycin and polymyxin B. +, Positive; –, negative; v, variable (<60 % of strains showed a positive reaction); v⁺, >60 % of strains showed a positive reaction; v[–], >60 % of strains showed a negative reaction. The reactions of *A. latericius* KMM 426^T are indicated in parentheses.

Characteristic	1	2
H ₂ S production	+	– (–)
Degradation of:		
Gelatin	+	– (–)
Tween 20	–	v ⁺ (–)
Tween 40	+	v [–] (–)
DNA	–	v (–)
Urea	–	+
Growth at 8 % NaCl	–	+
Acid from:		
Galactose	–	v ⁺ (+)
Glucose, lactose	–	+
Maltose, xylose	–	v ⁺ (+)
Rhamnose	–	v [–] (+)
Mannitol	–	v [–] (–)
Susceptibility to:		
Ampicillin, carbenicillin	–	+
Tetracycline	+	– (–)

of the DNA was 40.0 mol% (as determined by the thermal denaturation method).

To establish the precise taxonomic position of strain KMM 3674^T, its 16S rDNA sequence (1381 bp) was determined. Part of this determined sequence (1365 bp) was used for comparative analysis with published sequences of related representatives of the CFB phylum. Analysis revealed that strain KMM 3674^T belonged phylogenetically to the family *Flavobacteriaceae* and formed a distinct subline within the genus *Arenibacter* (Fig. 1). The level of 16S rDNA sequence similarity of strain KMM 3674^T with strains of the single species of the genus *Arenibacter*, *A. latericius*, was 94.9–95.0 %.

Similarities in physiological characteristics and fatty acid and menaquinone compositions support the inclusion of strain KMM 3674^T in the genus *Arenibacter*. However, strain KMM 3674^T differs from the only currently described

Fig. 1. Phylogenetic position of strain KMM 3674^T among marine species of the family Flavobacteriaceae on the basis of 16S rDNA sequence comparison. The phylogenetic tree was generated by the neighbour-joining method (Saitou & Nei, 1987). *Rhodothermus marinus* (X80994) was used as the outgroup. Numbers shown next to each node indicate the percentage bootstrap value of 1000 replicates (only 70 % or higher are cited). Bar, 0.02 genetic distance (K_{nuc}).

species of this genus, *A. latericius*, by positive reactions for gelatin hydrolysis and H₂S production, absence of growth at 8 % NaCl and urea degradation, lack of acid formation from carbohydrates, resistance to ampicillin and carbenicillin and susceptibility to tetracycline, as shown in Table 1. According to Stackebrandt & Goebel (1994), strains may belong to a separate species if their 16S rDNA sequences differ by >3 %, i.e. they have <97 % sequence similarity. The divergence of the 16S rDNA sequences of strain KMM 3674^T and the type strain of *A. latericius*, KMM 426^T, is 5 %. The DNA G + C contents of KMM 3674^T and *A. latericius* strains are 40.0 and 37.5–38.2 mol%, respectively. The phenotypic features mentioned (Table 1), in association with molecular differences, allow the differentiation of strain KMM 3674^T from strains of *A. latericius*.

Thus, we propose that strain KMM 3674^T should be placed in the genus *Arenibacter* as *Arenibacter troitsensis* sp. nov.

Description of *Arenibacter troitsensis* sp. nov.

Arenibacter troitsensis (tro.it.sen'sis. N.L. adj. *troitsensis* referring to Troitsa Bay, from where the organism was isolated).

Cells are Gram-negative, strictly aerobic with respiratory metabolism, chemo-organotrophic, non-motile and asporogenic rods, 0.4–0.7 µm wide and 3–5 µm long. Oxidase-, catalase- and alkaline phosphatase-positive. Colonies are circular, low convex, shiny with entire edges and 1–3 mm in diameter on Marine Agar 2216. Dark orange, non-diffusible pigments are produced. No growth is observed without Na⁺. Growth occurs at 1–6 % NaCl. Flexirubin pigments are absent. Growth occurs at 10–42 °C. Gelatin and Tween

40 are degraded. Agar, casein, starch, alginic acids, cellulose (CM-cellulose and filter paper), chitin, urea, Tween-20 and Tween-80 are not hydrolysed. No acid is formed from arabinose, galactose, glucose, lactose, maltose, melibiose, rhamnose, sucrose, xylose, adonitol, dulcitol, glycerol, inositol or mannitol. Arabinose, glucose, lactose, mannose and sucrose are utilized as sole sources of carbon and energy. Utilization of inositol, mannitol, sorbitol, citrate, fumarate and malonate is not observed. Nitrate reduction and H₂S production are positive. Indole and acetoin (Voges-Proskauer reaction) are not produced. Susceptible to oleandomycin, lincomycin and tetracycline. Resistant to ampicillin, carbenicillin, kanamycin, benzylpenicillin, neomycin, streptomycin, gentamicin and polymyxin B. Predominant cellular fatty acids are C_{15:0}, i-C_{15:1}, i-C_{15:0}, C_{16:1ω7} and i-C_{17:1}. Major isoprenoid quinone is MK-6. The G + C content of the DNA is 40.0 mol%.

The type strain is KMM 3674^T (=JCM 11736^T). Isolated from a bottom sediment sample from Troitsa Bay in the Gulf of Peter the Great, Sea of Japan.

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