

Arenibacter palladensis sp. nov., a novel marine bacterium isolated from the green alga *Ulva fenestrata*, and emended description of the genus *Arenibacter*

Olga I. Nedashkovskaya,¹ Marc Vancanneyt,² Ilse Cleenwerck,² Cindy Snauwaert,² Seung Bum Kim,³ Anatoly M. Lysenko,⁴ Lyudmila S. Shevchenko,¹ Kang Hyun Lee,⁵ Myung Soo Park,⁶ Galina M. Frolova,¹ Valery V. Mikhailov,¹ Kyung Sook Bae⁶ and Jean Swings²

Correspondence

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

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

²BCCM/LMG Bacteria Collection, Laboratory of Microbiology, Faculty of Sciences, Ghent University, Ledeganckstraat 35, B-9000 Ghent, Belgium

³Department of Microbiology, Chungnam National University, 220 Gung-dong, Yusong, Daejeon 305-764, Republic of Korea

⁴Institute of Microbiology of the Russian Academy of Sciences, Pr. 60 Let October 7/2, 117811 Moscow, Russia

⁵Department of Applied Microbiology, College of Agriculture and Life Sciences, Chungnam National University, 220 Gung-dong, Yuseong, Daejeon 305-764, Republic of Korea

⁶Korea Institute of Bioscience and Biotechnology, 52 Oun-dong, Yusong, Daejeon 305-333, Republic of Korea

The taxonomic position of three novel, marine, heterotrophic, aerobic, pigmented, gliding bacteria, isolated from the green alga *Ulva fenestrata* in the Sea of Japan, was determined. 16S rRNA gene sequence analysis revealed that the strains belong to the genus *Arenibacter*. The results of DNA–DNA hybridization experiments supported by phenotypic and chemotaxonomic data showed that the isolates represent a novel species of the genus *Arenibacter*, for which the name *Arenibacter palladensis* sp. nov. is proposed. The type strain is KMM 3961^T (= LMG 21972^T = CIP 108849^T).

The genus *Arenibacter* belongs to the family *Flavobacteriaceae* (Bernardet *et al.*, 2002) and currently comprises three species: *Arenibacter latericius*, *Arenibacter troitsensis* and *Arenibacter certesii* (Ivanova *et al.*, 2001; Nedashkovskaya *et al.*, 2003a, 2004). Members of the genus are Gram-negative, aerobic, heterotrophic and dark-orange-pigmented marine bacteria. These organisms were isolated from various marine environments, including bottom-sediment samples, the brown alga *Chorda filum*, the green alga *Ulva fenestrata* and the edible holothurian *Apostichopus japonicus*. The genera *Muricauda* (Bruns *et al.*, 2001) and *Zobellia* (Barbeyron *et al.*, 2001) are the closest phylogenetic relatives of the *Arenibacter* species. Novel phenotypic findings and improved determination of some previously

described phenotypic properties justify emended descriptions of the genus *Arenibacter* and *A. latericius*.

During June 2000 we isolated three novel strains, KMM 3961^T, KMM 3979 and KMM 3980, from the green alga *Ulva fenestrata*, which was collected in Pallada Bay, Gulf of Peter the Great, Sea of Japan. A polyphasic taxonomic study of the algal isolates, cultured on marine agar 2216 (Difco), indicated that they represent a novel species of the genus *Arenibacter*.

The phylogenetic position of strain KMM 3961^T was determined by analysis of the complete 16S rRNA gene sequence. Genomic DNA was prepared according to the protocol of Niemann *et al.* (1997). 16S rRNA gene amplification, purification and sequencing were performed as described by Vancanneyt *et al.* (2004) but with the following modifications: PCR-amplified 16S rRNA genes

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Arenibacter palladensis* KMM 3961^T is AJ575643.

were purified by using a NucleoFast 96 PCR Clean-up kit (Macherey-Nagel). Sequencing reactions were performed by using a BigDye terminator cycle sequencing kit (Applied Biosystems) and purified using a Montage SEQ₉₆ Sequencing Reaction Clean-up kit (Millipore). Electrophoresis of sequence reaction products was performed by using an ABI Prism 3100 genetic analyser (Applied Biosystems). Sequence assembly was performed using the program Auto-Assembler (Applied Biosystems). The 16S rRNA gene sequence (continuous stretch of 1476 bp) and sequences of strains retrieved from EMBL were aligned and a phylogenetic tree was constructed by the neighbour-joining method, using the BIONUMERICS software package, version 3.50 (Applied Maths). Unknown bases were excluded from the analyses. Bootstrapping analysis was undertaken to test the statistical reliability of the topology of the neighbour-joining tree: 500 bootstrap resamplings of the data were performed (Fig. 1). A comparative analysis revealed that strain KMM 3961^T is affiliated to the genus *Arenibacter*, a member of the family *Flavobacteriaceae* (Fig. 1). The levels of 16S rRNA gene sequence similarity between strains KMM 3961^T and *A. certesii* KMM 3941^T, *A. latericius* KMM 426^T and *A. troitsensis* KMM 3674^T were 94·8, 95·1 and 99·7 %, respectively.

The DNA G + C contents of strains KMM 3961^T, *A. certesii* KMM 3941^T, *A. latericius* KMM 426^T and *A. troitsensis* KMM 3674^T were determined. Strains were cultivated on marine agar for 24 h at 37 °C. DNA was extracted from

0·75–1·25 g cells (wet weight), using the DNA-extraction protocol of Wilson (1987), as modified by Cleenwerck *et al.* (2002). Cells were lysed in a Tris/EDTA buffer (10 mM Tris/HCl with up to 200 mM EDTA, pH 8·0) containing RNase A (Sigma), SDS (Serva) and proteinase K (Merck) to final concentrations of 400 µg ml⁻¹, 2 % (w/v) and 200 µg ml⁻¹, respectively. NaCl (5 M stock solution) and CTAB/NaCl solution (10 % w/v CTAB in 0·7 M NaCl) were added to final concentrations of 1 M and 13·3 %, v/v, respectively. For determination of the DNA G + C content, DNA was enzymically degraded into nucleosides as described by Mesbah *et al.* (1989). The nucleoside mixture obtained was then separated by HPLC using a Waters Symmetry Shield C8 column maintained at a temperature of 37 °C. The solvent was 0·02 M NH₄H₂PO₄ (pH 4·0) with 1·5 % acetonitrile. Non-methylated phage λ DNA (Sigma) was used as the calibration reference.

The DNA G + C contents for all strains tested were in the range 37–39 mol%. Slightly higher values (39–40 mol%) were obtained when the DNAs of strains KMM 3961^T, KMM 3979 and KMM 3980 were isolated using the method of Marmur (1961) and when the DNA G + C contents were determined by the thermal denaturation method (Marmur & Doty, 1962).

DNA–DNA hybridizations between strains KMM 3961^T, *A. certesii* KMM 3941^T, *A. latericius* KMM 426^T and *A. troitsensis* KMM 3674^T were performed; the DNA was

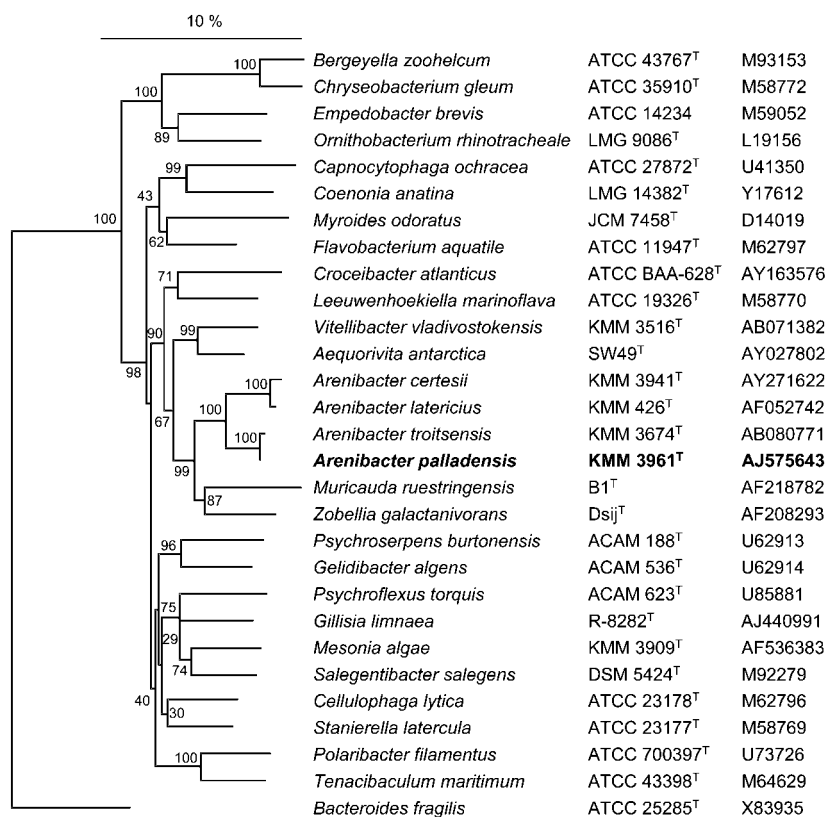


Fig. 1. Neighbour-joining evolutionary distance phylogenetic tree based on the 16S rRNA gene sequences of KMM 3961^T and representative members of related genera of the family *Flavobacteriaceae*. The topology of the tree was not changed in the least-squares or maximum-likelihood trees. Numbers at nodes indicate bootstrap values (%). Bar, 0·1 substitutions per nucleotide position.

prepared as described above. The microplate method was used as described by Ezaki *et al.* (1989) and Goris *et al.* (1998), using an HTS7000 BioAssay Reader (Perkin Elmer) for the fluorescence measurements. Biotinylated DNA was hybridized with single-stranded unlabelled DNA non-covalently bound to microplate wells. Hybridizations were performed at 35 °C in a hybridization mixture [2 × SSC, 5 × Denhardt's solution, 2.5 % dextran sulfate, 50 % formamide, denatured low-molecular-mass salmon sperm DNA (100 µg ml⁻¹) and biotinylated probe DNA (1250 ng ml⁻¹)]. Each hybridization experiment was performed in triplicate. A binding level of 62 % was found between strains KMM 3961^T and *A. troitsensis* KMM 3674^T, indicating that KMM 3961^T represents a separate species. The latter two strains had low values for binding (6–20 %) with *A. certesii* KMM 3941^T and *A. latericius* KMM 426^T. To determine the levels of DNA relatedness between strains KMM 3961^T, KMM 3979 and KMM 3980, DNA was isolated by the method of Marmur (1961) and DNA–DNA hybridizations were performed spectrophotometrically using the initial renaturation rate method described by De Ley *et al.* (1970). The levels of DNA–DNA binding between strains KMM 3961^T, KMM 3979 and KMM 3980 were found to be 96–99 %. The results of the DNA–DNA hybridization experiments indicate that the strains under study represent a separate and novel *Arenibacter* species (Wayne *et al.*, 1987).

To determine their whole-cell fatty acid profiles, strain KMM 3961^T, *A. certesii* strain KMM 3941^T, *A. latericius* strains KMM 426^T, KMM 3522, KMM 3523, KMM 3528 and KMM 3557 and *A. troitsensis* strain KMM 3674^T were grown at 25 °C for 48 h on marine agar. Analysis of the fatty acid methyl esters was carried out according to the standard protocol of the Microbial Identification System (Microbial ID). The predominant cellular fatty acids of KMM 3961^T were of the straight-chain unsaturated, branched-chain unsaturated and saturated types: iso-C_{15:0} (8.7 %), iso-C_{15:1} (12.7 %), C_{15:0} (15.0 %), iso-C_{17:0} 3-OH (17.4 %) and summed feature 3 (11.1 %; comprising iso-C_{15:0} 2-OH and/or C_{16:1ω7}) (Table 1). The presence of a significant amount of iso-C_{17:0} 3-OH (6.9–21.9 %) in all strains tested should be emphasized as it is one of the characteristic fatty acids of members of the family *Flavobacteriaceae*. Previously, Ivanova *et al.* (2001) had reported the absence of hydroxy fatty acids in *Arenibacter* strains.

Isoprenoid quinones were extracted from lyophilized cells and analysed as described by Akagawa-Matsushita *et al.* (1992). Menaquinones were detected by using monitoring at 270 nm and were identified by comparison with known quinones from the reference strain *Salegentibacter salegens* DSM 5424^T. The main isoprenoid quinone was MK-6.

The physiological and biochemical properties of strains KMM 3961^T, KMM 3979 and KMM 3980 were examined as described by Nedashkovskaya *et al.* (2003b, 2004). Physiological and biochemical properties of strain KMM 3961^T were also determined using API 20E, API 20NE and API ZYM galleries (bioMérieux) and the Biolog GN2

Table 1. Cellular fatty acid compositions (percentage content) of *Arenibacter* species

Taxa: 1, *A. palladensis* sp. nov. KMM 3961^T; 2, *A. certesii* KMM 3941^T; 3, *A. latericius* (range for four strains; value for the type strain in parentheses); 4, *A. troitsensis* KMM 3674^T. Only fatty acids accounting for more than 1.0 % for one of the strains are indicated. Summed feature 3 consisted of one or more of the following fatty acids (which could not be separated by the Microbial Identification System): iso-C_{15:0} 2-OH, C_{16:1ω7c} and C_{16:1ω7t}.

Fatty acid	1	2	3	4
iso-C _{15:0}	8.7	7.7	6.9–15.8 (8.1)	6.8
anteiso-C _{15:0}	3.3	6.3	4.8–13.5 (9.3)	3.2
iso-C _{15:1}	12.7	7.2	4.9–14.0 (14.0)	12.2
anteiso-C _{15:1}	0.5	0.8	0.6–2.9 (2.9)	0.6
C _{15:0}	15.0	11.5	4.2–16.0 (14.2)	13.6
C _{15:1ω6c}	2.6	1.9	1.0–2.3 (2.3)	1.2
iso-C _{16:0}	0.2	1.7	0.5–1.3 (0.5)	0.3
C _{16:0}	0.6	1.0	1.1–2.7 (1.9)	1.5
iso-C _{17:1ω9c}	4.0	4.7	2.2–4.6 (2.9)	5.3
C _{17:1ω8c}	0.5	2.4	0.5–2.0 (1.3)	0.9
C _{17:1ω6c}	1.4	3.0	0.7–2.9 (2.4)	1.1
C _{15:0} 2-OH	0.4	0.6	0.7–1.0 (0.6)	0.4
iso-C _{15:0} 3-OH	5.3	3.5	4.6–5.7 (5.6)	5.1
C _{15:0} 3-OH	2.2	0.6	0.0–1.4 (0)	1.6
iso-C _{16:0} 3-OH	1.6	7.2	2.1–5.7 (2.1)	2.2
C _{16:0} 3-OH	2.0	0.8	0.6–1.3 (1.3)	2.2
iso-C _{17:0} 3-OH	17.4	13.3	6.9–14.4 (6.9)	21.9
C _{17:0} 2-OH	1.0	3.8	2.1–5.1 (2.1)	1.7
Summed feature 3	11.1	13.5	9.8–11.9 (9.8)	9.6

Microplate system according to the manufacturers' instructions.

The physiological, morphological and biochemical characteristics of the strains studied are listed in the species description and in Table 2. Similarities in the phenotypic characteristics support the inclusion of strains KMM 3961^T, KMM 3979 and KMM 3980 in the genus *Arenibacter*. However, the three strains differ clearly from currently described *Arenibacter* species by their ability to move on substrate surfaces by means of gliding and to grow in media containing no sea water or Na⁺ ions. Also, the novel isolates cannot reduce nitrates to nitrites or form acid from D-lactose or L-raffinose, in contrast to the other *Arenibacter* strains tested in this study. Moreover, only strains KMM 3961^T, KMM 3979 and KMM 3980 produced acid from DL-xylose and were resistant to oleandomycin. The novel bacteria can be further differentiated from *A. latericius* and *A. certesii* by the absence of urea hydrolysis, by the lack of susceptibility to ampicillin and by the higher G + C content of the DNA (Table 2). Other phenotypic characteristics, such as growth at 8 % NaCl, the maximum growth temperature (38 °C), oxidation of D-galactose, D-glucose, D-melibiose and N-acetylglucosamine, resistance to tetracycline and the absence of Tween 40 hydrolysis and H₂S

Table 2. Phenotypic characteristics of *A. palladensis* sp. nov. and other *Arenibacter* species

Taxa: 1, *A. palladensis* (three strains); 2, *A. latericius* (five strains; results for the type strain in parentheses); 3, *A. certesii* KMM 3941^T; 4, *A. troitsensis* KMM 3674^T. All of the strains were positive for the following characteristics: respiratory-type metabolism, oxidase, catalase, acid and alkaline phosphatases, α - and β -galactosidases, α - and β -glucosidases, *N*-acetylglucosaminidase, esterase lipase (C8), leucine, valine and cystine arylamidases, trypsin, α -chymotrypsin, naphthol-AS-BI-phosphohydrolase, α -mannosidase and α -fucosidase activities, growth with 1–6 % NaCl and at 10–32 °C, acid formation from D-maltose, D-cellobiose and D-sucrose, utilization of L-arabinose, D-glucose, D-lactose and D-mannose, susceptibility to lincomycin and resistance to kanamycin, benzylpenicillin, gentamicin, neomycin, streptomycin and polymyxin B. All of the strains were negative for the following characteristics: requirement for organic growth factors, production of flexirubin-type pigments, indole and acetoin, degradation of agar, casein, starch, alginic acids, cellulose (CM-cellulose and filter paper) and chitin, acid production from L-arabinose, L-sorbose, adonitol, dulcitol, inositol, mannitol, malate, fumarate and citrate and utilization of inositol, mannitol, sorbitol, malonate and citrate. Abbreviations: +, positive; –, negative; V, variable (<60 % strains positive); v⁺, >60 % strains positive; v[–], >60 % strains negative.

Characteristic	1	2	3	4
Gliding motility	+	–	–	–
Na ⁺ requirement for growth	–	+	+	+
Nitrate reduction	–	+	+	+
H ₂ S production	–	–	–	+
Degradation of:				
Gelatin	–	–	–	+
DNA	–	v [–] (–)	–	–
Urea	–	+	+	–
Tween 20	–	v ⁺ (–)	–	–
Tween 40	v	v [–] (–)	–	+
Growth with:				
8 % NaCl	+	+	+	–
10 % NaCl	+	–	+	–
Growth at 42 °C	–	+	–	+
Acid production from:				
D-Galactose, D-glucose	+	+	+	–
D-Lactose, L- raffinose	–	+	+	+
D-Melibiose	+	v ⁺ (+)	+	–
L-Fucose	+	v ⁺ (–)	+	+
L-Rhamnose	+	v [–] (+)	–	–
DL-Xylose	+	–	–	–
Glycerol	–	+	–	–
N-Acetylglucosamine	+	v [–] (+)	+	–
Production of:				
Esterase (C4), lipase (C14)	+	–	–	–
β -Glucuronidase	+	–	–	+
Susceptibility to:				
Ampicillin	–	+	+	–
Carbenicillin	–	+	–	–
Oleandomycin	–	+	+	+
Tetracycline	–	–	–	+
DNA G + C content (mol%)	40	37–38	38	40

production also distinguish the novel isolates from the closest relative, *A. troitsensis* (Table 2).

The above-mentioned phenotypic features (Table 1), in association with molecular divergence, support strongly the differentiation of the strains studied from the *Arenibacter* species with validly published names.

Originally, the genus *Arenibacter* was described as consisting of non-gliding bacteria requiring Na⁺ ions for growth and unable to decompose gelatin (Ivanova *et al.*, 2001). In the course of our study of these novel algal isolates and *A. troitsensis* KMM 3674^T, several phenotypic traits were found to be helpful for the differentiation of *Arenibacter* species.

Thus, we propose the placement of strains KMM 3961^T, KMM 3979 and KMM 3980 in the genus *Arenibacter*, as *Arenibacter palladensis* sp. nov., and the emendation of the description of the genus *Arenibacter*.

Emended description of the genus *Arenibacter* Ivanova et al. 2001

This description is based on that of Ivanova et al. (2001). Some species may display gliding motility and grow without sea water or Na⁺ ions. Aerobic. Produce non-diffusible carotenoid pigments. Cytochrome oxidase-, catalase- and alkaline phosphatase-positive. The major respiratory quinone is MK-6. The predominant cellular fatty acids are straight-chain saturated and unsaturated and branched-chain unsaturated fatty acids C_{15:0}, iso-C_{15:0}, iso-C_{15:1}, iso-C₁₇ 3-OH and summed feature 3 (comprising iso-C_{15:0} 2-OH and/or C_{16:1}ω7). The main polar lipid is phosphatidylethanolamine. The type species is *Arenibacter latericius*.

Description of *Arenibacter palladensis* sp. nov.

Arenibacter palladensis (pal.la.den'sis. N.L. masc. adj. *palladensis* pertaining to Pallada Bay, where the first strains were isolated).

The main characteristics are the same as those given for the genus. In addition, cells range from 0.4 to 0.5 µm in width and from 1.6 to 2.3 µm in length, and move by means of gliding. On marine agar, colonies are 2–4 mm in diameter, circular with entire edges and dark orange in colour. Growth is observed at 4–38 °C. The optimal temperature for growth is 23–25 °C. Growth occurs at 0–10% NaCl. Does not hydrolyse agar, casein, gelatin, alginate, starch, Tween 20, DNA, urea, cellulose (CM-cellulose and filter paper) or chitin. Forms acid from D-glucose, D-lactose, D-maltose and D-sucrose, but not from L-arabinose, D-galactose, L-sorbose, N-acetylglucosamine, citrate, adonitol, dulcitol, glycerol, inositol or mannitol. Utilizes L-arabinose and D-mannose, but not mannitol, inositol, sorbitol, malonate or citrate. Nitrate is not reduced. Indole, H₂S and acetoin (Voges–Proskauer reaction), arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase are not produced. According to the results of testing with the Biolog GN2 Microplate system, the type strain utilizes dextrin, cellobiose, D-fructose, L-fucose, D-galactose, gentiobiose, α-D-glucose, α-lactose, α-D-lactose, lactulose, maltose, D-mannose, D-melibiose, methyl β-D-glucoside, D-raffinose, sucrose, D-trehalose, turanose, methylpyruvate, α-ketobutyric acid, DL-lactic acid, N-acetyl-D-glucosamine, L-glutamic acid, L-threonine and glucose 1-phosphate. Does not utilize α-cyclodextrin, Tween 40 or 80, adonitol, L-arabinose, D-arabitol, i-erythritol, myo-inositol, D-mannitol, psicose, L-rhamnose, xylitol, monomethyl succinate, acetic acid, citric acid, formic acid, D-galactonic acid lactone, D-gluconic acid, D-glucosaminic acid, D-glucuronic acid, α- and β-hydroxybutyric acids, p-hydroxyphenylacetic acid, itaconic acid, α-ketoglutaric acid, α-ketovaleric acid, malonic acid, propionic acid, quinic acid, D-saccharic acid,

sebacic acid, succinic acid, bromosuccinic acid, succinamic acid, glucuronamide, alaninamide, D-alanine, L-alanyl glycine, L-asparagine, glycyl L-glutamic acid, L-histidine, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-pyrroglutamic acid, D- and L-serine, DL-carnitine, γ-aminobutyric acid, uronic acid, inosine, uridine, thymidine, phenylethylamine, putrescine, 2-aminoethanol, 2,3-butane-diol, glycerol or glucose 6-phosphate. The predominant fatty acids in the type strain are straight-chain saturated and unsaturated and branched-chain unsaturated fatty acids C_{15:0} (15.0%), iso-C_{15:0} (8.7%), iso-C_{15:1} (12.7%), iso-C₁₇ 3-OH (17.4%) and summed feature 3 (11.1%; comprising iso-C_{15:0} 2-OH and/or C_{16:1}ω7). The G + C content of the DNA is 39–40 mol%.

The type strain is KMM 3961^T (=LMG 21972^T=CIP 108849^T), isolated from the green alga *U. fenestrata*, collected in Pallada Bay, Sea of Japan.

Emended description of *Arenibacter latericius* Ivanova et al. 2001

The description is as for the genus and is based on that of Ivanova et al. (2001). In addition, cells range from 0.4 to 0.6 µm in width and from 2.1 to 5.0 µm in length. Gliding motility not observed. Growth is detected at 10–42 °C and in 1–8% NaCl. Decomposes urea. Some strains may hydrolyse DNA and Tweens 20 and 40. Does not hydrolyse agar, casein, gelatin, starch, alginic acids, Tween 80, cellulose (CM-cellulose and filter paper) or chitin. Forms acid from D-cellobiose, D-galactose, D-glucose, D-lactose, D-maltose, L-raffinose, D-sucrose and glycerol. Can oxidize D-melibiose, L-fucose, L-rhamnose and N-acetylglucosamine. Does not produce acid from L-arabinose, L-sorbose, DL-xylose, adonitol, inositol, dulcitol, mannitol, malate, fumarate or citrate. According to the Biolog system, α-D-glucose, L-glutamic acid, L-ornithine, uridine, glycerol, DL-α-glycerol phosphate, glucose 1-phosphate, glucose 6-phosphate are utilized. Dextrin, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, cellobiose, D-fructose, α-D-lactose, α-D-lactose lactulose, glycogen, maltose, D-mannitol, D-mannose, D-melibiose, methyl β-D-glucoside, D-raffinose, sucrose, D-trehalose, DL-lactic acid, turanose, succinic acid, glucuronamide, alaninamide, L-alanine, L-alanyl-glycine, L-asparagine, L-aspartic acid, glycyl L-aspartic acid, glycyl L-glutamic acid, L-proline, D- and L-serine and L-threonine are weakly utilized. Susceptible to erythromycin, ampicillin, carbenicillin, oleandomycin, cephaloridin and lyncomycin. Not susceptible to kanamycin, benzylpenicillin, oxacillin, neomycin, streptomycin, gentamicin, polymyxin B or tetracycline. Hydrogen sulfide, indole and acetoin are not produced. Nitrates are reduced to nitrites. The cellular fatty acids are predominantly odd-numbered and iso-branched (about 70%): C_{15:0} (4.2–16.0%), iso-C_{15:0} (6.9–15.8%), anteiso-C_{15:0} (4.8–13.5%), iso-C_{15:1} (4.9–14.0%), iso-C_{15:0} 3-OH (4.6–5.7%), iso-C_{17:0} 3-OH (6.9–14.4%) and summed feature 3 (9.8–11.9%; comprising C_{16:1}ω7 and/or iso-C_{15:0} 2-OH).

The type strain is KMM 426^T (=VKM B-2137D^T=LMG 19693^T=CIP 106861^T).

Acknowledgements

This research was supported by grants from the Federal Agency for Science and Innovations of the Ministry for Education and Sciences of the Russian Federation [nos RI-26/109 (2-2.16) and 112/001/724], the Russian Foundation for Basic Research (no. 05-04-48211) and the Presidium of the Russian Academy of Sciences 'Molecular and Cell Biology'.

References

- Akagawa-Matsushita, M., Itoh, T., Katayama, Y., Kuraishi, H. & Yamasato, K. (1992). Isoprenoid quinone composition of some marine *Alteromonas*, *Marinomonas*, *Deleya*, *Pseudomonas* and *Shewanella* species. *J Gen Microbiol* **138**, 2275–2281.
- Barbeyron, T., L'Haridon, S., Corre, E., Kloareg, B. & Potin, P. (2001). *Zobellia galactanovorans* gen. nov., sp. nov., a marine species of *Flavobacteriaceae* isolated from red alga, and classification of [*Cytophaga*] *uliginosa* (ZoBell and Upham 1944) Reichenbach 1989 as *Zobellia uliginosa* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **51**, 985–997.
- Bernardet, J.-F., Nakagawa, Y. & Holmes, B. (2002). Proposed minimal standards for describing new taxa of the family *Flavobacteriaceae* and emended description of the family. *Int J Syst Evol Microbiol* **52**, 1049–1070.
- Bruns, A., Rohde, M. & Berthe-Corti, L. (2001). *Muricauda ruestringensis* gen. nov., sp. nov., a facultatively anaerobic, appendaged bacterium from German North Sea intertidal sediment. *Int J Syst Evol Microbiol* **51**, 1997–2006.
- Cleenwerck, I., Vandemeulebroecke, K., Janssens, D. & Swings, J. (2002). Re-examination of the genus *Acetobacter*, with descriptions of *Acetobacter cerevisiae* sp. nov. and *Acetobacter malorum* sp. nov. *Int J Syst Evol Microbiol* **52**, 1551–1558.
- De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in micro-dilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- Goris, J., Suzuki, K., De Vos, P., Nakase, T. & Kersters, K. (1998). Evaluation of a microplate DNA-DNA hybridization method compared with the initial renaturation method. *Can J Microbiol* **44**, 1148–1153.
- Ivanova, E. P., Nedashkovskaya, O. I., Chun, J. & 7 other authors (2001). *Arenibacter* gen. nov., new genus of the family *Flavobacteriaceae* and description of a new species, *Arenibacter latericius* sp. nov. *Int J Syst Evol Microbiol* **51**, 1987–1995.
- Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.
- Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.
- Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G + C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.
- Nedashkovskaya, O. I., Suzuki, M., Vysotskii, M. V. & Mikhailov, V. V. (2003a). *Arenibacter troitsensis* sp. nov., isolated from marine bottom sediment. *Int J Syst Evol Microbiol* **53**, 1287–1290.
- Nedashkovskaya, O. I., Suzuki, M., Vysotskii, M. V. & Mikhailov, V. V. (2003b). *Reichenbachia agariperforans* gen. nov., sp. nov., a novel marine bacterium in the phylum *Cytophaga-Flavobacterium-Bacteroides*. *Int J Syst Evol Microbiol* **53**, 81–85.
- Nedashkovskaya, O. I., Kim, S. B., Han, S. K., Lysenko, A. M., Mikhailov, V. V. & Bae, K. S. (2004). *Arenibacter certesii* sp. nov., a novel marine bacterium isolated from the green alga *Ulva fenestrata*. *Int J Syst Evol Microbiol* **54**, 1173–1176.
- Niemann, S., Puehler, A., Tichy, H.-V., Simon, R. & Selbitschka, W. (1997). Evaluation of the resolving power of three different DNA fingerprinting methods to discriminate among isolates of a natural *Rhizobium meliloti* population. *J Appl Microbiol* **82**, 477–484.
- Vancanneyt, M., Mengaud, J., Cleenwerck, I. & 7 other authors (2004). Reclassification of *Lactobacillus kefirgranum* Takizawa *et al.* 1994 as *Lactobacillus kefirnofaciens* subsp. *kefirgranum* subsp. nov. and emended description of *L. kefirnofaciens* Fujisawa *et al.* 1988. *Int J Syst Evol Microbiol* **54**, 551–556.
- Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). International Committee on Bacterial Systematics. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.
- Wilson, K. (1987). Preparation of genomic DNA from bacteria. In *Current Protocols in Molecular Biology*, pp. 2.4.1–2.4.5. Edited by F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl. New York: Green Publishing and Wiley-Interscience.